

Tree-soil-water relations under elevated CO₂ – following the flow.

By

Susan Elizabeth Quick



A thesis submitted to the University of Birmingham for the degree of

DOCTOR OF PHILOSOPHY

School of Geography, Earth and Environmental Sciences

College of Life and Environmental Sciences

The University of Birmingham

January 2025

University of Birmingham Research Archive e-theses repository



This unpublished thesis/dissertation is under a Creative Commons Attribution 4.0 International (CC BY 4.0) licence.

You are free to:

Share — copy and redistribute the material in any medium or format

Adapt — remix, transform, and build upon the material for any purpose, even commercially.

The licensor cannot revoke these freedoms as long as you follow the license terms.

Under the following terms:



Attribution — You must give appropriate credit, provide a link to the license, and indicate if changes were made. You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.

No additional restrictions — You may not apply legal terms or technological measures that legally restrict others from doing anything the license permits.

Notices:

You do not have to comply with the license for elements of the material in the public domain or where your use is permitted by an applicable exception or limitation.

No warranties are given. The license may not give you all of the permissions necessary for your intended use. For example, other rights such as publicity, privacy, or moral rights may limit how you use the material.

Unless otherwise stated, any material in this thesis/dissertation that is cited to a third-party source is not included in the terms of this licence. Please refer to the original source(s) for licencing conditions of any quotes, images or other material cited to a third party.

List of publications and media proceedings

Journal articles accepted/ in review for publication:

This Ph.D. resulted in one first-author paper that has been published (Chapter 3). Four coauthor publications have also been contributed to.

Chapter 3: **Quick, S. E.** , Curioni, G., Harper, N.J., Krause, S. and MacKenzie, A.R., 2025. Water usage of old-growth oak at elevated CO₂ in the FACE (Free-Air CO₂ Enrichment) of climate change. *Biogeosciences*, 22(6), pp.1557-1581.<https://doi.org/10.5194/bg-22-1557-2025>

Acknowledgements of attributions for collaborative work are described in section vii.

Other co-author publications not directly reported in this thesis are as follows.

Co-author publications:

Hagan Brown, W., Gloor, E., Fyfe, R., MacKenzie, A. R., Harper, N. J., Ganderton, P., Hart, K., Curioni, G., Quick, S., Davidson, S. J., Yetton, E., Diehl, J. L., and Fauset, S.: Elevated CO₂ Increases the Canopy Temperature of Mature *Quercus robur* (Pedunculate Oak), (2025), *Global Change Biology* 31, no. 11: e70565. <https://doi.org/10.1111/gcb.70565>.

Kemppinen, J., Lembrechts, J. J., Van Meerbeek, K., Carnicer, J., Chardon, N. I., Kardol, P., Lenoir, J., Liu, D., Maclean, I., Pergl, J., Saccone, P., Senior, R. A., Shen, T., Słowińska, S., Vandvik, V., von Oppen, J., Aalto, J., Ayalew, B., Bates, O., Bertelsmeier, C., Bertrand, R., Beugnon, R., Borderieux, J., Brûna, J., Buckley, L., Bujan, J., Casanova-Katny, A., Christiansen, D. M., Collart, F., De Lombaerde, E., De

Pauw, K., Depauw, L., Di Musciano, M., Díaz Borrego, R., Díaz-Calafat, J., Ellis-Soto, D., Esteban, R., de Jong, G. F., Gallois, E., Garcia, M. B., Gillerot, L., Greiser, C., Gril, E., Haesen, S., Hampe, A., Hedwall, P.-O., Hes, G., Hespanhol, H., Hoffrén, R., Hylander, K., Jiménez-Alfaro, B., Jucker, T., Klimes, D., Kolstela, J., Kopecký, M., Kovács, B., Maeda, E. E., Máliš, F., Man, M., Mathiak, C., Meineri, E., Naujokaitis-Lewis, I., Nijs, I., Normand, S., Nuñez, M., Orczewska, A., Peña-Aguilera, P., Pincebourde, S., Plichta, R., **Quick, S.**, Renault, D., Ricci, L., Rissanen, T., Segura-Hernández, L., Selvi, F., Serra-Diaz, J. M., Soifer, L., Spicher, F., Svenning, J.-C., Tamian, A., Thomaes, A., Thoonen, M., Trew, B., Van de Vondel, S., van den Brink, L., Vangansbeke, P., Verdonck, S., Vitkova, M., Vives-Inglá, M., von Schmalensee, L., Wang, R., Wild, J., Williamson, J., Zellweger, F., Zhou, X., Zuza, E. J., and De Frenne, P.: Microclimate, an important part of ecology and biogeography, (2024) *Glob. Ecol. Biogeogr.*, n/a, e13834, <https://doi.org/10.1111/geb.13834>, 2024.

MacKenzie, A. R., Krause, S., Hart, K. M., Thomas, R. M., Blaen, P. J., Hamilton, R. L., Curioni, G., **Quick, S. E.**, Kourmouli, A., Hannah, D. M., Comer-Warner, S. A., Brekenfeld, N., Ullah, S., and Press, M. C.: BIFoR FACE: Water–soil–vegetation–atmosphere data from a temperate deciduous forest catchment, including under elevated CO₂, *Hydrol. Process.* (2021), 35, e14096, <https://doi.org/10.1002/hyp.14096>.

Rabbai, A., Wendt, D. E., Curioni, G., **Quick, S. E.**, MacKenzie, A. R., Hannah, D. M., Kettridge, N., Ullah, S., Hart, K. M., and Krause, S.: Soil moisture and temperature dynamics in juvenile and mature forest as a result of tree growth, hydrometeorological forcings, and drought, *Hydrol. Process.* (2023), 37, e14919, <https://doi.org/10.1002/hyp.14919>.

In review:

Gardner, A, Rob MacKenzie, A. R., Shoaib, A. M., Krause, S., Larsen, J., Quick, S.E., van Wijngaarden, K., Gauthey, A. (2025), Elevated CO₂ buffers negative impacts of heat stress on growth of 180 years-old *Quercus robur*, *Plant Physiology*. (In review)

Promotional Interviews and TV filming:

- Interview with Professor Alice Roberts (2021)
- BBC Gardener's World (2021)

i. Abstract

The climate background to increasing atmospheric carbon dioxide levels alongside other greenhouse gases is described. The effects of these climatic drivers and the broad picture of other related climate impact is outlined. Proportions of carbon-cycle emissions and sinks within the landscape are noted. The relationships between the Water cycle, Carbon cycle and Energy cycle are considered, in particular the water, carbon, energy balance approach used within global climate models.

The role of forests in contributing to emissions and as a sink for carbon is described as a background to the Birmingham Institute of Forest Research Free-air CO₂ enrichment (BIFoR FACE) experiment is described. This project, juxtaposed amongst other related research projects, contributes novel research topics embracing plant-water related themes within this large experiment. The Soil-Plant-Atmosphere continuum model is used to focus the thesis work as a whole.

Firstly, it tackles the enhancement of knowledge concerning daytime transpiration of large (>10cm DBH) mature trees of *Quercus*, *Acer* and *Crataegus* species, under climatic changes within the BIFoR FACE experiment across the three treatment types: elevated carbon dioxide (eCO₂), ambient carbon dioxide (aCO₂), no-infrastructure control (*Ghost*). The measurement of stem xylem sap flow (as a proxy for whole canopy transpiration) in these trees produces both new processed data relating to daytime tree water usage and also sub-daily sap flux data demonstrating tree xylem dynamics under the three experimental conditions across seven seasons of treatment from year1 (2017) to year 7 (2023).

Secondly soil water volumetric water content (VWC) and temperature data are collected for the same seven seasons, enabling relations between soil and plant to

be explored. This exploration is facilitated by using the relative extractable water transformation of soil-water data down to one metre soil depth. Again findings and results from the differing timescales (30-minute, day, month, season, year) are considered and compared with the tree-water results.

Finally leaf measurements of stomatal conductances (enabling leaf transpiration calculation) and leaf temperatures are presented enabling comparison of the three tree species differences and similarities. Sub-daily dynamic patterns of leaf transpiration are compared with the sap flux results for oak and extraction of potential storage water usage available to the leaves is reported for this species.

The broad nature of this study highlights some benefits and limitations of the new knowledge which can be extracted from our manipulation experiment and illustrates how lateral thinking can assist in making effective use of novel data.

ii. Dedication

Many a genius has been slow of growth.

Oaks that flourish for a thousand years do not spring up into beauty like a reed.

George Henry Lewes, (A Spanish Drama, ch. 2)

To the memory of my sister Aileen,

mother Margaret Eileen Mary (nee Murphy)

and my father Reginald Gordon.

*To all those who cannot reach their true potential due to
prejudice, lack of education, disability, disease or deprivation.*

iii. Acknowledgments

Firstly a thank you to the subject of my research, the trees in the BIFoR FACE woods. I asked your permission to drill very fine holes in your stems, to core a few of you for wood characteristics analysis and to give me examples of your leaves, in-situ and cut-twig, to measure. You may not hear this thanks, but this study could not take place without you, I hope you are not too injured by my actions.

My project was based at the BIFoR FACE facility, whose infrastructure and research are supported by the JABBS Foundation, the University of Birmingham, the John Horseman Trust, the Ecological Continuity Trust, and NERC (grant NE/S015833/1). I would also like to thank the additional NERC projects (see Chapters 3, 4, 5), the University of Birmingham (especially the IGI and the PGR Travel Fund) and the British Ecological Society Travel Fund for funding various training and travel aspects of this research.

The whole BIFoR FACE team have helped me to achieve this thesis indirectly, but support from my fellow PhD student dinosaurs (to quote Angeliki Kourmouli), i.e. Aileen Baird, Liam Crowley, Anna Gardner, Angeliki, and Clare Ziegler, plus many of the other co-students recruited during my period of study, has meant my expedition was not endured alone.

Next, I would like to thank my two supervisors for their guidance and support throughout this PhD. I firstly thank Professor A. Rob MacKenzie for accepting me as suitable to undertake a doctoral research project and helping me define the work. Also for patiently guiding me through my learning obstacles and acting as a true mentor to me throughout this PhD study. He consistently made himself available for my

questions, and provided feedback on drafts, despite my lack of academic scientific research background and my insistence on taking on much more than could be completed in one doctoral project. For that, and for sticking by me until the end, I will be forever grateful.

I would like to thank Professor Stefan Krause for his professional sense of humour, his wise guidance, encouragement and unwavering confidence in me, always making sure I was on the right track. Also for sharing his discipline knowledge and bringing me back to earth when I went astray (in the woods).

I would like to extend my sincere thanks to the BIFoR FACE Operations team past and present. Special thanks goes to Nicholas Harper for his technical knowledge which contributed to the success of the sap measurement trial. Secondly for all those involved in helping me to learn how to: a) take tree cores, especially Peter Miles and also with advice from Professor Neil Loader (Swansea University), b) gain appropriate leaf samples, including assistance for in-situ measurement of Ghost trees, especially the arborists and Gael Denny, c) gain access to the canopy through the canopy access system (CAS) or by use of ladders – the whole set of Ops team climbers at the time (i.e. Nick Harper, Peter Miles, Robert Grzesik, Gael Denny, Tom Downes, Kris Hart). For help with software issues and data management, Giulio Curioni and Furkan Tekinay.

For facilitation of help from many sources, for encouraging self-belief and for highly professional management of the FACE facility, I would like to thank Dr Kris Hart, the Operations Manager. For giving me her time, patience, guidance, enthusiasm and support, Deanne Brettell, BIFoR Project Officer.

My many invaluable friendships, support and inspiration from inside and outside the University of Birmingham and the BIFoR FACE team over the past eight plus years have seen me through. COVID-19 was a challenge to mental and physical health which none of us could have easily predicted, but which brought new support networks into play, putting our fragile lives into perspective in these years of climate emergency. As my good friend Eileen said, we are the survivors. We need to act to prevent more casualties.

To my husband, Paul Burke, who, despite his challenge to the cohesion-tension theory, always generously supported my efforts to gain sufficient understanding of biology, forestry and ecology to complete my project. He proof-read early draft chapters to improve the English. He also acted as senior technician to get cabling extensions completed for my oak tree sap measurement campaign, gave me an electronic microscope for looking at samples and lent me essential soldering equipment, cables and miscellanea when stuck in lock-down. He is the love of my life, now for fifty plus years. To the remainder of my family and close friends, especially my academic son, Dr Tom Quick and my outstanding factory manager of a daughter, Alison Burnett, who is also a wonderful loving mother to two boys. Your patience when I was 'too busy to come and see you' has been amazing.

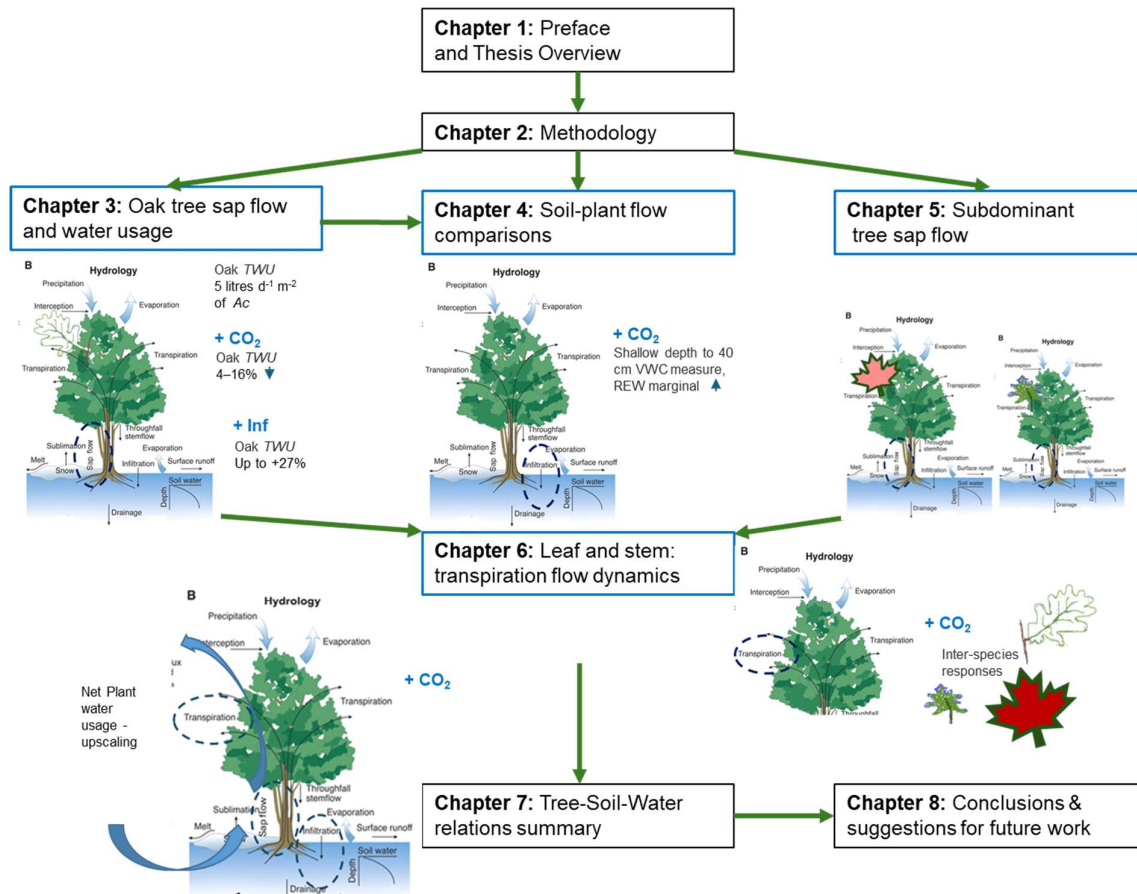
Conference presentations:

- The Birmingham Institute of Forest Research Annual Meeting (2017, 2019, 2020, 2021, 2022, 2023, 2024)
- University of Birmingham postgraduate, HEFi and early career (2017, 2018, 2019, 2024)
- HydroEco2017
- Royal Meteorological Society (2018)
- IUFRO Forests and Water, Chile (2018)
- British Ecology Society Annual Conference (2018, 2021, 2023)
- Ecological Continuity Trust + BES Plants Soils Ecosystems (2019)
- New Phytologist, Early Career (2019)
- IUFRO World Congress (2019)
- Association of Applied Biologists Trees for Future (2021)
- European Geosciences Union (2021, 2022, 2023, 2024)
- Microclimate Ecology and Biogeography (2022)

Other presentations and key outreach:

- Trees in Time e-lecture (2023).
- Understanding Memory of UK Treescapes for Better Resilience and Adaptation (MEMBRA) project Cycles, with artists from the Walking Forest.

iv. Graphical abstract



v. **Table of Contents**

Chapter 1 Preface and Thesis Overview	1
Introduction and motivation	1
The Climate Emergency	1
How Forests respond.....	3
The theory of plant-water usage and transpiration.....	5
Tree-soil-atmosphere water relationships.	8
Mechanistic models of plant hydraulic function.	9
Background and context of FACE experiments	13
Research focus	16
Aims and objectives	17
Project Aims.....	17
Project Objectives.....	17
Thesis overview	18
Main novel contributions	20
References Chapter 1	22
Chapter 2 : Methodology	33
2.1 BIFoR FACE Experiment	33
2.2 Overall methodology	34
2.3 Field Methods.....	35
Environmental and BIFoR FACE measurements.....	37

Soil measurements	38
Sap flow measurement.	42
Tree trait measurements.....	44
Leaf measurement of in-situ and cut-twigg parameters	45
2.4 Methods for laboratory analysis	46
2.5 Methods for data exploration and statistical analysis	46
References Chapters 2	48
Chapter 3 This study Oak tree sap flow and water usage	51
Revised items.	52
Published document follows (25 pages).....	55
References Chapter 3.	56
Chapter 4 : Soil-plant flow comparisons.	73
1 Introduction.....	76
2 Methods.....	82
3 Results	98
4 Discussion and conclusion.	107
References Chapter 4	117
Chapter 5 : Subdominant tree sap flow	127
1 Introduction.....	128
2 Methods.....	134
3 Results	146

4	Discussion	148
	References Chapter 5.	150
	Chapter 6 : Leaf and stem: transpiration flow dynamics	156
1	Introduction.....	158
2	Materials and Methods	164
3	Results	172
4	Discussion	181
5	Concluding summary and further work	188
	Chapter 7 : Tree-Soil-Water relations summary.	202
	Thesis Summary.	202
	Drawing together plant water flow themes	203
	Key findings	206
	Water usage changes in responses to eCO ₂ and due to FACE infrastructure ..	206
	Soil-water dynamics.....	207
	Transpiration derived from leaf and stem water flow measurements.....	209
	Benefits, Limitations and Opportunities.....	210
	Benefits.....	210
	Limitations.....	211
	Opportunities	213
	References Chapter 7	217
	Chapter 8 : Conclusions & suggestions for future work.....	220

Future work	220
Long term effects of eCO ₂ on tree responses	220
Inter-species comparisons	221
Cross and inter forest scaling	221
Development of data: System relations	222
Lessons learnt with respect to monitoring plant and soil hydraulic variables	222
Concluding remarks	223
References Chapter 8	224
Chapter 9 : References	226
Chapter 10 : Appendices	260
Appendix 1: Chapter 1 Supplementary Material.	A1
First page extracts of coauthor publications.	A1
Appendix 2: Chapter Two Supplementary Material.	A6
2-A: Maps of Arrays showing details of precipitation, throughfall, soil moisture/ temperature measurement transducer positioning and of soil sampling areas.	A6
2-B: Detailed method for sap flow probeset installations	A11
2-C: Detailed method for Tree core sampling and processing.	A15
2-D: Detailed method for stomatal conductance and leaf temperature measurement using porometry.	A35
2-E: Report justifying drilling of mature trees	A46

Appendix 3: Chapter 3 Supplementary Material	A58
Appendix 4: Chapter 4 Supplementary Material	A85
Appendix 5: Chapter 6 Supplementary Material	A104

vi. List of Figures

Chapter 1

Figure 1- 1: Global carbon budget as a function of time, for fossil CO₂ emissions (E_{FOS} , including a small sink from cement carbonation; grey) and emissions from land-use change (E_{LUC} ; brown), as well as their partitioning among the atmosphere (G_{ATM} ; cyan), ocean (S_{OCEAN} ; blue), and land (S_{LAND} ; green). Panel shows the cumulative flux (the sum of all prior annual fluxes) since the year 1850. Adapted from Friedlingstein et al., (2023), Fig. 2(b))1

Figure 1- 2: Schematic representation of the overall perturbation of the global carbon cycle caused by anthropogenic activities, averaged globally for the decade 2011–2020. See legends for the corresponding arrows and units. The uncertainty in the atmospheric CO₂ growth rate is very small (0.02 GtC yr⁻¹) and is neglected for the figure. The anthropogenic perturbation occurs on top of an active carbon cycle, with fluxes and stocks represented in the background and taken from Canadell et al. (2022) for all numbers, except for the carbon stocks in coasts which is from a literature review of coastal marine sediments (Price and Warren, 2016). Adapted from Friedlingstein et al., (2023), Fig.2.2

Figure 1- 3: Schematic of the land water balance (left) and land energy balance (right) for a given surface soil layer. Left: dS/dt refers to the change in water content within the layer (soil moisture, surface water, snow; depending on the depth of the layer), P is the precipitation, E is the evapotranspiration, R_s is the surface runoff, and R_g is the drainage. Right: dH/dt refers to the change of energy within the same layer, include all water storage components i.e. temperature change, phase changes associated with soil freezing/melting or snow melt), R_n is the net radiation, λE is the

latent heat flux, SH is the sensible heat flux, and G is the ground heat flux to deeper layers. (based on Fig.4 from Seneviratne <i>et al.</i> , 2010).....	3
Figure 1- 4: The current generation of climate models treats the biosphere and atmosphere as a coupled system. Land surface parameterizations represent the biogeophysics, biogeochemistry, and biogeography of terrestrial ecosystems. (A) Surface energy fluxes and (B) the hydrologic cycle. These are the core biogeophysical processes Circled dotted lines indicate parameters of interest in this thesis. Many models also include (C) the carbon cycle and (D) vegetation dynamics so that plant ecosystems respond to climate change. Some models also include (E) land use and (F) urbanization to represent human alteration of the biosphere. (Based on Bonan, 2008, Fig.2).....	4
Figure 1- 5: To show key aspects of the vadose zone in respect of soil moisture zones and tree-water usage via the root zone. The saturated and unsaturated soil A and B provide distinct soil moisture contributions. From Fig.2 Seneviratne <i>et al.</i> , (2010)	11
Figure 1- 6: (adapted from McElrone <i>et al.</i> (2013) Fig. 4 A: Representation of the water transport pathways along the soil-plant-atmosphere continuum (SPAC). (A) Water moves from areas of high water potential (i.e. close to zero in the soil) to low water potential (i.e., air outside the leaves). Details of the Cohesion-Tension mechanism are illustrated with the inset panels (A), where tension is generated by the evaporation of water molecules during leaf transpiration (1) and is transmitted down the continuous, cohesive water columns (2) through the xylem and out the roots to the soil (3).....	12
Figure 1- 7: Outline of BNL type FACE system (BIFoR, 2024).....	16

Figure 1- 8: Water cycle flow sequence.	18
--	----

Chapter 2

Figure 2-1: Key plant hydraulic related measurements. (a) dominant, (b) subdominant trees. 34	
---	--

Figure 2-2: To show instrumentation status at end 2017 and from 2019, along with zone of the respective instrumentation. Green highlighted items were still being implemented by the author from 2017.....	37
--	----

Figure 2-3: Photo of soil moisture and throughfall set-up near to an oak in a typical array.....	39
--	----

Figure 2-4: installation of soil moisture profile probes	40
--	----

Figure 2-5: Photos of soil temperature installation near to an oak in a typical array..	40
---	----

Figure2-6: Soil sampling of short soil cores.....	41
---	----

Figure 2-7: Sap flow trial - installation in Array 9.	43
--	----

Figure 2-8: Methods of measuring tree size including tape measure for circumference, callipers for stem diameter E-W and N-S and use of clinometer and laser distance device to identify canopy extent (canopy radius) at the four cardinal compass points ((Hemery, Savill and Pryor, 2005)). Average canopy diameter and stem diameter (calculated from circumference measured as above) are combined to calculate average canopy spread diameter and approximate canopy area. Stem height of subdominant trees was measured using the clinometer and laser distance device. Height of oaks was estimated during canopy access in relation to tower height.	45
--	----

Chapter 3

(No table of figures)

Chapter 4

Figure 4- 1: BIFoR FACE Site details showing position of all Arrays and example soil-water and tree-water monitoring points in a three example treatment arrays: Array 5 (eCO₂), Array 6 (eCO₂), Array 7 (*Ghost*).84

Figure 4- 2: Daily precipitation, daily average VWC with ribbon +/-sd by treatment and daily average REW with ribbon +/- sd by treatment, for all shallow soil-moisture data (10-12 cm depth). Top (a), (b), (c) show key period 2018-2021 hydraulic years using REW_{yr} where each hydraulic year is calculated separately for field capacity and wilt point.. Bottom (d), (e), (f) shows complete hydraulic year range 2017-2023 used for REW_{7yr}. where an overall seven-year field capacity and wilt point are extracted. Black box indicates a longer drought period in 2018. Red box indicates a longer hot drought period in 2022. Hydraulic year start (Black dotted lines) 2017-2023 are shown. Red dotted lines indicate start of treatment season in each year.90

Figure 4- 3: (previous two pages) To show correlation between CS655 and Enviroscan shallow VWC, (a) and REW data (b) using hydrological year theta_{ma} and theta_{min}. (a) for VWC and (b) for REW. Seasons 3 to 5 are shown (2019-2021).93

Figure 4- 4: Enviroscan profile for 2019 to 0.4 m depth (i.e. 10cm, 20 cm, 40 cm depth). Data is shown as mean daily value by treatment with +/- std deviation. Graphics are shown against daily precipitation (below). Comparison of VWC with REW in fine root depths in 2019 season. (a) selected infrastructure array positions giving reliable data (A1, A3, A4, A5) are visualised alongside all

Ghost array data with VWC (left) and REW_{yr} (right). Averaged data include all rain period data (i.e. $REW > 1$). (b) selected infrastructure array positions giving reliable data (A1, A3, A4, A5) are visualised with VWC (left) and REW_{5yr} (right). Averaged data exclude rain periods giving $REW > 1$	94
Figure 4- 5: Correlation of daily REW_{yr} data from Enviroscan and Delta-T type profile sensors across treatment season months (labels at top) for each coincident nearest tree position during season 6 (2022). Enviroscan 40cm and 60cm depth data were used to derive 50cm data (depth labels on right).	95
Figure 4- 6: To show range of REW_{5yr} (mean and 95%-iles) across the 5 hydraulic years of the study 2018-2022 when sufficient sensors were working reliably. Horizontal facet shows depths in metres and vertical facet shows hydraulic years. Transducer position are within selected arrays (x-axis labels). Values of $REW_{5yr} > 1$ have been omitted.	99
Figure 4- 7: Visual comparison of Enviroscan infrastructure treatments fine root depth data by box and whisker plots during treatment seasons 2019-2021 for probes giving reliable data (A1, A3, A4, A5). Top facet labels indicate month, side facets indicate sensor depth (to 40cm only). Top row (a) shows VWC. Bottom row (b)shows REW_{5yr} . REW data include all rain period data giving $REW > 1$ so y-axis scales differ between years (e.g. 2021).	100
Figure 4- 8: Showing box and whisker daily REW_{7yr} plots for every month of years 2018-2023 differentiated by treatment to end of treatment season in 2023. Autumn (Nov-Dec) 2017 is omitted but forms a part of hydraulic year for 2018. Rain days are included except where they result in $REW_{7yr} > 1$	102

Figure 4- 9: Histograms and density plots for CS daily REW_{7yr} data for all hydraulic years 2017-2023 both (left) for combined all-year data and (right) for individual hydraulic years. Daily REW_{7yr} data >1 have been removed.	103
Figure 4- 10: Showing shallow VWC drought responses during the longest June-July drought periods. For (a) mid-VWC values (y-axis) during VWC decrease, versus VWC decrease slope ($\% d^{-1}$) during drought (x-axis with suppressed zero), and bottom, (b) average precipitation rate (mm) (y-axis) against VWC decrease slope ($\% d^{-1}$) during drought (x-axis with suppressed zero). Sub-panels are facets of seasons 2 to 7 (2018 to 2023) in both cases.	104
Figure 4- 11: Cumulative mean normalised tree water usage ($TWUn$), (litres $d^{-1} mm^{-1}$), for each of three seasons 2019-2021	105
Figure 4- 12: All three years of interest, 2019, 2020, 2021. Correlative scatterplots REW_{10cm} against $TWUn$. Top figures, (a), (b), (c), are all shallow sensors, middle, (d), (e), (f), are just infrastructure tree and soil data, bottom (g), (h), (i), are no-infrastructure data. (a) to (f) have suppressed y-axis to circa $0.7 l d^{-1} mm^{-1}$.....	106

Chapter 5

Figure 5- 1: Showing pre-build habitat survey results. Note array positions have been changed for build. The figure is based on Table 2 and Figure 2 from WSP Phase 1 Botanical survey A9RCB9B 2014 (BIFoR., 2014).....	136
Figure 5- 2: Initial infrastructure array species survey results for larger trees > 10 cm DBH shown as proportion (%) of total large trees. T indicates all such trees in eCO_2 arrays. T + C indicates all such trees in infrastructure arrays.	

Note some mistaken species identity occurred for hazel trees during first survey.....	137
Figure 5- 3: Sycamore woodslices showing diffuse nature and asymmetry. ..	142
Figure 5- 4: Stage 6 output diurnal sap flux (l s^{-1}) in monthly periods across whole years for two sycamore trees with long probesets. Years 2019-2023 (including treatment seasons 3 to 7) are shown starting at the bottom with 2019 (season 3).....	145
Figure 5- 5: Example sap flux results from individual (E-facing and W-facing) long probesets for three sycamore and one hawthorn tree during July in years 2019 to 2021. Top graphics (orange) are for eCO_2 tree probesets and bottom (green) are for aCO_2 tree probesets.....	146
Figure 5- 6: Example sap flux results from individual (E-facing and W-facing) long probesets for three sycamore and one hawthorn tree (A4_9982) during July in years 2019, 2022 and 2023. Top graphics (orange) are for eCO_2 tree probesets and bottom (green) are for aCO_2 tree probesets.....	147
Figure 5- 7: (based on (Ávila-Lovera, Winter and Goldsmith, 2023), Fig. 2) Showing conceptual representation of the key plant–water relation traits typically analysed. Trait abbreviations: stomatal length (SL), stomatal density (SD), stomatal conductance (g_s), leaf hydraulic conductance (K_{leaf}), vein density (VD), leaf cavitation resistance ($P_{50\text{leaf}}$), leaf area (LA), leaf thickness (LT), leaf turgor loss point (Ψ_{tlp}), relative water content at turgor loss point (RWCT_{tlp}), stem hydraulic conductivity (K_h), stem cavitation resistance ($P_{50\text{stem}}$), and Huber value (HV).....	148

Chapter 6

Figure 6- 1: Schematic showing a) an extract from a model of water flows to/ from the dominant canopy and b) detail of leaf transpiration contribution to the plant -water flow.....163

Figure 6- 2: Environmental daytime canopy parameters across years 2019-2021. Top row: Maximum (yellow) mean (orange) and minimum (brown) daily PAR. 2nd row: maximum T_{atop} , dark red, minimum RH_{top} , green.169

Figure 6- 3: Leaf transpiration ($\text{mg m}^{-2} \text{ s}^{-1}$). Oak panels: (a) infrastructure ($a\text{CO}_2$ and $e\text{CO}_2$) dominant oak data derived from in-situ measurements, all three seasons 2019-2021, (b) oak infrastructure in-situ measurements July and August 2019-2021, and (c) derived from all cut-twig measurements, three seasons 2019-2021 in all treatments. Subdominant hawthorn and sycamore bottom-canopy in-situ leaf transpiration data box and whisker plots from 2020 and 2021 are shown similarly in (d) separated by treatment and species. Note maximum transpiration (y-axis) is $250 \text{ mg H}_2\text{O m}^{-2} \text{ s}^{-1}$ for (a) and (b), but $80 \text{ mg H}_2\text{O m}^{-2} \text{ s}^{-1}$ for (c) and (d). For (a) and (b) Wilcoxon rank sum test results are embedded. Box and whisker plots show median and interquartile range (IQR, 25%ile to 75%ile) with whiskers calculated as $1.5 \times \text{IQR}$ from the hinge and points for outliers. Mean values are calculated from the entire range of data and shown as spots (pink).172

Figure 6- 4: Box and whisker plots showing median and interquartile range (IQR, 25%ile to 75%ile) with whiskers calculated as $1.5 \times \text{IQR}$ from the hinge and points for outliers. Mean values (panels (a), (b) and (c), are calculated from the entire range of data and shown as spots (pink). Comparison of middle-day

infrastructure (aCO₂ and eCO₂) in-situ oak leaf transpiration results through (a) season and (b) July and August with previous whole-tree normalised daylight tree water usage results ((c) season and (d) July)) derived from 30-minute sap flux data (based on Quick et al. 2025, in supplementary information Fig. S 8).

.....178

Figure 6- 5: Comparison of oak (a), (c) middle-day whole-tree transpiration (i.e. sapflux) to (b), (d) middle-day leaf-transpiration for days in mid-season (July & August) during 2020 (a) & (b) and 2021 (c) & (d).180

Figure 6- 6: Comparison between (a) sap flux results from Meinzer et al., (2004) and (b) leaf (top two panels) and sap fluxes of transpiration from BIFoR FACE showing potential characteristics of morning slope and levelling. Data for (b) is taken from 2021 results shown Figure 6-5.181

Appendices

(No table of figures)

vii. List of Tables

Chapter 1

Table 1- 1: Transpiration efficiency/ Water use efficiency. Definitions at differing sensing scales.....	6
--	---

Chapter 2

Table 2- 1: Key core parameters and their instrumentation types.	36
---	----

Chapter 3 (excludes Tables in Publication)

Table 3- 1: Itemised typos and errors relating to publication Quick et al. (2025).	52
---	----

Table 3- 2: Showing the steps taken to determine the best GLMM model for sap flux treatment comparisons. Table 3- 3.....	55
--	----

Chapter 4

Table 4- 1: Instrumentation types and sampling details for soil-water and environmental variables used in this study.	85
--	----

Table 4- 2: Enviroscan infrastructure positions utilised for analysis and all Ghost positions showing failures in particular treatment seasons. OK indicates working sensor, F indicates a failure of one or more depth positions (N.B. A7 probeset had no data from 1 m depth throughout.	96
--	-----------

Table 4- 3: Results from shallow CS sensors. One-way ANOVA for CO ₂ effects. F(df, value), Pr(>F and % difference summary for eCO ₂ REW _{7yr} compared with aCO ₂ REW _{7yr} in years 2018-2023. Daily values are compared, calculated from the entire hydraulic year and for leaf-on season April–October as shown. Bold typeface indicates p-value <0.05.	101
--	-----

Chapter 5

Table 5- 1: Species fitted with dendrometer bands (i.e. greater than 10cm DBH) sited within the new array boundaries. Numbers of trees (% of all species in brackets, average DBH, total biomass are shown as at summer 2023.

Infrastructure figures are taken from Norby *et al.*, 2024. 138

Table 5- 2: Raw and processed data as of January 2025. Note, one hawthorn tree is multi-stemmed with two stems monitored. 138

Table 5- 3: Subdominant trees selected for sap flow monitoring. Long probes have four thermocouples radially into the tree, short have two radially into the tree. Two probesets are fitted to each stem at East-facing and West-facing positions. 138

Table 5- 4: Sycamore 1st leaf, 1st leaf-tint and calculated leaf-on season lengths for seasons 2017 to 2022 based on physical observations of first of such events across the experimental woodland..... 139

Table 5- 5: Calculations stages 1 to 7 showing flow of data processing to obtain TWU from time to heat balance t_z from all differential HPC probeset thermocouple radial positions (from Quick *et al.*, 2025)..... 143

Chapter 6

Table 6- 1: Wilcoxon rank sum test for effects of eCO_2 on oak-leaf transpiration during 2019 to 2021. Whole season and July-Aug only infrastructure (eCO_2 and aCO_2) leaf data are tested for differences. Transpiration and stomatal conductance were separately tested. In-situ and cut-twigs data are included. 173

Table 6- 2: Wilcoxon rank sum test for effects of eCO_2 in 2019 to 2021 from in-situ leaf transpiration data only (top eight rows) compared with ANOVA results

from sap flux data (mean eCO_2 TWU_n, compared with mean aCO_2 TWU_n) in same seasons (bottom four rows). For Wilcoxon tests whole-season and July-Aug only infrastructure (eCO_2 and aCO_2) leaf data are tested for differences.

.....175

Table 6- 3: In-situ Infrastructure only (i.e. combined aCO_2 and eCO_2) oak data linear model for leaf transpiration versus top-canopy PAR. Top four rows show results for each season and combined results for all years. Bottom four rows show results for each mid-season (July-August), and combined results for all years.....175

Table 6- 4: Wilcoxon rank sum test for effects of eCO_2 on subdominant-leaf in-situ bottom-canopy transpiration during 2020 to 2021. For sycamore (left side of table) whole-season and July-Aug eCO_2 versus all control (i.e. aCO_2 and *ghost*) leaf data are tested for differences. For hawthorn all data from years 2020-2021 are combined by treatment (right of table) as no control data were captured during 2020.177

Chapters 7 & 8, Appendices

(No Table of Tables)

viii. List of Symbols

Symbol	Description	Units used in this thesis
A_c	Projected canopy area (area of ground covered by a plant canopy)	m^2
$ASVP$	Air saturated vapour pressure	kPa
A_{sw}	Cross-sectional sapwood area *	cm^2
A_z	Annular ring cross-sectional area at thermocouple z	cm^2
E	leaf transpiration	$mmol\ m^{-2}\ s^{-1}$ or $mg\ H_2O\ m^{-2}\ s^{-1}$
F_L	volume fraction of water element of xylem woody matrix	unitless
F_M	volume fraction of wood element of xylem woody matrix	unitless
g_s	stomatal conductance	$mol\ m^{-2}\ s^{-1}$
G_s	canopy stomatal conductance	$mm\ s^{-1}$
H	heartwood radius	m
J	Sap flux density	$m\ s^{-1}$
J_z	Point sap flux density across xylem sapwood area at measurement point. Unit derivation: $m^3\ (water)\ m^{-2}(xylem\ sapwood\ area)\ s^{-1}$	$m\ s^{-1}$
$LSVP$	leaf saturated vapour pressure	kPa
p	Barometric/ (ambient, top) pressure	hPa
P	Precipitation	mm
P_r	Local precipitation (outside forest).	mm
P_{fs}	Throughfall estimate within treatment season April–Oct	%
P_{is}	Interception estimate within treatment season April–Oct	%
PAR	Photo-synthetically Active Radiation	$\mu mol\ of\ photons\ m^{-2}\ s^{-1}$
Q_p	Probeset volumetric sap flux (across sapwood)	litres s^{-1}

Symbol	Description	Units used in this thesis
Q_T	Whole tree sap flux density	litres s ⁻¹
R	cambium radius	m
R_b	Stem radius	mm
R_n	Net Radiation	Watt m ⁻²
RH_{bot}	Relative humidity (average of Met tower bottom)	%
RH_{top}	Relative humidity (average of Met tower top)	%
r_z	radius of measurement point within sap transducer (z =1 to 4).	m
SVP	Saturated vapour pressure	kPa
T	Temperature	°C
T_{a_bot}	Air temperature at bottom of Met Towers	°C
T_{a_top}	Air temperature at top of Met Towers	°C
T_{air}	Air temperature (°C)	°C
$T_{airFACE}$	Air Temp (Av) - top of FACE towers	°C
T_d	Soil Temperature (depths d=10, 20, 40, 60 cm)	°C
T_L	Leaf temperature (°C)	°C
T_{ss}	Surface soil temperature	°C
TG	Total solar radiation	Watt m ⁻²
TWU	Tree diurnal (dawn to dusk) water usage per day	litres d ⁻¹
$\overline{TWU}$	Monthly mean TWU	litres d ⁻¹
TWU_n	Tree diurnal (dawn to dusk) water usage per day normalised by stem radius at the point of probeset insertion, R_b (mm).	litres d ⁻¹ mm ⁻¹
$\overline{TWU}_n$	Monthly mean TWUn	litres d ⁻¹ mm ⁻¹

Symbol	Description	Units used in this thesis
t_z	Time to heat balance point for one thermocouple pair position (z =1 to 4) in the xylem sap Compensated Heat Pulse (CMP) probeset data	seconds
V_c	Wound compensated heat velocity	m s ⁻¹
VPD_{air}	Vapour pressure deficit (VPD) air	kPa
VPD_L	VPD leaf	kPa
V_s	Raw heat velocity (uncompensated)	mm s ⁻¹
X_d	Vertical distance between heater probe and upper (downstream) sap sensor probe	mm
X_u	Vertical distance between heater probe and lower (upstream) sap sensor probe	mm
θ_{12}	Volumetric water content (VWC) at depth 12cm (CS probes)	%
θ_d	VWC - Enviroscan (depths d = 10, 20, 40, 60, 100cm)	%
θ_{DT}	Volumetric water content - Delta-T (depths d = 20, 50, 100cm)	%
θ_{max}	VWC maxima during no rain – field capacity	%
θ_{min}	VWC minima – permanent wilt point (within leaf-on season)	%

ix. List of Other Abbreviations

Abbreviation	(Note: parameter abbreviations are covered in Symbols list)
A	Assimilation
aCO ₂	Ambient atmospheric CO ₂ level (μmol mol ⁻¹) in infrastructure arrays
adjusted R ²	adjusted coefficient of determination
ANOVA	Analysis of variance
a.s.l.	Above sea level
ASW	$\theta_{max} - \theta_{min}$ available soil water (%)
AWC	Available (soil) water content
BIFoR	Birmingham Institute of Forest Research
CAS	Canopy Access System
Ch.	Chapter or chapters
Conditional R ²	variance explained by the whole model (e.g. ANOVA or glmm)
CI	Confidence interval
CS	Campbell Scientific (trading name)
CT	Cohesion-tension theory
DBH	Diameter at breast height
df	degrees of freedom
doy or DOY	Day of year
eCO ₂	elevated CO ₂ (+150 μmol mol ⁻¹ above ambient in this study) in arrays
EF	exponential filter
FACE	Free-air CO ₂ enrichment
GHG	Greenhouse gases
Ghost	Label for experimental arrays without infrastructure or treatment. Also called 'undisturbed' within BIFoR FACE.
GtC	Gigatonnes of Carbon
Glmm or GLMM	Generalised linear mixed effect model
GPP	Gross primary productivity
gwc	Gravimetric water content
IR	Infra-red
IRGA	Infra-red gas analyser
ITE	Instantaneous transpiration efficiency
LAI	leaf area index
Marginal R ²	variance explained by the fixed effects (e.g. in a glmm model)

Abbreviation	(Note: parameter abbreviations are covered in Symbols list)
Met	meteorological
p	Significance (statistics)
POME	principle of maximum entropy
PPFD or Q	Photosynthetic photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
REW	Relative extractable water
SD	Standard deviation
SE	Standard error
SI	Supplementary Information <i>or</i> International System of Units
SPAC	Soil-Plant-Water Continuum
SWUE	Soil water use efficiency
t	student's t-test statistic, <i>or</i> time
TE	Transpiration efficiency
TH	Tree Height
TWD	Tree water deficit
USO	Unified stomatal optimisation (theory)
VPD	Vapour pressure deficit
VWC or vwc	Volumetric water content
WUE	Water use efficiency

x. Authors Declaration

I, Susan Elizabeth Quick, declare that this thesis entitled 'Tree-soil-water relations under elevated CO₂ – following the flow.' and the work presented are both my own and have been generated by me as the result of my own original research. I confirm that:

- This work was done wholly while in candidature for a doctoral degree at the University of Birmingham.
- Where I have consulted the published work of others, this has clearly been attributed.
- I have acknowledged all main sources of help.
- Where this thesis is based on collaborative work with others, I have made clear what was contributed by others and what I have contributed myself.
- Where chapters of this work have been published or submitted for publication, this has been clearly stated.

Signed:

A handwritten signature in black ink that reads "Susan Quick". The signature is written in a cursive, flowing style.

Susan E. Quick

Date: 24/01/2025

xi The presentation of this thesis

Chapters 3, 4 and 6 are written primarily in the style of journal manuscripts (Ch.3 manuscript is published) each with an Appendix, for supporting information not wholly within the publication scope. I am the lead author on each, with coauthor contributions specified in each instance at the beginning of each for each accepted or drafted document. The publication and submission status of each of these Chapters at thesis submission date is stated at the beginning of each.

Chapters 4 and 5 are at an early stage for a potential future publication. Some of Chapter 5 data are used in Chapter 6. The data are offered as a potential contribution to discussions and research endeavours in the field by other authors.

The work contained in these chapters and elsewhere in this thesis is substantially my own.

Author acknowledgements:

This thesis presents a hybrid 'alternative format' with chapter 3, where I am first author, accepted as peer reviewed research article and chapters 4 and 6 formatted and drafted in preparation as contributions for submission to undecided peer review journals.

Coauthors contributed to the accepted paper where I am first author, as outlined at within Chapter 3 and in the preliminary section of this thesis

xii Acknowledgement of collaborative work

Chapter 3: Oak tree sap flow and water usage

Author contributions: Susan E. Quick designed the study, surveyed and selected the individual trees, installed the instrumentation, collected the manual data (herself) and the logged data (in conjunction with the FACE Operations team). Nicholas Harper provided technical advice on installation. Susan E. Quick analysed the sap data (with

assistance from Giulio Curioni and Furkan Tekinay on raw sap data concatenation) and visualisation and designed/ performed the statistical analyses. Giulio Curioni/ Furkan Tekinay processed the environmental data. Susan E. Quick wrote the chapter. Giulio Curioni, Stefan Krause and A. Rob MacKenzie reviewed the chapter. Susan E. Quick edited the chapter.

Chapter 5: Subdominant tree sap flow

Author contributions: Susan E. Quick collected and analysed the data. Susan E. Quick wrote the chapter. A. Rob MacKenzie reviewed the chapter. Susan E. Quick edited the chapter.

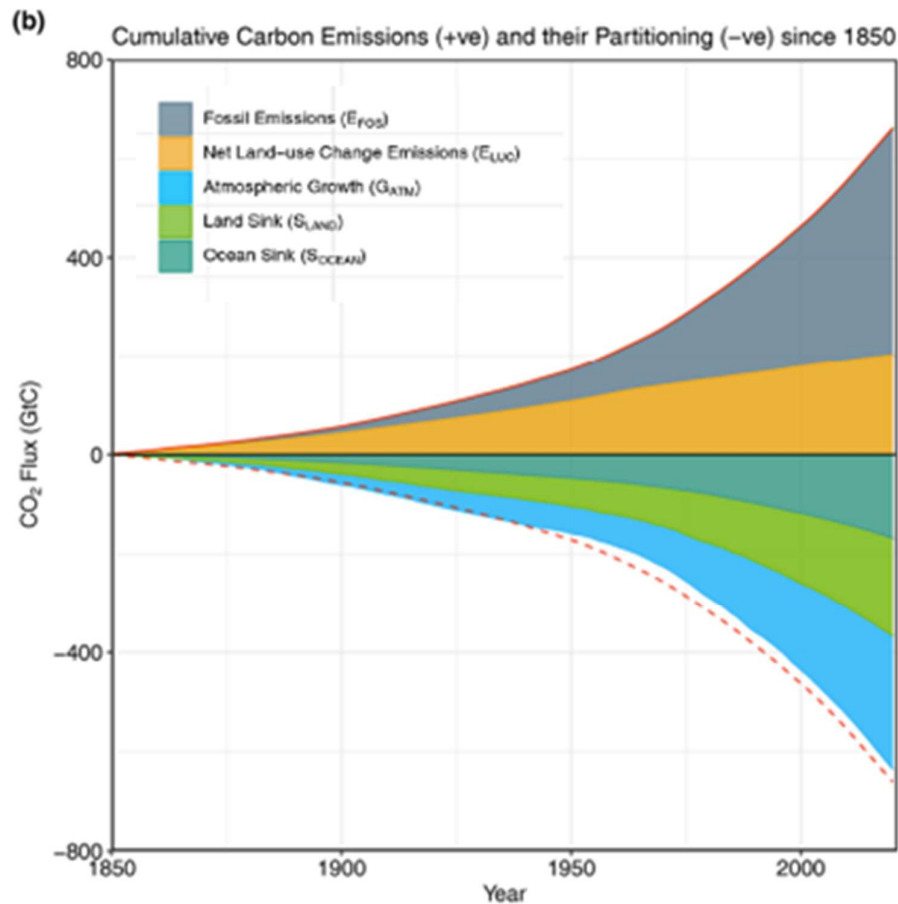
Chapter 6: Leaf and stem: transpiration flow dynamics

Author contributions: Susan E. Quick collected and analysed the data. Susan E. Quick wrote the chapter. A. Rob MacKenzie and Anna Gardner reviewed the chapter. Susan E. Quick edited the chapter.

Chapter 1 Preface and Thesis Overview

Introduction and motivation

The Climate Emergency



- 5 Figure 1- 1: Global carbon budget as a function of time, for fossil CO_2 emissions (E_{FOS} , including a small sink from cement carbonation; grey) and emissions from land-use change (E_{LUC} ; brown), as well as their partitioning among the atmosphere (G_{ATM} ; cyan), ocean (S_{OCEAN} ; blue), and land (S_{LAND} ; green). Panel shows the cumulative flux (the sum of all prior annual fluxes) since the year 1850. Adapted from Friedlingstein et al., (2023), Fig. 2(b))

- Forests provide a large proportion of land-based carbon storage (~ 450 GtC flux stock)
- 10 which acts as a sink for about a third of the total CO_2 emissions to atmosphere (Fig.1-1, Fig.1-2). Water security and management under current and future climate changes also benefit greatly from the services provided by forests. The biodiversity of existing

old growth forests alongside species succession also affects the provision of these essential services. Rising atmospheric CO₂ levels, alongside increase in other greenhouse gases (GHGs), are a direct cause of global heating. Atmospheric CO₂ levels, driven primarily by anthropogenic emissions, were at about 405 $\mu\text{mol mol}^{-1}$ monthly average December 2016 and currently (December 2024) amount to a monthly average of about 424 $\mu\text{mol mol}^{-1}$ (NOAA ESRL, 2024). Historically CO₂ concentrations were much lower prior to the industrial revolution ranging from 200 $\mu\text{mol mol}^{-1}$, before

The global carbon cycle

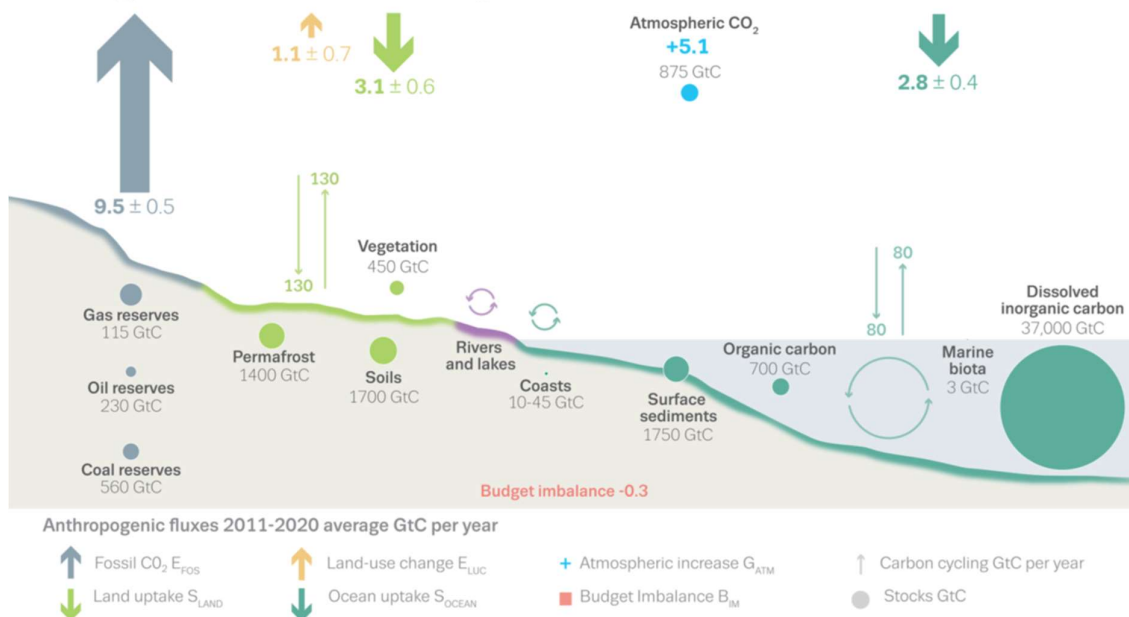
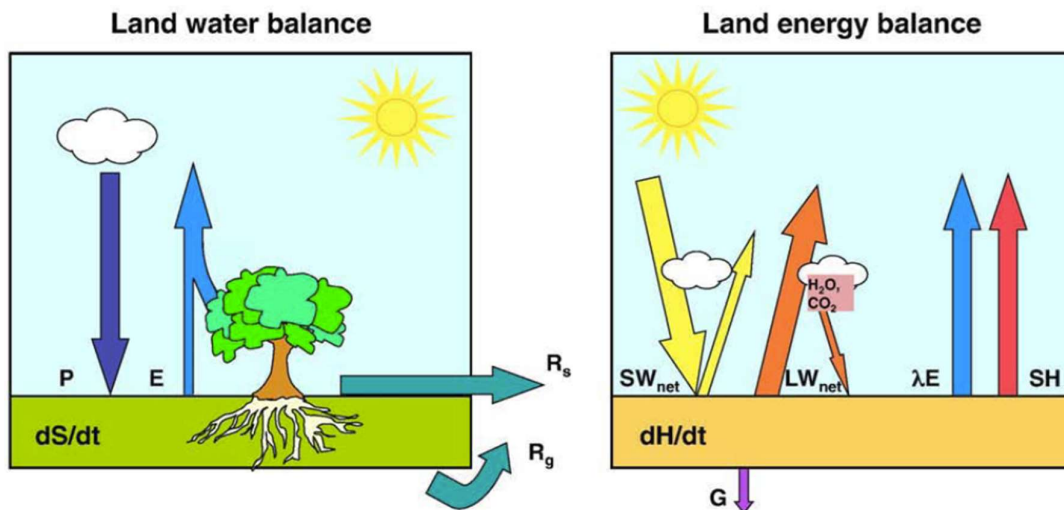


Figure 1- 2: Schematic representation of the overall perturbation of the global carbon cycle caused by anthropogenic activities, averaged globally for the decade 2011–2020. See legends for the corresponding arrows and units. The uncertainty in the atmospheric CO₂ growth rate is very small (0.02 GtC yr⁻¹) and is neglected for the figure. The anthropogenic perturbation occurs on top of an active carbon cycle, with fluxes and stocks represented in the background and taken from Canadell et al. (2022) for all numbers, except for the carbon stocks in coasts which is from a literature review of coastal marine sediments (Price and Warren, 2016). Adapted from Friedlingstein et al., (2023), Fig.2.

the 18th century (i.e. pre-industrial) and to 280 $\mu\text{mol mol}^{-1}$ by 1850 (IPCC, 2023). The effect has been, in conjunction with that of other GHGs (including e.g. methane and water vapour), to cause a rise of global annual temperature of 1.1 °C.

30 **How Forests respond**

Forests have shown their resilience to climate impacts despite increasing air temperature, longer droughts, drier winters and more frequent storms. Within forests and across land surfaces, carbon, water and energy cycles are closely knit (Friedlingstein *et al.*, 2023; Seneviratne *et al.*, 2010 , Figs.1-2 and 1-3) and untangling
 35 the interactions of changes to atmospheric CO₂ and associated climatic changes can be challenging, in particular in respect of carbon storage and the effects of water shortage or excess. Temperate old growth oak forests contribute to forest carbon stocks globally as well as the carbon sink, they also act locally to slow water run-off,



40 Figure 1- 3: Schematic of the land water balance (left) and land energy balance (right) for a given surface soil layer. Left: dS/dt refers to the change in water content within the layer (soil moisture, surface water, snow; depending on the depth of the layer), P is the precipitation, E is the evapotranspiration, R_s is the surface runoff, and R_g is the drainage. Right: dH/dt refers to the change of energy within the same layer, include all water storage components i.e. temperature change, phase changes associated with soil freezing/melting or snow melt), R_n is the net radiation, λE is the latent heat flux, SH is the sensible heat flux, and G is the ground heat flux to deeper layers. (based on Fig.4 from Seneviratne *et al.*, 2010).
 45

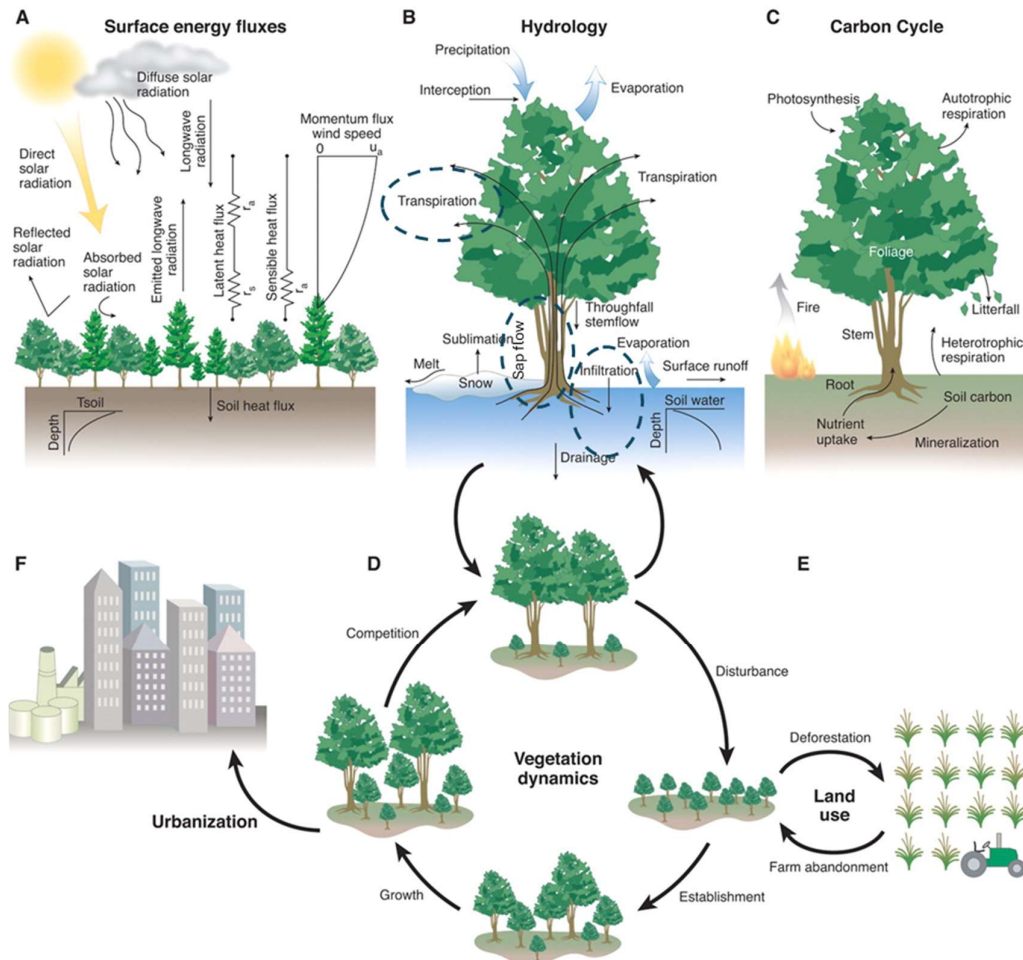


Figure 1- 4: The current generation of climate models treats the biosphere and atmosphere as a coupled system. Land surface parameterizations represent the biogeophysics, biogeochemistry, and biogeography of terrestrial ecosystems. (A) Surface energy fluxes and (B) the hydrologic cycle. These are the core biogeophysical processes. Circled dotted lines indicate parameters of interest in this thesis. Many models also include (C) the carbon cycle and (D) vegetation dynamics so that plant ecosystems respond to climate change. Some models also include (E) land use and (F) urbanization to represent human alteration of the biosphere. (Based on Bonan, 2008, Fig.2)

assisting in flood mitigation and planet cooling (Ellison *et al.*, 2017; Ellison, Pokorný and Wild, 2024). The hydrological cycles processes involved at forest scale which are incorporated into climate models are shown in Fig. 1-4.

This thesis addresses some gaps in data and analysis of effects on mature trees' responses to current and future climate-related drivers, as identified within previous studies. Effects are explored under experimental atmospheric CO₂ increases within a Free-Air Carbon-dioxide Enrichment (FACE) experiment to advance our knowledge of forest water cycling and reduce the uncertainties in climate change projections (De

Kauwe *et al.*, 2013; Medlyn *et al.*, 2015), enabling the effects of multiple limiting resources—light, nutrients, and water—to be better understood (Walker *et al.*, 2015). The findings inform approaches to reafforestation, forest preservation and environmental changes and deciduous forest management as well as feeding
65 vegetation and climatic models with valuable data (Bonan, 2008). The work pays particular attention to tree water usage, transpiration and tree-soil-water relations by considering water inputs, water flow and water vapour outputs within this experimental forest FACE study.

The theory of plant-water usage and transpiration

70 The study of plant-water requires some knowledge of the theoretical and experimental approaches which have resulted in our current understanding of photosynthesis, water-movement, water-usage and transpiration. In respect of plant-water, Stephen Hales, a British botanist, who described his experimental evidence of ‘the rise of water in plant’ in 1727, is considered to be the father of plant physiology (Hales’ Vegetable
75 Staticks (1727) cited by King, 2011). Later work by, for example Julius von Sachs, a German biologist, in his book *Handbuch der Experimental Physiologie der Pflanzen* (1865) (cited by *Britannica*, no date), expanded Hales’ work, describing in more detail the movement of water from soil to roots (imbibing), stem and leaf (perspiration, now called transpiration). The late experimental confirmation of the
80 1980s cohesion-tension theory (CT) (originally suggested in the 19th century) governing the flow of xylem sap and its link to transpiration has been discussed in detail in Brown, (2013), who also tackles the complex warming and cooling effects of transpiration related to evaporation and to greenhouse gases. Venturas, Sperry and Hacke, (2017) looked further at limitations of CT, summarising current explanations

85 (including liquid-vapour phase change of water molecules, and the effects of nucleation sites) and summarising research into the causes of embolization and cavitation.

Energy (from solar light) is necessary for photosynthesis in green organisms (see e.g. 18th century experiments of De Saussure described in King, 2011) enabling exchange of atmospheric CO₂ for emitted oxygen and water vapour (transpiration). In the leaf the
 90 CO₂ and water are assimilated and converted into sugars. Sir John Bennet Lawes (Lawes, 1850) developed the concept of transpiration efficiency (see also Table 1-1) establishing the link between dry matter accumulation and transpiration. Combining previous gas diffusion theory (Fick's law) and plant photosynthesis knowledge, in 1900

Definitions	References
General: Water use efficiency (WUE, also called transpiration efficiency - TE) indicates the ability of plants to limit transpiration loss by control of stomata opening & closing. It is defined at different scales: a) leaf, b) whole plant, c) stand/ landscape area.	
a) Leaf i) the ratio between net CO ₂ assimilation (A) and stomatal conductance to H ₂ O (g _s), known as intrinsic water use efficiency (iWUE), ii) the ratio between A and transpiration (E), (i.e. ratio of water loss to carbon gain (rate of carbon uptake per unit of water lost A/E)) known as instantaneous WUE	a) i) (Guerrieri <i>et al.</i> , 2016); ii) ((Hubick and Farquhar, 1989), see field limitations pp.522-523); (Donovan and Ehleringer, 1994)
b) from flux towers: GPP (gross primary productivity)/ET(evapotranspiration) defined as ecosystem water use efficiency, WUE _e	b) (Amatya, 2016); (Law <i>et al.</i> , 2002) (need for longer term studies); (Beer <i>et al.</i> , 2009); (Keenan <i>et al.</i> , 2013)
c) soil water use efficiency (SWUE), based on gross primary production and surface soil moisture derived from remote sensing products (ECV-SM) '	c) (He <i>et al.</i> , 2017)

Table 1- 1: Transpiration efficiency/ Water use efficiency. Definitions at differing sensing scales.

95 scientists Brown and Escombe (Brown and Escombe, 1900) published their theory of
movement of gases through small pores proven through experimentation with plant
stomata affirming the process of static diffusion within the leaf. Further explanation of
the theoretical and empirical evidence leading to expanded knowledge of mechanistic
plant-water systems can be found within a wealth of literature. For example Brodribb,
100 (2009) gives a modern summary of theory and evidence leading to the conclusion that,
under normal water-availability conditions, vascular plants' hydraulic and
photosynthetic systems must be interlinked. By measuring Tree-Water-Deficit (TWD,
defined by Zweifel, Zimmermann and Newbery, 2005), the whole-tree response to
water shortages, it has also been possible to determine a tree's response to drought
105 stress. Though laboratory and field experiments alongside microbiological advances
(e.g. studying stomatal closure under adverse water availability conditions, or analysis
of plant-hormones) have further developed our plant-water knowledge to the present
time, the above fundamental principles still govern our approach.

Of note for interpretation of studies at leaf scale is the dynamic hysteresis exhibited by
110 C₃ plant stomata as part of their water efficiency response under large step (400 to 700
mol CO₂ mol⁻¹) changes in CO₂ concentrations and to steps in incident
Photosynthetically Active Radiation (PAR). A faster response has been found for
stomatal closure during sudden rises (both for CO₂ concentration and PAR steps)
compared with slower reopening (as CO₂ concentration or PAR decreases). The time-
115 related stomatal responses for oak for PAR steps are in the order of 7 (opening) to 12
(closing) minutes (Drake, Gonzàlez-Meler and Long, 1997; Vico *et al.*, 2011; Vodnik
et al., 2013; Woods and Turner, 1971).

The measurement of heat flow through the xylem as a proxy for sap (liquid) flow developed in the 1930s (Huber, 1932) and was further refined over the following decades (e.g. Marshall, 1958). These methods are widely used, especially in woody plants, both to determine optimum plant watering, and to further study plant ecohydrology (Poyatos et al., 2016). Steppe *et al.*, (2015) introduced a number of suggestions to improve the monitoring of xylem sap, in particular to ensuring capture of low and reverse levels of flow. These measurement developments were reviewed in a pre-fieldwork project report (see Tables in App.2-E to Ch. 2) but sensors selection was outwith this project. More detailed topic-specific sensor theoretical background is contained in Ch.2 and other chapters i.e. three – oak-sap, four – soil-water, five – subdominant-sap, six – leaf-water.

Tree-soil-atmosphere water relationships.

The soil–plant–atmosphere continuum (SPAC) was described by Philip (1966) and has subsequently been the focus of research into whole plant hydraulics functioning in three component strands namely:

- soil-water physics characterised in terms of tension, plant-water hydraulics;
- physiology characterised in terms of diffusion pressure deficit (often renamed plant-water potential);
- water exchange processes between plant and air, especially transpiration, characterised by both leaf-water and meteorological/ environmental (e.g. air temperature, relative humidity (RH), vapour pressure deficit (VPD)) drivers.

In parallel with the SPAC development, which included consideration of the complex water-energy interfaces within plants, other researchers (Cowan and Farquhar, 1977;

Ellsworth, 1999; Keenan, Sabaté and Gracia, 2008) focused their attention on the carbon-water cycle interfaces within forests. In addition there have been many catchment scale studies which incorporate forests as an integral element, but which do not unpack the detailed water-energy-carbon cycle interactions (Zhang, Dawes and Walker, 2001; McDonnell, 2014).

Carbon-energy factors (e.g. incident solar energy, air and leaf temperatures, chemical energy transform emissions and related factors) which are inherent in driving photosynthesis cannot be ignored in the complexity of the SPAC. A synthesis of the integrated leaf-level response to these changes on a global basis was prepared by Wang and Wang, (2021), whereas Philip, (1966) had also recognised the important influential effects which, for example, plant age, light and CO₂, might have on the overall SPAC. These principles have stood the test of time with more recent work (e.g. Murray *et al.*, 2019; Lavergne *et al.*, 2020; Hu *et al.*, 2023; Peters *et al.*, 2023) highlighting further contributions to determining influences on leaf-level responses.

155 **Mechanistic models of plant hydraulic function.**

Some pivotal reviews concerning plant hydraulic function and modelling assist in determining a focused, knowledgeable approach. Mencuccini, (2003) considered four aspects of plant-water flow (hydraulic conductance) namely: whole-plant to leaf-area scaling; links between leaf gas exchange and leaf xylem-flow; short-term whole-plant water-flow regulation under ample water; and long-term structural acclimation of xylem water-flow to changing environmental conditions. This was pivotal in identifying why a consideration of whole-plant processes related to water transport is required. In contrast, research concentrating on individual elements of a plant (e.g. leaf, branch,

stem) were progressing (e.g. (Wullschlegel and Norby, 2001; Sánchez-Pérez *et al.*,
165 2008; Novick *et al.*, 2009; Donohue *et al.*, 2017)).

Landsberg, Waring and Ryan, (2017) presented a comprehensive summary of the
approaches to tree hydraulics used in the modelling and analysis of tree-water
relations. For example, they explained these theories (mostly using the analogy of
Ohm's law) and described the concepts of flows across potential gradients, using
170 terminology related to the laws of physics such as resistance, conductance and
potential (e.g. stomatal conductance, water potential) to describe tree-water flow.

Some notable gaps in theoretical description, such as the interactions between
phloem and xylem, and their effects on tree water relationships, were identified in this
study, but Landsberg, Waring and Ryan, (2017) considered it unlikely that findings in
175 this particular gap would challenge the overall tree-water relations paradigm. Rather
they warned against putting efforts into research which might give limited returns, and
highlighted area where real progress might be likely. Examples of promising returns
included: plant hydraulic conductance in the context of interplant interactions, nutrition
and adaptation outlined in Mencuccini's (2003) review; water stored in soils, including
180 more investigations into soil type and wetness, including tree species and tree-root
systems; and climatic thresholds causing (tree) mortality, such as drought, high
temperatures and vapour pressure deficit extremes.

Landsberg, Waring and Ryan's (2017) review did point to some gaps in the 'normal
science' plant hydraulics environment which are of relevance to this project. Of special
185 note for future work in tree-water relations was the need to refocus on climatic
thresholds and inter-species competitive advantages during e.g. drought, high

temperatures and vapour pressure deficits. One such need is to study inter-species responses in respect of subdominant trees within a mixed forest. In the context of soil-water characteristics and tree rooting depths, they highlighted that spatial variation measurement uncertainties might reduce confidence in obtaining predictions of (tree) water use during acclimation. In this context it is useful to explore whether the balance of use of soil-water by the dominant and subdominant species could be influenced by eCO_2 .

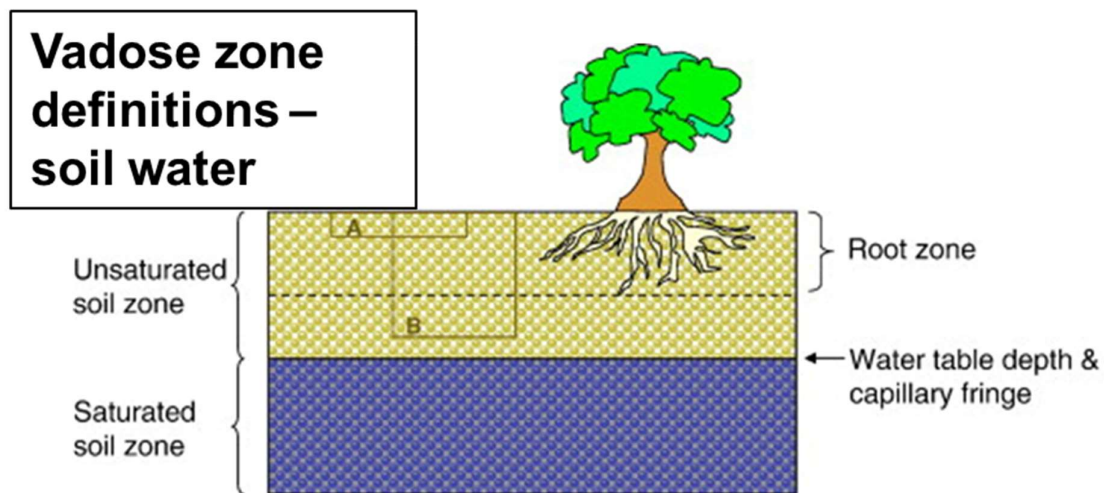


Figure 1- 5: To show key aspects of the vadose zone in respect of soil moisture zones and tree-water usage via the root zone. The saturated and unsaturated soil A and B provide distinct soil moisture contributions. From Fig.2 Seneviratne et al., (2010)

Landsberg, Waring and Ryan (2017) gave particular attention to understanding the vadose zone, as it is mostly from within this depth that tree roots can obtain their essential water sources. A good illustration of the scenario is shown in Fig. 1-5. There were also problems of predicting the pace of contrasting fast and slow processes. Extensive field work and experimentation, from field trials to stand simulations, up to the coupling of vegetation ecosystem models with global climate models, were required to take the plant hydraulics scientific understanding to a higher level. Finally,

205

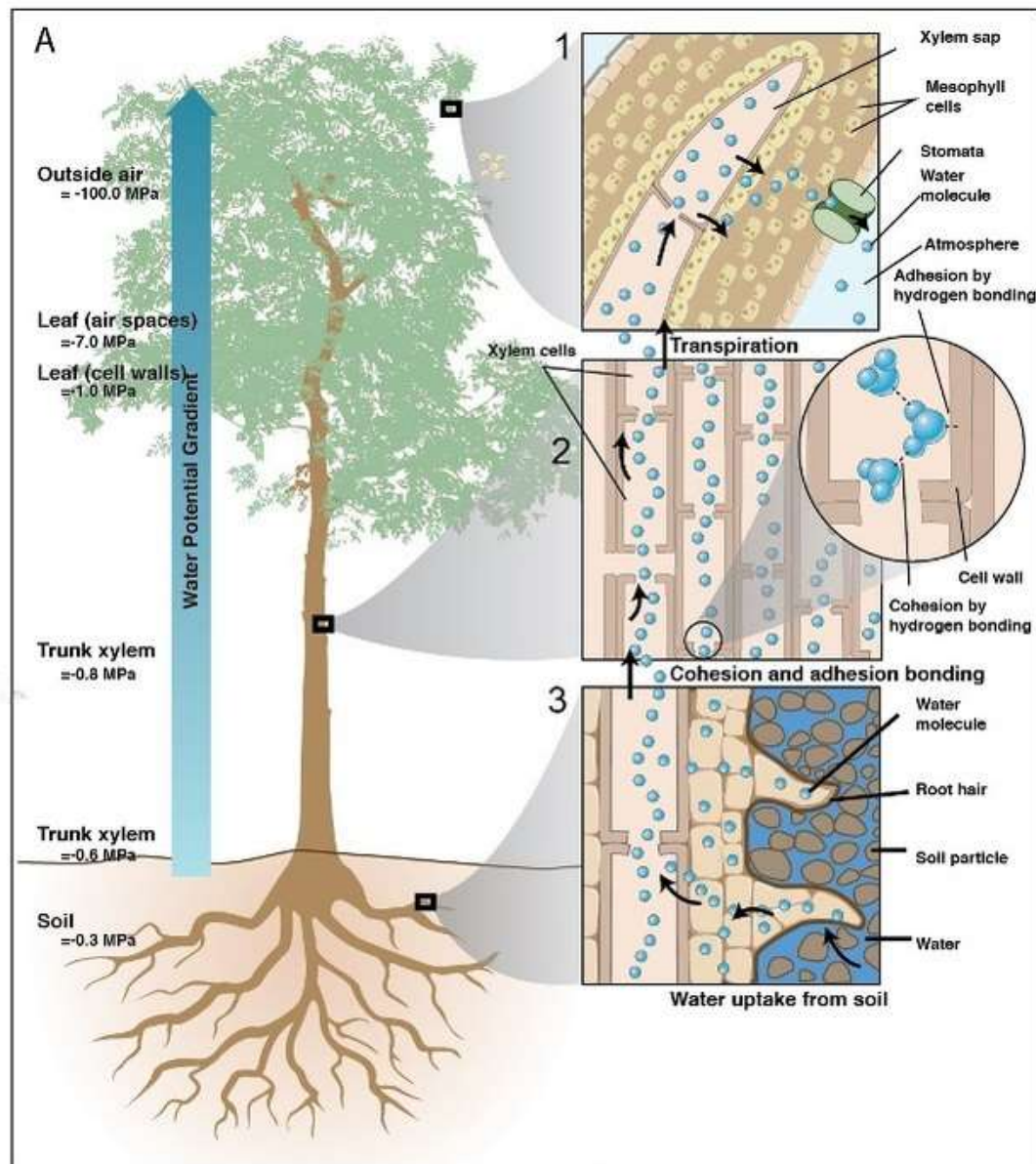


Figure 1- 6: (adapted from McElrone et al. (2013) Fig. 4 A: Representation of the water transport pathways along the soil-plant-atmosphere continuum (SPAC). (A) Water moves from areas of high water potential (i.e. close to zero in the soil) to low water potential (i.e., air outside the leaves). Details of the Cohesion-Tension mechanism are illustrated with the inset panels (A), where tension is generated by the evaporation of water molecules during leaf transpiration (1) and is transmitted down the continuous, cohesive water columns (2) through the xylem and out the roots to the soil (3).

210

this review saw that fruitful opportunities existed for tree physiologists interacting seamlessly with geneticists, insect/disease physiologists and ecologists working on the effects of climate change. These latter more complex issues might follow on from my project using the project data in future collaborations.

The analysis (Landsberg, Waring and Ryan, 2017) failed to consider how trees might adapt to the changing climate in respect of atmospheric changes, which may add complexity to our understanding of carbon-water dynamics. Keenan, Sabaté and Gracia (2008) in contrast, considered that many interactions between species, soil and atmospheric processes are still poorly understood within the plant hydraulic framework and recommended, amongst other things, focus on belowground biomass, species-specific responses to climate change factors such as eCO_2 , drought and the role of acclimation. A recent Tansley Insight (Li, 2024) also points to the deficiencies in focus on underground effects of elevated CO_2 . A diagram (Fig.1-6, McElrone *et al.*, 2013), highlights key aspects of our current SPAC understanding.

Background and context of FACE experiments

Leakey *et al.*, (2009) in their review of carbon, water and nitrogen relations in plants under increasing CO_2 , promoted the importance of longer-term experiments to determine, e.g. acclimation. The need for FACE experiments had arisen as a means of studying the effects of rising atmospheric CO_2 levels on plants in natural environments alongside the study of changing atmospheric feedback mechanisms under these future environmental conditions. This new approach had been deemed necessary to minimise the effects of confinement, e.g. in laboratory chambers, greenhouses or open-topped chambers, where the walls affected the environment

235 surrounding the plants (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007;
DOE., 2020). Controlled treatment conditions (of CO_2 , sometimes with nested
controlled watering or nutrients) are important in providing repeatable multiple season
responses. This is particularly important for considering effects on long-lived trees and
to accommodate season-to-season environmental variation for annual field crops
240 (Ainsworth and Long, 2021).

Looking back on two decades of FACE field crop and forest experiments, Leakey *et al.*, (2009) considered both the limitation and value of controlled laboratory and chamber-based measurements in comparison to free-air, for comparison of leaf, tree and forest-scale water usage. We were reminded that some FACE experiments
245 incorporated irrigation, so soil water effects could not be tested. By allowing treatment of plants under field conditions at a realistic scale, FACE experiments provided an important reality check on previous controlled CO_2 environment (e.g. open-topped chamber) findings.

As ambient atmospheric CO_2 increases the conditions under which gases are
250 exchanged within tree canopies will also change. Ainsworth and Long, (2005) in their meta-analysis of 15 years of FACE experiments compared expected changes in differing species under eCO_2 . Early FACE and pre-FACE (i.e. environmental enclosure) experimental findings showed consistent reduction in stomatal conductance and transpiration, improved water-use efficiency, higher rates of
255 photosynthesis, and increased light-use efficiency (Drake, Gonzalez-Meler and Long, 1997) across different field crops, but low temperature was shown to decrease these effects. FACE experimental results were not as conclusive as pre-FACE in demonstrating these efficiency improvements. Functional groups differed in their

response to FACE. The 15-year meta-analysis showed that trees under eCO₂ in dry
260 conditions were generally more responsive than field crops, showing an average 47%
stimulation in A_{sat} , low photosynthetic acclimation and lesser increase in leaf
carbohydrates. Whilst there was significant increase in LAI in the young and rapidly
growing trees, no change in LAI had been detected in field crops grown under FACE
and few mature tree experiments had taken place. A second meta-analysis (Ainsworth
265 and Long, 2021) updated these findings for 18 C₃ field crop studies. CO₂ elevation of
ca. 200 ppm caused a ca. 18% increase in yield under non-stress dry conditions.
Warming by ca. 2°C reduced the average increase to 10%. Drought stress and ample
nitrogen availability enhanced the eCO₂ effect (Norby *et al.*, 2016).

Whilst early FACE studies in crops refined the method for short herbs, the FACE
270 mechanisms needed to change for large plants (i.e. shrubs and trees). Considerable
development effort was required to apply the FACE application technology to forested
plantations. Forest FACE experiments studying plant hydraulics in a temperate climate
have been undertaken predominantly with young plantations, with the exception of
WEB-FACE (Keel *et al.*, 2007; Leuzinger *et al.*, 2005; Leuzinger and Körner, 2007)
275 and more recently EUC-FACE (Gimeno *et al.*, 2016; Norby *et al.*, 2016). The WEB-
FACE approach used pure CO₂ delivered into the plant canopy through small bore
pipes, whereas the Brookhaven National Laboratory (BNL) designed system (Fig.1-7)
allowed a mixture of pure CO₂ added to ambient air to be delivered through the canopy,
drifting in with localised wind currents (Hendrey *et al.*, 1999; Hart *et al.*, 2020). The
280 latter system was developed for the remaining second generation FACE experiments
in forests.

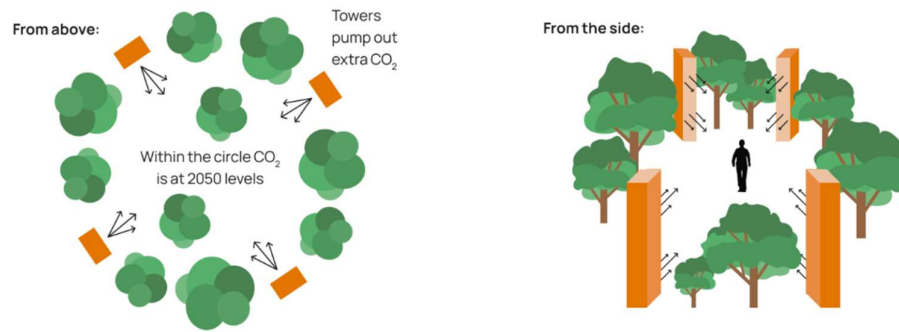


Figure 1- 7: Outline of BNL type FACE system (BIFoR, 2024)

Holtum and Winter, (2003) studied effects of high frequency FACE system CO₂ fluctuations using young tropical trees and speculated that fluctuations may diminish the response of photosynthesis to eCO₂. In contrast Hendrey *et al.*, (1999) found no difference between constant and fluctuating eCO₂ on wholechain photosynthetic electron transport for CO₂ oscillations of a half-cycle of 30 s or less, including most of the fluctuations observed in FACE systems. Allen *et al.*, (2020) confirmed an effect of FACE fluctuations on small plants and devised a correction factor. Whilst a number of refinements have been made to the FACE forest systems to improve consistency, to this author's knowledge little research has considered the effects of FACE infrastructure when comparing trees exposed to the resulting ambient and elevated mixtures used in experiments to undisturbed vegetation with no infrastructure (see (Harper, 2023)).

295 **Research focus**

The above principles, and the need to expedite appropriate data collection within the FACE experimental environment (MacKenzie *et al.*, 2021), led my initial focus on monitoring of plant-water flow. I considered how to test the effects of elevated carbon dioxide (eCO₂) on plant-water flows, within this complexity of drivers, whilst using the best knowledge base and practices concerning these plant hydraulics mechanisms.

Given the known areas of uncertainty, I focused my research on the three elements of SPAC so as to tease out the key elements driving flow of water, from inputs at tree roots to outputs at leaves and canopy.

Aims and objectives

305 **Project Aims**

This project aims to address gaps in ecohydrological research to predict mature trees' response to future increased levels of atmospheric CO₂. It also provides extended empirical data of value for other more general studies of forest ecohydrology or microenvironments. The broad aim was to measure components of the water cycle at
310 key points through the soil–plant–atmosphere continuum (SPAC), within the defined boundaries (i.e. from atmospheric boundary to water table level). All measurements were from the Birmingham Institute of Forest Research (BIFoR) FACE research woodland, facilitating scientific comparisons between all three treatments. This investigation was mainly focused on a tree-based approach and especially on the
315 dominant species in the research woods, namely oak.

Project Objectives

The main objectives of this study were confirmed in 2019 as follows:

- To take a systems view of water dependencies at BIFoR FACE experiment within a temperate oak-hazel woodland under eCO₂.
- 320 • To investigate the role of water and water vapour at the interfaces between the woodland vegetation, i.e. trees, shrubs and ground cover; the sub-surface environment, i.e. soil, rock, water table; and at the atmospheric interface.
- To consider vegetation species abundance, structure, age, vulnerability and provenance.

325 The original objectives from 2017 had been refined and streamlined to be more
Specific, Measurable, Achievable, Realistic and Time-bound (SMART) within the
restrictions imposed by study for this award.

Thesis overview

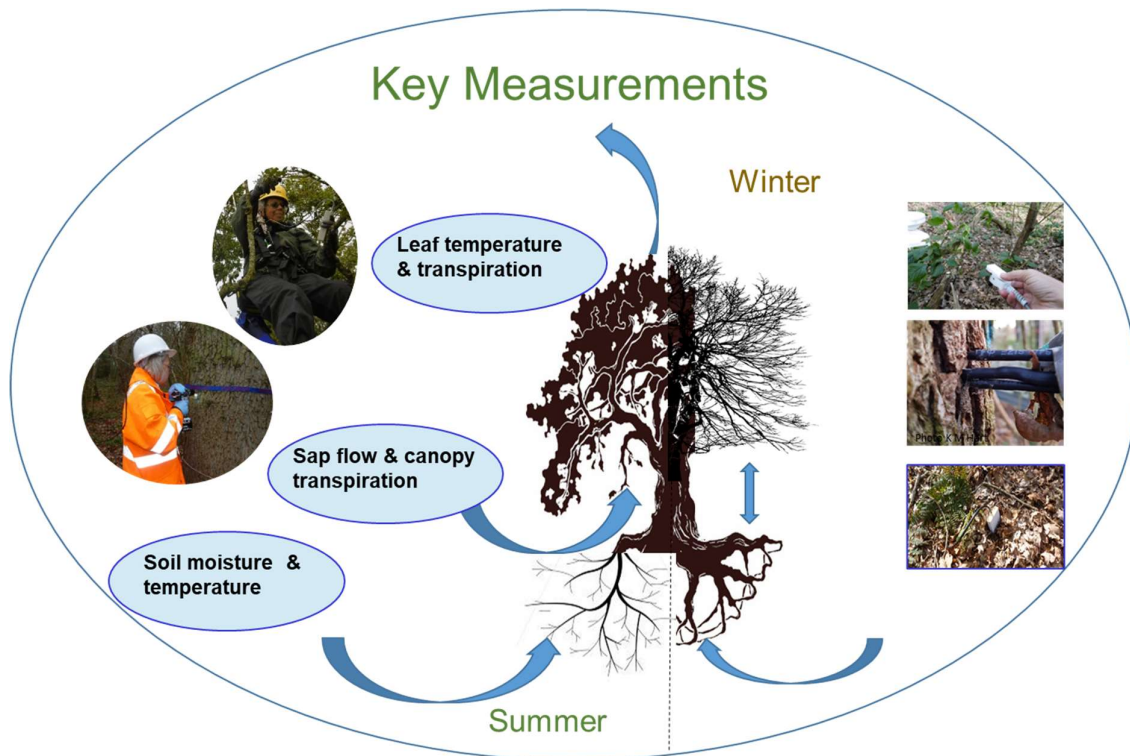


Figure 1- 8: Water cycle flow sequence.

330 This thesis comprises three main themes (Fig.1-8, figure repeated in Chapter 3
publication supplement): tree-water flow data (from xylem sap measurement) which
forms the core of the project; soil-water flow (determined by volumetric water content
(VWC) measurement down to 1 metre depth) fed by throughfall precipitation; and leaf
– atmospheric water vapour flow (from measures of leaf stomatal conductance/
335 transpiration). The work is presented as a water cycle flow sequence, all based on
empirical data concerning BIFoR FACE experimental woodland and the three
treatment categories there-in.

All themes consider the environmental parameters most pertinent to that theme. Data processed during analysis for the first theme are reassessed within the other two themes to examine the flow relationships.

The first theme investigates tree stem sap flow for both old growth oak and subdominant species sycamore and hawthorn, in a temperate forest free-air carbon dioxide enrichment (FACE) experimental facility. The species mixtures present have determined the measurement approach. Daytime tree water usage for oak during the treatment periods April to October over a five year period 2017-2021 are reported in Ch.3 which includes a publication (Quick *et al.*, 2025). Next, comparisons of oak tree sap flow results with those from subdominant trees are made, contributing to understanding of inter-species water usage within this experimental forest.

These chapters (Chapter 3 and Chapter 5) provide methods of normalisation of water usage data using tree size parameters (e.g. stem bark radius , canopy spread) for each species studied. This facilitates comparison of water usage (driven by photosynthesis) for within-species and between-species trees under the three treatment conditions. The overarching goal of these broad chapters is to provide forest-based multi-year long-term data to contribute to improvement of vegetation and water-carbon forest models.

The second theme (Chapter 4) investigates plant-water sources from soil including consideration of atmospheric inputs (precipitation/ throughfall) and drivers (e.g. soil water potential differing by depth). The study looks in addition at the challenges posed by uncalibrated and differing-technology based transducers for soil moisture measurement. The effects of drought are considered here.

The third theme (Chapter 6) investigates the flow of water and water vapour exchange between leaf and atmospheric for a subset of oak, sycamore and hawthorn trees present in the BIFoR FACE experiment. Stomatal conductance and leaf temperature data collected from in-situ and cut-twigs leaves in this forest under the three experimental conditions are used to calculate and compare within-day transpiration with that derived from the stem as sap flux (a proxy for whole-tree transpiration).

Leaf transpiration is calculated from leaf measurements VPD (i.e. leaf temperature, RH and air temperature) at appropriate heights in three species, the dominant oak and two subdominant species hawthorn and sycamore.

This theme also shows how control patch data can demonstrate within day variation of leaf-level transpiration response given confounding factors of incident light, air temperature and relative humidity. All trees studied are assumed to experience similar air flow conditions and average light levels at a particular canopy height within the forest as these are not measured specifically in proximity to the sample leaves.

Comparison of oak middle-day sap flux values with leaf transpiration, normalising by median, when synchronising daytime dynamics was used to assess tree water storage.

Main novel contributions

- Data collection from mature trees >10cm diameter at breast height (DBH) under FACE and related control conditions.
- Inclusion of semi-natural forest control patches as well as infrastructure control, an added dimension compared with other forest FACE experiments.
- Comparisons: Multi-species contributions to soil-water/ tree-water flow demands both seasonally and within the daylight hours.

- Whole-tree water usage normalisation to enable oak trees of differing dimensions
385 to be compared within and across treatment types.
- Consideration of relative extractable water (annually calculated) as a means of
smoothing and normalising the heterogeneity of VWC between treatment types
and soil depths. Also to enable merging of data from differing transducer types.
- Comparison of leaf-level transpiration (calculated from stomatal conductance and
390 leaf temperature and environmental parameters) to canopy-level transpiration (i.e.
per-tree sap flux) results to deduce daily characteristics and fluctuations in
atmospheric demands and the relationship to tree size.

During my PhD, I contributed to SoilTemp global database project (url:
<https://www.soiltempproject.com> later renamed <https://www.meb-network.com> and
395 see [Lembrechts *et al.*, \(2020\)](#)).

During the PhD studentship I contributed to publications not wholly contained in this
thesis, including: Hagan Brown *et al.*, (2025) (DOI:
<http://dx.doi.org/10.1111/gcb.70565>), Kemppinen *et al.*, (2024) (DOI:
<https://doi.org/10.1111/geb.13834>), MacKenzie *et al.*, (2021) (DOI:
400 <https://doi.org/10.1002/hyp.14096>) and Rabbai *et al.*, (2023) (DOI:
<https://doi.org/10.1002/hyp.14919>). The first pages of these publications are included
in Appendix 1, and the full open-access documents are available via the DOI links.

References Chapter 1

- 405 Ainsworth, E. A. and Long, S. P. (2005) 'What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂', *New Phytologist*, 165(2), pp. 351–372. doi: 10.1111/j.1469-8137.2004.01224.x.
- Ainsworth, E. A. and Long, S. P. (2021) '30 years of free-air carbon dioxide enrichment (FACE): What have we learned about future crop productivity and its potential for adaptation?', *Global Change Biology*, 27(1), pp. 27–49. doi: 10.1111/gcb.15375.
- Ainsworth, E. A. and Rogers, A. (2007) 'The response of photosynthesis and stomatal conductance to rising [CO₂]: Mechanisms and environmental interactions', *Plant, Cell and Environment*, pp. 258–270. doi: 10.1111/j.1365-3040.2007.01641.x.
- 415 Allen, L. H. et al. (2020) 'Fluctuations of CO₂ in Free-Air CO₂ Enrichment (FACE) depress plant photosynthesis, growth, and yield', *Agricultural and Forest Meteorology*, 284, p. 107899. doi: <https://doi.org/10.1016/j.agrformet.2020.107899>.
- Amatya, D. (Ed.). (2016) *Forest hydrology : processes, management and assessment*. GEN, Boston, MA: CAB International,. doi: 10.1079/9781780646602.0000.
- 420 Beer, C. et al. (2009) 'Temporal and among-site variability of inherent water use efficiency at the ecosystem level', *Global Biogeochemical Cycles*. John Wiley & Sons, Ltd, 23(2). doi: 10.1029/2008GB003233.
- BIFoR (2024) 'The Forest Time-Machine', Old Joe, Autumn. Available at: <https://www.oldjoe.co.uk/article/forest-time-machine>.

425 Bonan, G. B. (2008) 'Forests and climate change: Forcings, feedbacks, and the climate benefits of forests', *Science*, 320(5882), pp. 1444–1449. doi: 10.1126/science.1155121.

Britannica (no date). Available at: <https://www.britannica.com/biography/Julius-von-Sachs#ref34690> (Accessed: 2 June 2025).

430 Brodribb, T. J. (2009) 'Xylem hydraulic physiology: The functional backbone of terrestrial plant productivity', *Plant Science*, 177(4), pp. 245–251. doi: <https://doi.org/10.1016/j.plantsci.2009.06.001>.

Brown, H. R. (2013) 'The theory of the rise of sap in trees: some historical and conceptual remarks', *Physics in Perspective*. Springer, 15, pp. 320–358.

435 Brown, H. T. and Escombe, F. (1900) 'Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants.', *Philosophical Transactions of Royal Society of London*, B 193:, pp. 223–291. doi: 10.1093/oxfordjournals.aob.a088791.

Cowan, I. R. and Farquhar, G. D. (1977) 'Stomatal function in relation to leaf
440 metabolism and environment', *Symposia of the society for experimental biology*, (February 1977).

DOE., U. S. (2020) U.S. Department of Energy Free-Air CO₂ Enrichment Experiments: FACE Results, Lessons, and Legacy, No Title. doi: 10.2172/1615612.

Donohue, R. J. et al. (2017) 'A simple hypothesis of how leaf and canopy-level
445 transpiration and assimilation respond to elevated CO₂ reveals distinct response

patterns between disturbed and undisturbed vegetation', *Journal of Geophysical Research: Biogeosciences*, 122(1), pp. 168–184. doi: 10.1002/2016JG003505.

Donovan, L. A. and Ehleringer, J. R. (1994) 'Water stress and use of summer precipitation in a Great Basin shrub community', *Functional Ecology*. JSTOR, pp. 289–
450 297. doi: 10.2307/2389821.

Drake, B. G., Gonzalez-Meler, M. A. and Long, S. P. (1997) 'More Efficient Plants: A Consequence of Rising Atmospheric CO₂?', *Annual Review of Plant Physiology and Plant Molecular Biology*, 48, pp. 609-639. doi: 10.1146/annurev.arplant.48.1.609.

Ellison, D. et al. (2017) 'Trees, forests and water: Cool insights for a hot world', *Global*
455 *Environmental Change*. doi: 10.1016/j.gloenvcha.2017.01.002.

Ellison, D., Pokorný, J. and Wild, M. (2024) 'Even cooler insights: On the power of forests to (water the Earth and) cool the planet', *Global Change Biology*. John Wiley & Sons, Ltd, 30(2), p. e17195. doi: 10.1111/gcb.17195.

Ellsworth, D. S. (1999) 'CO₂ enrichment in a maturing pine forest: Are CO₂ exchange
460 and water status in the canopy affected?', *Plant, Cell and Environment*, 22(5), pp. 461–472. doi: 10.1046/j.1365-3040.1999.00433.x.

Friedlingstein, P. et al. (2023) 'Global Carbon Budget 2023', *Earth System Science Data*, 15(12), pp. 5301–5369. doi: 10.5194/essd-15-5301-2023.

Gimeno, T. E. et al. (2016) 'Conserved stomatal behaviour under elevated CO₂ and
465 varying water availability in a mature woodland', *Functional Ecology*, 30(5), pp. 700–709. doi: 10.1111/1365-2435.12532.

Guerrieri, R. et al. (2016) 'Evapotranspiration and water use efficiency in relation to climate and canopy nitrogen in U.S. forests', *Journal of Geophysical Research: Biogeosciences*, 121(10), pp. 2610–2629. doi: 10.1002/2016JG003415.

470 Hagan Brown, W. et al. (2025) 'Elevated CO₂ Increases the Canopy Temperature of Mature *Quercus robur* (Pedunculate Oak)', *Global Change Biology*. John Wiley & Sons, Ltd, 31(11), p. e70565. doi: <https://doi.org/10.1111/gcb.70565>.

Harper, N. J. (2023) Are the baseline control spaces at the BIFoR Free Air Carbon Enrichment experiment being polluted? University of Birmingham.

475 Hart, K. M. et al. (2020) 'Characteristics of free air carbon dioxide enrichment of a northern temperate mature forest', *Global Change Biology*, 26(2), pp. 1023–1037. doi: 10.1111/gcb.14786.

He, B. et al. (2017) 'A new indicator of ecosystem water use efficiency based on surface soil moisture retrieved from remote sensing', *Ecological Indicators*, 75, pp. 10–
480 16. doi: 10.1016/j.ecolind.2016.12.017.

Hendrey, G. et al. (1999) 'A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO₂', *Global Change Biology*, 5, pp. 293–309. doi: 10.1046/j.1365-2486.1999.00228.x.

Holtum, J. A. M. and Winter, K. (2003) 'Photosynthetic CO₂ uptake in seedlings of two
485 tropical tree species exposed to oscillating elevated concentrations of CO₂.', *Planta*, Nov(218(1)), pp. 152–8. doi: 10.1007/s00425-003-1089-1.

Hu, Y. et al. (2023) 'Tree-level stomatal regulation is more closely related to xylem hydraulic traits than to leaf photosynthetic traits across diverse tree species',

Agricultural and Forest Meteorology, 329, p. 109291. doi:
490 10.1016/j.agrformet.2022.109291.

Hubick, K. and Farquhar, G. (1989) 'Carbon isotope discrimination and photosynthesis', Annual review of plant physiology and plant molecular biology.

IPCC (2023) 'Technical Summary', in Intergovernmental Panel on Climate Change (IPCC) (ed.) Climate Change 2021 – The Physical Science Basis: Working Group I
495 Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press, pp. 35–144. doi: DOI: 10.1017/9781009157896.002.

De Kauwe, M. G. et al. (2013) 'Forest water use and water use efficiency at elevated CO₂: A model-data intercomparison at two contrasting temperate forest FACE sites',
500 Global Change Biology, 19(6), pp. 1759–1779. doi: 10.1111/gcb.12164.

Keel, S. G. et al. (2007) 'Stomatal conductance in mature deciduous forest trees exposed to elevated CO₂', Trees - Structure and Function, 21(2), pp. 151–159. doi: 10.1007/s00468-006-0106-y.

Keenan, T. F. et al. (2013) 'Increase in forest water-use efficiency as atmospheric
505 carbon dioxide concentrations rise', Nature, 499(7458), pp. 324–327. doi: 10.1038/nature12291.

Keenan, T., Sabaté, S. and Gracia, C. (2008) 'Forest Eco-physiological Models and Carbon Sequestration BT - Managing Forest Ecosystems: The Challenge of Climate Change', in Bravo, F. et al. (eds). Dordrecht: Springer Netherlands, pp. 83–102. doi:
510 10.1007/978-1-4020-8343-3_5.

Kemppinen, J. et al. (2024) 'Microclimate, an important part of ecology and biogeography', *Global Ecology and Biogeography*. John Wiley & Sons, Ltd, n/a(n/a), p. e13834. doi: 10.1111/geb.13834.

King, J. (2011) *Reaching for the Sun: How Plants Work*. 2nd edn. Cambridge: Cambridge University Press. doi: 10.1017/CBO9780511973895.

Landsberg, J., Waring, R. and Ryan, M. (2017) 'Water relations in tree physiology: where to from here?', *Tree Physiology*, 37(1), pp. 18–32. doi: 10.1093/treephys/tpw102.

Lavergne, A. et al. (2020) 'Impacts of soil water stress on the acclimated stomatal limitation of photosynthesis: Insights from stable carbon isotope data', *Global Change Biology*. John Wiley & Sons, Ltd, 26(12)(7158–7172). doi: <https://doi.org/10.1111/gcb.15364>.

Law, B. E. et al. (2002) 'Environmental controls over carbon dioxide and water vapor exchange of terrestrial vegetation', *Agricultural and Forest Meteorology*, 113(1), pp. 97–120. doi: 10.1016/S0168-1923(02)00104-1.

Lawes, J. B. (1850) *Experimental Investigation Into the Amount of Water Given Off by Plants During Their Growth: Especially in Relation to the Fixation and Source of Their Various Constituents*. Clowes.

Leakey, A. D. B. et al. (2009) 'Elevated CO₂ effects on plant carbon, nitrogen, and water relations: Six important lessons from FACE', *Journal of Experimental Botany*, 60(10), pp. 2859–2876. doi: 10.1093/jxb/erp096.

- Lembrechts, J. J. et al. (2020) 'SoilTemp: A global database of near-surface temperature', *Global Change Biology*. John Wiley & Sons, Ltd, 26(11), pp. 6616–6629. doi: 10.1111/gcb.15123.
- 535 Leuzinger, S. et al. (2005) 'Responses of deciduous forest trees to severe drought in Central Europe', *Tree Physiology*, 25(6), pp. 641–650. doi: 10.1093/treephys/25.6.641.
- Leuzinger, S. and Körner, C. (2007) 'Water savings in mature deciduous forest trees under elevated CO₂', *Global Change Biology*. John Wiley & Sons, Ltd, 13(12), pp. 2498–2508. doi: 10.1111/j.1365-2486.2007.01467.x.
- 540 Li, Y. (2024) 'Climate feedback from plant physiological responses to increasing atmospheric CO in Earth system models', *New Phytologist*. John Wiley & Sons, Ltd, n/a(n/a). doi: 10.1111/nph.20184.
- MacKenzie, A. R. et al. (2021) 'BIFoR FACE: Water–soil–vegetation–atmosphere data from a temperate deciduous forest catchment, including under elevated CO₂', *Hydrological Processes*. John Wiley & Sons, Ltd, 35(3), p. e14096. doi: 10.1002/hyp.14096.
- 545 McDonnell, J. J. (2014) 'The two water worlds hypothesis: ecohydrological separation of water between streams and trees?', *Wiley Interdisciplinary Reviews: Water*. doi: 10.1002/wat2.1027.
- 550 McElrone, A. J. et al. (2013) *Water Uptake and Transport in Vascular Plants.*, Nature Education Knowledge. Available at: <https://www.scirp.org/reference/referencespapers?referenceid=3416499> (Accessed: 17 November 2025).

Medlyn, B. E. et al. (2015) 'Using ecosystem experiments to improve vegetation
555 models', *Nature Clim. Change*. Nature Publishing Group, a division of Macmillan
Publishers Limited. All Rights Reserved., 5(6), pp. 528–534. doi:
10.1038/nclimate2621.

Mencuccini, M. (2003) 'The ecological significance of long-distance water transport:
short-term regulation, long-term acclimation and the hydraulic costs of stature across
560 plant life forms', *Plant, Cell & Environment*. John Wiley & Sons, Ltd, 26(1), pp. 163–
182. doi: 10.1046/j.1365-3040.2003.00991.x.

Murray, M. et al. (2019) 'Convergence in Maximum Stomatal Conductance of C3
Woody Angiosperms in Natural Ecosystems Across Bioclimatic Zones ', *Frontiers in
Plant Science* , p. 558. Available at:
565 <https://www.frontiersin.org/article/10.3389/fpls.2019.00558>.

NOAA ESRL (2024) at Mauna Loa, Hawaii, United States (MLO). Available at:
<https://gml.noaa.gov/ccgg/trends/mlo.html> (Accessed: 10 December 2024).

Norby, R. J. et al. (2016) 'Model-data synthesis for the next generation of forest free-
air CO₂ enrichment (FACE) experiments', *New Phytologist*, 209(1), pp. 17–28. doi:
570 10.1111/nph.13593.

Novick, K. et al. (2009) 'The relationship between reference canopy conductance and
simplified hydraulic architecture', *Advances in Water Resources*. doi:
10.1016/j.advwatres.2009.02.004.

Peters, R. L. et al. (2023) 'Daytime stomatal regulation in mature temperate trees
575 prioritizes stem rehydration at night', *New Phytologist*. John Wiley & Sons, Ltd,
n/a(n/a). doi: <https://doi.org/10.1111/nph.18964>.

Philip, J. R. (1966) 'Plant Water Relations: Some Physical Aspects', *Annual Review of Plant Physiology. Annual Reviews*, 17(1), pp. 245–268. doi: 10.1146/annurev.pp.17.060166.001333.

580 Quick, S. E. et al. (2025) 'Water usage of old-growth oak at elevated CO₂ in the FACE (Free-Air CO₂ Enrichment) of climate change', *Biogeosciences. Copernicus Publications Göttingen, Germany*, 22(6), pp. 1557–1581. doi: 10.5194/bg-22-1557-2025.

585 Rabbai, A. et al. (2023) 'Soil moisture and temperature dynamics in juvenile and mature forest as a result of tree growth, hydrometeorological forcings, and drought', *Hydrological Processes. John Wiley & Sons, Ltd*, 37(6), p. e14919. doi: 10.1002/hyp.14919.

Sánchez-Pérez, J. M. et al. (2008) 'Water uptake by trees in a riparian hardwood forest (Rhine floodplain, France)', *Hydrological Processes*, 22(3), pp. 366–375. doi: 590 10.1002/hyp.6604.

Seneviratne, S. I. et al. (2010) 'Investigating soil moisture–climate interactions in a changing climate: A review', *Earth-Science Reviews*, 99(3), pp. 125–161. doi: 10.1016/j.earscirev.2010.02.004.

595 Steppe, K. et al. (2015) 'Sap flow as a key trait in the understanding of plant hydraulic functioning', *Tree Physiology*, 35(4), pp. 341–345. doi: 10.1093/treephys/tpv033.

Venturas, M. D., Sperry, J. S. and Hacke, U. G. (2017) 'Plant xylem hydraulics: What we understand, current research, and future challenges', *Journal of Integrative Plant Biology. Blackwell Publishing Ltd.*, pp. 356–389. doi: 10.1111/jipb.12534.

- Vico, G. et al. (2011) 'Effects of stomatal delays on the economics of leaf gas exchange under intermittent light regimes', *New Phytologist*. John Wiley & Sons, Ltd, 192(3), pp. 640–652. doi: 10.1111/j.1469-8137.2011.03847.x.
- Vodnik, D. et al. (2013) 'Interspecific variability of plant stomatal response to step changes of [CO₂]', *Environmental and Experimental Botany*, 88, pp. 107–112. doi: 10.1016/j.envexpbot.2011.12.029.
- Walker, A. P. et al. (2015) 'Predicting long-term carbon sequestration in response to CO₂ enrichment: How and why do current ecosystem models differ?', *Global Biogeochemical Cycles*. John Wiley & Sons, Ltd, 29(4), pp. 476–495. doi: 10.1002/2014GB004995.
- Wang, Z. and Wang, C. (2021) 'Responses of tree leaf gas exchange to elevated CO₂ combined with changes in temperature and water availability: A global synthesis', *Global Ecology and Biogeography*, (July), pp. 1–13. doi: 10.1111/geb.13394.
- Woods, D. B. and Turner, N. C. (1971) 'Stomatal response to changing light by four tree species of varying shade tolerance', *New Phytologist*. John Wiley & Sons, Ltd, 70(1), pp. 77–84. doi: 10.1111/j.1469-8137.1971.tb02512.x.
- Wullschleger, S. D. and Norby, R. J. (2001) 'Sap velocity and canopy transpiration in a sweetgum stand exposed to free-air CO₂ enrichment (FACE)', *New Phytologist*. John Wiley & Sons, Ltd, 150(2), pp. 489–498. doi: 10.1046/j.1469-8137.2001.00094.x.
- Zhang, L., Dawes, W. R. and Walker, G. R. (2001) 'Response of mean annual evapotranspiration to vegetation changes at catchment scale', *Water Resources Research*. John Wiley & Sons, Ltd, 37(3), pp. 701–708. doi: 10.1029/2000WR900325.

Zweifel, R., Zimmermann, L. and Newbery, D. M. (2005) 'Modeling tree water deficit from microclimate: an approach to quantifying drought stress', *Tree Physiology*, 25(2), pp. 147–156. doi: 10.1093/treephys/25.2.147.

Chapter 2 : Methodology

This chapter describes the methodology, materials and methods used on this project. Firstly an overview of the BIFoR FACE experiment is given. Then a general description of the overall methodology for this project, followed by details concerning measurements and methods used in the study.

2.1 BIFoR FACE Experiment

A brief description of BIFoR FACE experiment is as follows. The BIFoR FACE forested experimental facility is located within Mill Haft, a mature temperate deciduous woodland, in Staffordshire (52.801°N, 2.301°W), United Kingdom adjacent to Norbury Junction and a part of Norbury Park Estate. The total BIFoR FACE experimental area covers 7.3 ha within an estate size of 19.1 ha, the whole constituting an arable mosaic with increasing young plantation areas. The BIFoR FACE forest is a circa 1840 plantation of dominant oak standards (*Quercus robur* L.), with unknown plantation dates for the hazel (*Corylus avellana* L.) coppice. Naturally propagated larger trees *Acer pseudoplatanus* (sycamore), *Crataegus monogyna* (hawthorn), *Ilex aquafolium* (holly) and smaller numbers of woody plants of other orders (e.g. *Ulmus* (elm), *Fraxinus* (ash)) of varying ages up to circa 100 years old, are also present. It has remained largely unmanaged for the past 30 to 40 years other than usage for shooting pheasant.

Detailed descriptions about the site, the FACE facility and the broad experimental approach can be found at Hart et al., (2020) and MacKenzie *et al.*, (2021). In summary, there are nine experimental arrays ~30m in diameter, each containing ~six live oak trees: three with infrastructure injecting eCO₂ (+150 parts per million by volume (ppmv)), three infrastructure controls injecting aCO₂ (~410 to 430 μmol mol⁻¹ for 2017–

2021, inclusive), and three *ghost* (no-treatment, no-infrastructure) controls. Figures showing the experimental array layout are shown in Ch.3 and Ch 4.

2.2 Overall methodology

All three experimental conditions (eCO_2 , aCO_2 and *ghost*) were considered. Whilst infrastructure (eCO_2 , aCO_2) array trees were the main focus, the investigation was designed to use *ghost* control tree results as both a baseline and a crosscheck against which any experimental FACE effects could be referenced.

Figure 2-1(a) shows the main flow paths of water and water vapour around an example oak tree along with key measurement points of interest for this project. Whilst monitoring was focused on oak trees, adjacent subdominant trees are also shown in Fig.2-1(b) to illustrate the mixed-forest measurements taken. Monitoring methods for assessing water flow (soil-stem-leaf-atmosphere) were considered to determine practical limitations when applied to forest treescapes. This was not focused on instrumentation selection (which had already been completed before this project commenced) but rather to tease out any potential limitations or additions which could be considered within the duration of the project.

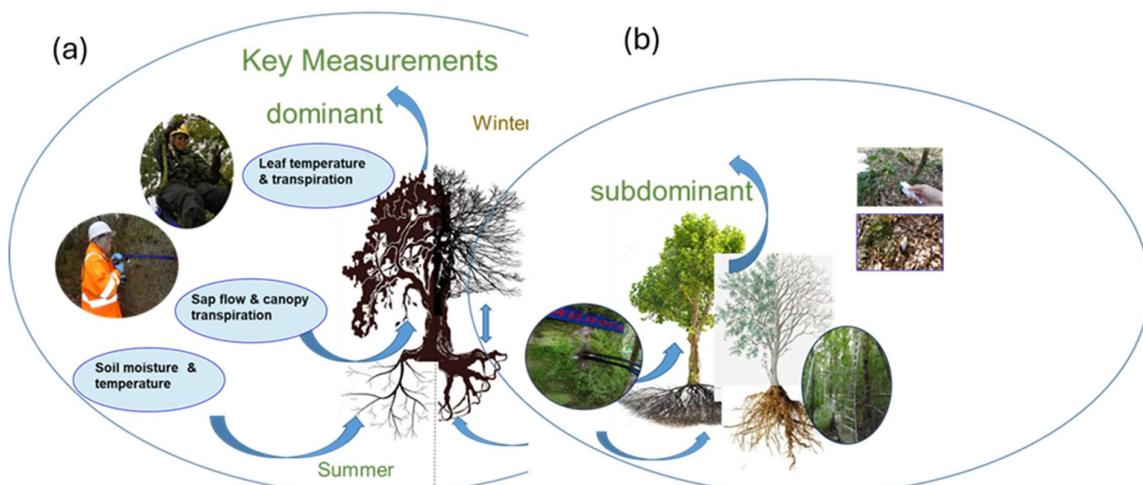


Figure 2- 1: Key plant hydraulic related measurements. (a) dominant, (b) subdominant trees.

665 Firstly, below-ground instrumentation (e.g. for measuring soil moisture) was extended to ensure some data from *ghost* arrays. Next, a sap flow trial was planned for installed in oak trees within *ghost* arrays with extensions to eCO_2 and aCO_2 oaks in just two arrays minimising any irreversible damage to stems.

Manually collected data (e.g. tree size, stomatal conductances, leaf temperatures) 670 required for this project were collected up to 2022 after which core data sources (e.g. from stem dendrometers) were used. In-situ leaf-level measurement was instigated following a trial of the approach on cut-twigs from *ghost* oak trees.

BIFoR FACE Operations team provided supporting engineering and meteorological instrumentation (FACE system, Met Towers, Flux tower). Data curation, including 675 overviewing and reporting of logged data quality, was managed by a Data manager (Giulio Curion 2019-2023, Furkan Tekinay 2024-) for some of this project's duration and prior to this by each researcher or professional nominee. The author checked remotely for failures of logged data and maintained the sap flow instrumentation (e.g. probesets, enclosures, heater boxes) on-site. Liaison with the Operations team 680 enabled maintenance of handheld (e.g. porometer), underground (soil moisture etc.) and core instrumentation.

2.3 Field Methods

An overview of the zones of measurement for this study is shown in Fig.2-2. Details of key core parameters measured and instrumentation types are indicated in Table 2-1 685 with more details in following Chapters 3 to 6. The arrangement of core research instrumentation in arrays (excluding FACE instrumentation mounted on towers, separately logged) as well as soil sampling sites are shown in Appendix 2-A.

Description	Make	Model	Position	Parameter	Symbol	Where	Units	Logger rate
Shallow soil-moisture. Depth, d = 12 cm, water content reflectometry (WCR)	Campbell Scientific [Logan, USA]	CS655 $\pm 3\%$ v/v for typical soils	Inserted from ground level	Volumetric water content (VWC) + Surface soil temperature	θ_{12} T_{ss}	Various all Arrays 1-9	% $^{\circ}\text{C}$	At least every 30 mins
Soil-moisture profile probes (2016 onwards – all nine arrays)	Enviro-scan-Sentek	RT6	Adjacent to oak	VWC (depths d = 10, 20, 40, 60, 100 cm)	θ_d	A 1-9 (not all active)	%	At least every 30 mins
Soil-moisture profile probes (2022 onwards infrastructure arrays)	Delta-T	WET-150	Adjacent to oak & Campbell probe position	Volumetric water content (depths d = 20, 50, 100 cm)	θ_{DT}	A1-6	%	At least every 10 mins
Soil temperature profile probes	Campbell Scientific [Logan, USA]	T107	Adjacent to oak	Soil Temperature (depths d=10, 20, 40, 60 cm)	T_d	A 1-9	$^{\circ}\text{C}$	30 mins (Av. per sampling period)
Tipping bucket gauges	Campbell Scientific	ARG-100	Ground level	throughfall precipitation - accumulative	P_{fs}	all Arrays 1-9	mm	30 mins
FACE Array tower	Hukseflux pyranometer	LP02-03	Top canopy (c20 m)	hemispherical solar radiation (Av)	TG	A 1-6	Watt m^{-2}	10 secs
	Campbell Sci	T107	Top canopy (c20 m)	Air Temp (Av)	$T_{airFACE}$	A 1-6	$^{\circ}\text{C}$	10 secs
Met Tower environmental	Kipp & Zonen (Delft; The Netherlands)	NR Lite 2	~23 m	Net Radiation	R_n	4 x Met Towers (mean)	Watt m^{-2}	
	LI-COR Biosciences (Lincoln; USA)	LI-190R Quantum Sensor	~23 m	Photo-synthetically Active Radiation	PAR	4 x Met Towers (mean)	$\mu\text{mol of photons m}^{-2} \text{s}^{-1}$	
	Vaisala	HMP15 5RH + DTR503	1.4 m (bottom) above ground	Relative Humidity/ Temperature	RH_{bot} And $T_{a_{bot}}$	4 x Met Towers (mean)	% and $^{\circ}\text{C}$	30 mins.
	Vaisala	HMP15 5RH + DTR503	~25 m (top) above ground	Relative Humidity/ Temperature	RH_{top} And $T_{a_{top}}$	4 x Met Towers (mean)	% and $^{\circ}\text{C}$	30 mins.
	Vaisala (Helsinki; Finland)	PTB210 by	~25 m (top)	Barometric/ (ambient, top) pressure	p	4 x Met Towers (mean)	hPa	
	Texas Electronics (Dallas; Texas)	TR-525M	2 m above ground	Precipitation - accumulative	P	4 x Met Towers (mean)	mm	15 mins

Table 2- 1: Key core parameters and their instrumentation types.

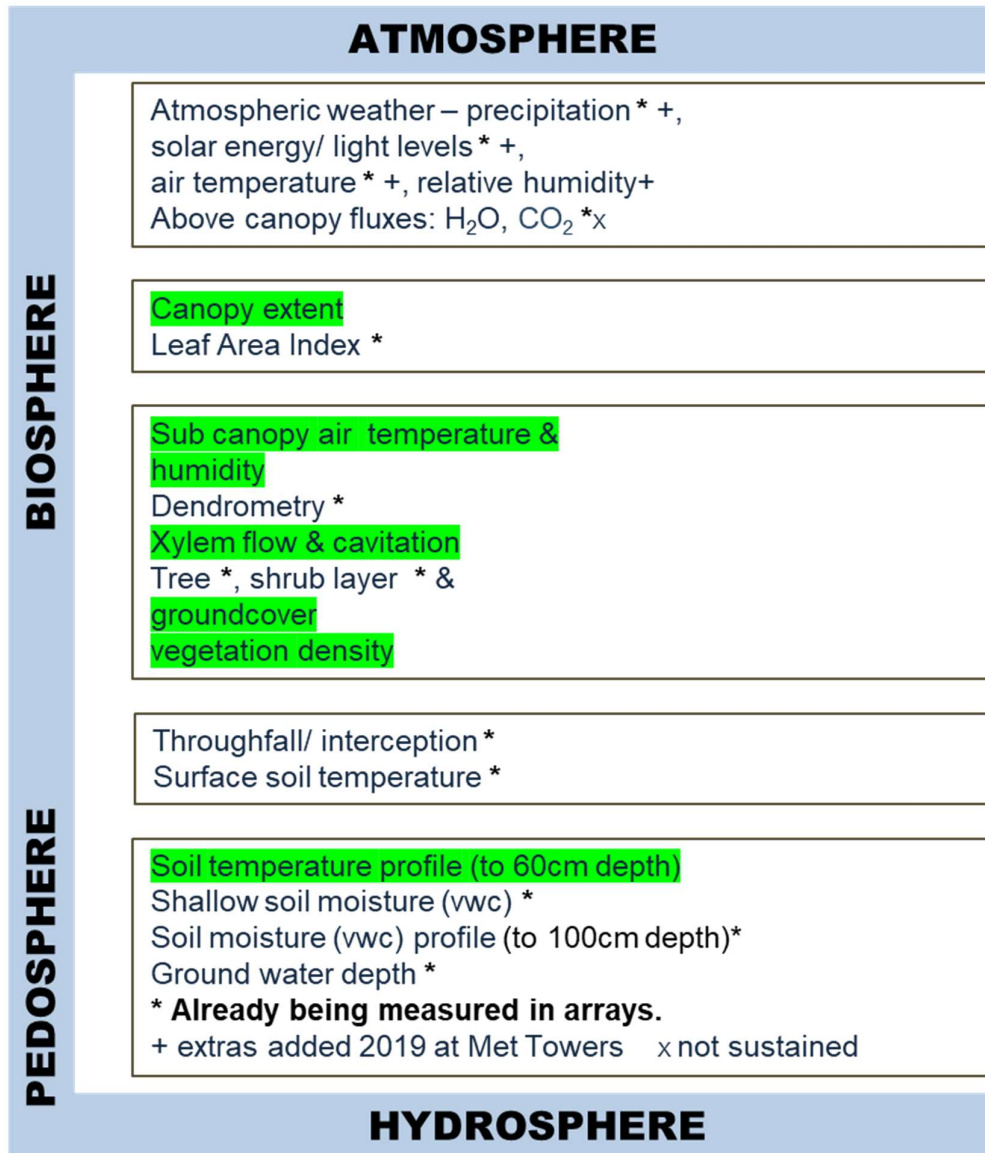


Figure 2- 2: To show instrumentation status at end 2017 and from 2019, along with zone of the respective instrumentation. Green highlighted items were still being implemented by the author from 2017.

Restrictions (especially during 2020-2021) meant measurements of leaf-water potential and intrinsic WUE were not undertaken within this project.

Environmental and BIFoR FACE measurements

Weather parameters – i.e. precipitation, throughfall, air temperature, relative humidity (RH), light levels (total and PAR), were measured using core instrumentation described in Table 2-1 which shows positions/ heights and frequency of measurement.

Precipitation monitoring has been well refined during the long period of meteorological station data recording, but the adequacy of throughfall monitoring by use of point collection is debatable. For this reason (and so as to expedite collection of more critical water flow-related data) this project did not pursue any improvements to the single and dual point monitoring using tipping-bucket type collection instigated as a part of the core instrumentation at Birmingham Institute of Forest Research (BIFoR) FACE.

Soil measurements

Key core data (e.g. soil moisture) were recorded by networked loggers. Soil measurements are focused on the vadose zone (i.e. above the water table, which varied in depth across the site from ~1 to ~5 m depth). Rather than attempt to study other diverse processes which occur within the soil, both when it is adequately moist and when drought occurs, my study focused on the water sources which could both be measured and which would potentially be utilised by the dominant oak species in the research forest.

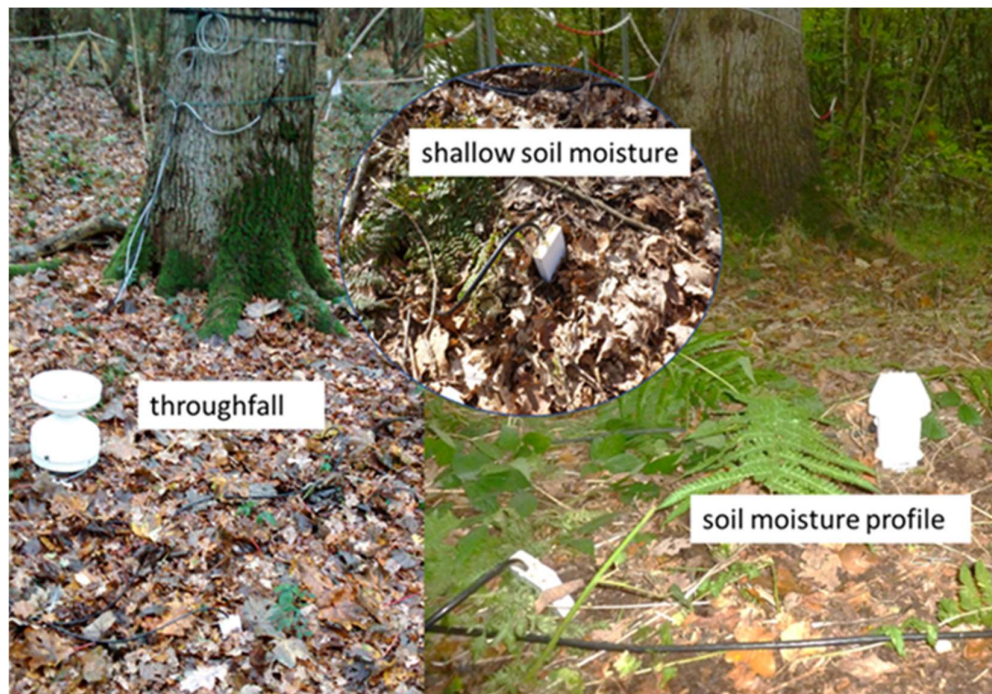
Continuous measures of soil moisture (volumetric water content (VWC, %)), from shallow (Table 2-1: CS655), profile to one metre depth (Table 2-1: Enviroscan-Sentek RT6) probesets and soil temperature (T_d , °C) probes (Table 2-1: T107), were taken at least every 30 minutes and logged in Campbell Scientific (CS) array loggers. Whilst the technology used for the shallow probesets was water content reflectometry (WCR), that of the profile probesets utilised capacitance at set depths.

Soil was analysed from regular soil sampling regimes (Handy, n.d.; Kourmouli, 2022) giving values of gravimetric water content (gwc), horizon depths, other soil

characteristics (e.g. bulk density) and fine root length/ density (Handy *et al.*, in prep;
720 Ziegler *et al.*, 2023) at depths to one metre.

For this study, the typical soil instrumentation set up was within 3 m of an oak tree as shown in Fig. 2-3. One method of Enviroscan soil moisture profile probe installation is shown in Fig. 2-4. Figure 2-5 shows soil temperature installation near to an oak in a typical array and Fig. 2-6 illustrates an early short-cores soil sampling campaign.

725 This project considered the reliability and accuracy of the pre-selected soil moisture sensor types (Table 2-1). The calibration of shallow soil moisture devices has been extensively reported (e.g. Dorigo *et al.*, 2013; Walker, Willgoose and Kalma, 2004), but their limitations of use within treescapes is still open to further refinement due to the known heterogeneity of soil types and root content of the environments presented.



730 Figure 2- 3: Photo of soil moisture and throughfall set-up near to an oak in a typical array.



Figure 2- 4: installation of soil moisture profile probes



Figure 2- 5: Photos of soil temperature installation near to an oak in a typical array.



735 **Figure 2-6: Soil sampling of short soil cores.**

For comparative purposes the suppliers of both probe types recommend that factory calibration (which for Enviroscan RT6 in sands, loam and clay loams gives an error of <0.01 coefficient of variation (CV) and for CS655 error $\pm 3\%$) should suffice. Site calibration (not undertaken in this instance) either in laboratory or in-situ is described

740 in the manufacturers documents (e.g. Sentek, 2011). Reliability of the profile Enviroscan probesets (random measurement failures) was of particular concern. Many obstacles to successful consistent soil moisture profile monitoring were present, not least the problems of installation, contact with the soil,

and drying/ cracking of soils during drought. This is discussed later (Ch. 4) alongside
745 sensor calibration methods and normalisation techniques. Discussion of solutions in
the field to overcome these obstacles by application of differing measurement
technology are beyond the scope of this study.

Sap flow measurement.

The measurement of heat flow through the xylem as a proxy for sap flow developed in
750 the 1930s (Huber, (1932, cited in Poyatos *et al.*, 2020, p.2613) and was further refined
over the following decades (e.g. Marshall, 1958). These methods are widely used,
especially in woody plants, both to determine optimum plant watering, and to study
plant ecohydrology (Poyatos *et al.*, 2016). Steppe *et al.*, (2015) introduced a number
of suggestions to improve the monitoring of xylem sap, in particular to ensuring capture
755 of low (e.g. nocturnal) and reverse levels of flow. I noted these aspects in a pre-
fieldwork project report (see Tables in App. 2-E) concerning the application of the
Tranzflo compensation heat pulse (HPC) probesets which were available to the
project. Additional shorter Tranzflo HPC probesets were acquired for monitoring
smaller diameter (10 to 25 cm diameter trees). The limitation of the selected
760 instrumentation for monitoring large trees is reported later as part of Ch.3.

A trial of sap flow measurement was instigated in winter 2016-2017 in one oak at the
edge of Array 9 (Fig. 2-7). In summer 2017 the trial was extended to include a total of
ten trees in Arrays 1 (eCO_2), 3 (aCO_2), 7, 8 and 9 (all *ghost* arrays). All trail sap flow
data were captured on stand-alone loggers with multiplexers (CR1000 and AM25T,
765 Campbell Scientific, Logan, Utah, USA) with remote visualisation available for logged
data facilitating failure detection and rectification. Daytime measurement was targeted
but HPC data was triggered every 30 minutes. The rollout to two oak trees in all arrays

with data captured on networked logger-multiplexers (one per array) followed. Data for subdominant trees' sap flow were collected on the same networked loggers.

770 Relocating and reprogramming of the nine networked logger-multiplexers (one per array) was necessary to enable sap flow measurement rollout. Some earthing problems were encountered in early installations in the infrastructure arrays. These were overcome by ensuring an earth spike was present adjacent to all array CS loggers. Rollout to two oaks in all arrays, entailing manufacture of extension leads and
775 using extension enclosures mounted on the ground to accommodate trees more than three metres from the loggers, was completed in 2018. Corrections to four faulty probeset connections were made at the end of the 2019 season.



Figure 2- 7: Sap flow trial - installation in Array 9.

780 Selection of the 18 oak trees was undertaken using the criteria described in Ch. 3. Each tree had two probesets facing east and west. The installation method and design of probesets is briefly described in Appendix 2-B and in Ch.3 and Ch.5.

Three subdominant species with stems > 10 cm diameter were planned to be included in the sap flow study following the successful trial of oak. Subdominant trees were also
785 selected for sap flow monitoring based on stem size and nearness to loggers. Following a species survey it was determined that there was unequal distribution of the most abundant subdominant species (i.e. sycamore, hawthorn and hazel) trees across the experimental arrays. Delays to this project (limited access during the COVID pandemic) also meant no hazel installations were included. The three potential
790 subdominant species were subsequently reduced to two species (sycamore, hawthorn) as a result. Sycamore and hawthorn selected as part of this project were not present in all arrays. Further details are described in Chapters 5 and 6.

Tree trait measurements

Methods for measurement of macro physical traits (see Ch.3, Ch.5 and Ch.6) for all
795 oaks and subdominant trees forming part of the study are shown in outline in Fig.2-6.

A list of all trees along with their installation/ commissioning date and other starting tree size parameters is contained in Appendix 4 (oak) and Appendix 6 (subdominant). Measurements relating to woodcores are described in Appendix 2-C to this chapter, also in Ch. 3 (Appendix 3). Data from dendrobands were used to update and check
800 manually-collected tree circumference measures (i.e. after field work was completed

in 2022) – those data are mostly published in Norby *et al.*, (2024). These checks reduced the likelihood of systematic error.

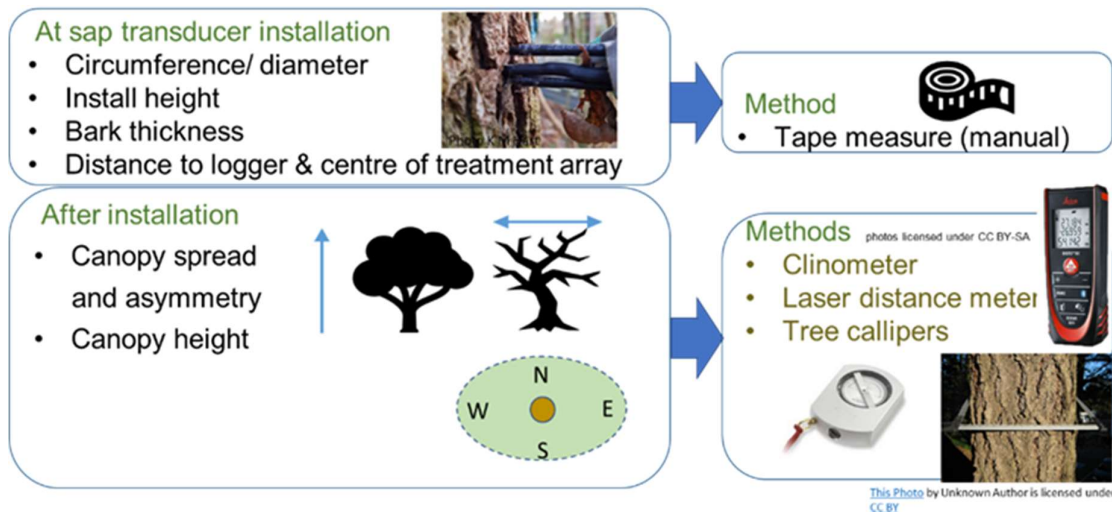


Figure 2- 8: Methods of measuring tree size including tape measure for circumference, callipers for stem diameter E-W and N-S and use of clinometer and laser distance device to identify canopy extent (canopy radius) at the four cardinal compass points ((Hemery, Savill and Pryor, 2005)). Average canopy diameter and stem diameter (calculated from circumference measured as above) are combined to calculate average canopy spread diameter and approximate canopy area. Stem height of subdominant trees was measured using the clinometer and laser distance device. Height of oaks was estimated during canopy access in relation to tower height.

Leaf measurement of in-situ and cut-twig parameters

The measurement of leaf parameters is most commonly undertaken by chamber devices (infra-red gas analyser (IRGA) or leaf porometers (Toro, Flexas and Escalona, 2019; Zhang and Zhang, 2019). Here a simple steady state porometer (Decagon, SC1) porometer (measuring stomatal conductance and temperature) was used (described in more detail in Chapter 5) and transpiration calculated from these and additional environmental parameters. The porometer's design has the advantage of small size, intuitive manual operation and high reliability. Water vapour released through the stomata sets up a relative humidity (RH) gradient along the chamber. The instrument monitors RH at two points along the flux path and, once the flux gradient reaches a steady state, calculates and displays the leaf diffusion conductance to water vapour (the reciprocal of resistance). Leaf temperatures were independently measured by

infrared laser in most cases (Ch.6) as this minimises the systematic error of leaf heating during measurement.

As described in Chapter 6, the Canopy Access System (CAS) enabled in-situ top
825 canopy measurements of stomatal conductance and abaxial leaf temperatures to be
taken in a small number (one per array) of sap flow fitted oak trees from 2019 to 2021
approximately once a month through the treatment season. In *ghost* arrays no canopy
access system was in place so there was reliance on arborists to collect cut-twigs from
oak, with measurements taken promptly after cutting. For the bottom canopy of sap
830 flow fitted subdominants (sycamore and hawthorn), similar measurements were taken
either directly from the ground or using an access ladder or deer platform. The method
is outlined briefly in Appendix 2-D.

2.4 Methods for laboratory analysis

One method of laboratory analysis was used as an integral part of the processing of
835 raw sap flow data, namely the weighing, drying and physical measurement (including
water displacement) of woodcores. This was required to calculate the woody matrix
characteristics used in sap flux calculations described in detail in Appendix 2-C.

2.5 Methods for data exploration and statistical analysis

Three main graphical methods were used to visually examine data, namely: time series
840 line and bar graphics, box and whisker graphics, and frequency distribution graphics.
Normalisation of data (e.g. sap flux, normalised by tree size to enable comparison
between different size trees), or conversion to normalised units (e.g. volumetric water
content data to relative extractable water) was necessary to enable treatment
comparisons to be undertaken.

845 Statistical analysis methods included ANOVA, non-parametric methods (e.g. for sap flux) and use of generalised linear mixed effect models (glmmms) for non-linear repeated-measures water usage data (Chapter 3).

Further details of all methods as used are listed in the relevant chapters.

References Chapters 2

- 850 Dorigo, W. A. et al. (2013) 'Global Automated Quality Control of In Situ Soil Moisture Data from the International Soil Moisture Network', *Vadose Zone Journal*. John Wiley & Sons, Ltd, 12(3), p. vzj2012.0097. doi: 10.2136/vzj2012.0097.
- Handy, G. et al. (no date) 're Roots'.
- Hart, K. M. et al. (2020) 'Characteristics of free air carbon dioxide enrichment of a
855 northern temperate mature forest', *Global Change Biology*, 26(2), pp. 1023–1037. doi: 10.1111/gcb.14786.
- Hemery, G. E., Savill, P. S. and Pryor, S. N. (2005) 'Applications of the crown diameter–stem diameter relationship for different species of broadleaved trees', *Forest Ecology and Management*, 215(1), pp. 285–294. doi:
860 <https://doi.org/10.1016/j.foreco.2005.05.016>.
- Kourmouli, A. (2022) Soil respiration and nutrient dynamics at a mature temperate woodland - the BIFoR FACE experiment. University of Birmingham. Available at: <https://etheses.bham.ac.uk/id/eprint/11835/>.
- Mackenzie, A. R. et al. (2021) 'BIFoR FACE: Water–soil–vegetation–atmosphere
865 data from a temperate deciduous forest catchment, including under elevated CO₂', *Hydrological Processes*. John Wiley & Sons, Ltd, 35(3), p. e14096. doi: 10.1002/hyp.14096.
- Marshall, D. C. (1958) 'Measurement of Sap Flow in Conifers by Heat Transport.', *Plant Physiology*, 33(6), pp. 385 LP – 396. doi: 10.1104/pp.33.6.385.

870 Norby, R. J. et al. (2024) 'Enhanced woody biomass production in a mature
temperate forest under elevated CO₂', *Nature Climate Change*. doi: 10.1038/s41558-
024-02090-3.

Poyatos, R. et al. (2016) 'SAPFLUXNET: towards a global database of sap flow
measurements', *Tree Physiology*, 36(12), pp. 1449–1455. doi:
875 10.1093/treephys/tpw110.

Poyatos, R. et al. (2020) 'Global transpiration data from sap flow measurements: the
SAPFLUXNET database', *Earth System Science Data*. doi: 10.5194/essd-2020-227.

Sentek (2011) Calibration of Sentek soil moisture sensors. V2.0. Sentek Sensor
Technologies. doi: info:doi/.

880 Steppe, K. et al. (2015) 'Sap flow as a key trait in the understanding of plant hydraulic
functioning', *Tree Physiology*, 35(4), pp. 341–345. doi: 10.1093/treephys/tpv033.

Toro, G., Flexas, J. and Escalona, J. M. (2019) 'Contrasting leaf porometer and infra-
red gas analyser methodologies: an old paradigm about the stomatal conductance
measurement', *Theoretical and Experimental Plant Physiology*, 31(4), pp. 483–492.
885 doi: 10.1007/s40626-019-00161-x.

Walker, J. P., Willgoose, G. R. and Kalma, J. D. (2004) 'In situ measurement of soil
moisture: a comparison of techniques', *Journal of Hydrology*, 293(1), pp. 85–99. doi:
10.1016/j.jhydrol.2004.01.008.

Zhang, L. and Zhang, S. (2019) 'The quantitative impact of different leaf temperature
890 determination on computed values of stomatal conductance and internal CO₂

concentrations', *Agricultural and Forest Meteorology*, 279, p. 107700. doi:
10.1016/j.agrformet.2019.107700.

Ziegler, C. et al. (2023) 'Quantification and uncertainty of root growth stimulation by
elevated CO₂ in a mature temperate deciduous forest', *Science of The Total*
895 *Environment*, 854, p. 158661. doi: <https://doi.org/10.1016/j.scitotenv.2022.158661>.

Chapter 3 This study Oak tree sap flow and water usage

900 This study (Quick *et al.*, 2025) has been published in the journal *Biogeosciences* (<https://www.biogeosciences.net/>). The published article and its supplement are available at the following link:

<https://bg.copernicus.org/articles/22/1557/2025/>

The published versions are licenced under CC BY version 4.0
905 (<https://creativecommons.org/licenses/by/4.0/>).

The published article is also embedded within this chapter.

Additional work (see Table 3-1) has been completed to correct errors identified in the publication. A reworded text plus captions for section 3.2.1 and the main correction (as Table 2- revised data version) incorporating the revised linear model corrections, the
910 corrected number of data points and degrees of freedom (df), a revised caption and corrected units for the intercept. A correction has consequently been made to Table S5 of the published version of the supplement which is included in Appendix 3.

The corrected SE and adjusted R^2 (aR^2) figures for Fig. 3(e) can be read from Table 2- revised data version (i.e. SE = 0.41 , aR^2 = 0.50).

915 Table 3-2 has additionally been prepared to clarify the development of generalised linear mixed effect models (GLMMs) and selection of the best models.

Item	Section/ Table/ Figure	Error type, Revision impact
1	3.2.1 text concerning data (tree-year points) and treatment- specific models. Table 2 caption (sentence 3) re-worded.	Minor text changes – embedded in thesis.
2	Table 2: Intercept units (Litres per day)	typo
3	Table 2 (df corrections): July 2018, df = 4 July 2021 df = 15 July 2017-2021 df =55	Typos plus model interpretation error.
4	Table 2 (linear model data): July 2017 –2021 data re-analysed	Data points for 2019 (one aCO ₂ tree-year point) were incorrect in linear model calculation. Minor impact on this model only, not on consequent findings.
5	Figure 3 (e), correct tabulation of SE and adj. R ² . See corrected Table 2	Data points for one tree-year were omitted in linear model. Minor impact on this model only, not on consequent findings.

Table 3- 1: Itemised typos and errors relating to publication Quick et al. (2025).

920 **Revised items.**

Clause and Table caption numbering is as per published document. Changes are underlined and sidelined.

‘ **3.2.1 *TWU* as a function of stem radius**

Example relationships between R_b , (mm) and monthly mean TWU ($\overline{TWU}$, litres d⁻¹)

925 for all trees with both probesets working were first analysed for 2019 summer months. July was used for comparison as typically it exhibits maximum $\overline{TWU}$. The hypothesis that $\overline{TWU}$ is a function of R_b was tested by a simple regression model. The best fit model for the combined 2019 data across all treatments was a simple linear fit; quadratic fits were tested and rejected. During July 2019, due to two

930 probesets malfunctioning, *Ghost* tree $\overline{TWU}$ results did not include trees as large as the largest in the infrastructure arrays as explained in Methods above. Data from six,

14, 18 & 17 trees were subsequently used for each July in years 2018, 2019, 2020 & 2021 respectively.

(revised Table 2 data)

	slope	SE	R ²	Adj. R ²	intercept	t	df	p
	(litres per day per millimetre)				(litres d ⁻¹)			
Jul-18	3.7	0.742	0.86	0.83	-721	5.011	4	p<0.01
Jul-19	3.3	0.417	0.82	0.81	-623	7.846	12	p<0.001
Jul-20	2.2	0.448	0.54	0.52	-300	4.995	16	p<0.001
Jul-21	2.6	0.618	0.58	0.55	-355	4.251	15	p<0.001
Aug-19	2.9	0.312	0.88	0.87	-540	9.3	12	p<0.001
July 2017-2021	3.107	0.410	0.52	0.50	-549	7.573	55	p<0.001

Table 2: Linear regression model parameters for July mean *TWU* ($\overline{TWU}$, litres d⁻¹) versus stem radius at insertion point (R_b , mm). August 2019 is also shown. Final row shows model statistics for July all years 2017-2021, for 57 tree-year points. The table does not discriminate $\overline{TWU}$ in respect of treatment. See Appendix A Table A1 for statistical abbreviations.

All years 2017–2021 of July data are shown in Fig. 3, illustrating the differences by year (Table 2) and treatment. The shorter regression for the *Ghost* array trees (Figs. 3(a) and (b)) has a smaller slope than infrastructure (*eCO₂* and *aCO₂*) array trees which exhibit similar slopes. Table 2 lists July model slopes for years 2018–2021 all treatments combined. The slopes are within +20%, -30%, giving a mean slope of 3.1 ± 0.4 litres d⁻¹ mm⁻¹, for $274 \text{ mm} \leq R_b \leq 465 \text{ mm}$, although the steepest slope (July 2018) and the shallowest slope (July 2020) differ by more than their combined standard errors and so may represent different relationships. The intercept of the linear regression is not physically meaningful as we are only considering a relationship for trees of R_b between 0.25 and 0.5 m. The results confirm the recent study by Schoppach et al., (2021) (and supporting reference (Hassler et al., 2018)) in respect of the relationship between DBH and the water usage of oak.

Figure 3: Mean July TWU ($\overline{TWU}$, litres d⁻¹) versus stem radius Rb (m) at measurement height is shown for the three treatment types in years 2017–2021. (a), (b) show Ghost (no-infrastructure-no-treatment) trees all years. (c), (d) show infrastructure arrays for treatment (eCO₂) and infrastructure control (aCO₂), years 2018–2021. (b) and (d) show treatment regression lines for all years combined (e) shows points for all years with single regression line. Rb is linearly proportional to $\overline{TWU}$ (mean slope 3.1 ± 0.4 litres d⁻¹ mm⁻¹). (a) and (c) error bars show sd. (e) additionally shows significance (***) p<0.001, SE and adjusted R2 (aR2) values.

The slopes of the three treatments in July for all years' data were also compared to determine differences due to treatment (Table S5). There is 10% difference between infrastructure treatment trees' slopes (Table S5 and Fig. 3(d): aCO₂ slope = 3.94, SE = 1.21 litres d⁻¹ mm⁻¹; eCO₂ slope = 3.56, SE = 0.30 litres d⁻¹ mm⁻¹), which is not statistically significant given the standard error on the slopes. Overall, for infrastructure treatment trees (eCO₂ and aCO₂; Fig. 3(d)), the slope is greater than for no-infrastructure Ghost array trees (slope = 1.2, SE = 0.47 litres d⁻¹ mm⁻¹) (Fig. 3(b)) and the magnitude of $\overline{TWU}$ for all infrastructure trees is greater for a given size. ‘

End of corrected Table 2 and text.

Step	<u>Models run</u>
	Note year 2020 is shown as an example
1.	Run a glm model (model_0) – no repeat measures modelled e.g. <code>glm(formula = normWtr ~ CO2, family = Gamma(link = "log"), data = sapflux2_2020)</code>
2.	Run a (model_1) glmer (generalised linear mixed effects) model, family = Gamma (link = "log") with tree as a random effect. e.g. <code>model_1_2020 <- glmer(normWtr ~ CO2 + (1 treelabel), family = Gamma(link = "log"), na.action=na.exclude, data=sapflux2_2020) # tree only - you want to be able to include the structure of the groupings.</code>
3.	Develop model 1 to see if Array or Array/ tree nesting is better than just tree: model_1array e.g. <code>modelG_1_2020array <- glmer(normWtr ~ CO2 + (1 array), family = Gamma(link = "log"), na.action=na.exclude, data=sapflux2_2020)</code> model_1array_tree e.g. <code>modelG_1_2020array_tree <- glmer(normWtr ~ CO2 + (1 array treelabel), family = Gamma(link = "log"), na.action=na.exclude, data=sapflux2_2020)# tree nested within array</code> model_1 was marginally better, so kept simplest model structure
4.	Develop models further using DOY as a time factor (for longitudinal effects)., N.B. DOY as a fixed factor crashed on these models e.g. <code>modelG_3_2020 <- glmer(normWtr ~ CO2 + DOY + (1 treelabel).</code> modelG_4 e.g. <code>modelG_4_2020 <- glmer(normWtr ~ CO2 + (1 DOY) + (1 treelabel) , family = Gamma(link = "log"), na.action=na.exclude, data=sapflux2_2020)# with DOY as a random effect</code> models 5 and 6 were not run as they crashed with fixed DOY. E.g. <code>modelG_5_2020 <- glmer(normWtr ~ CO2+DOY + (1 DOY) + (1 treelabel)</code> <code>modelGamma_6 <- glmer(normWtr ~ CO2*DOY + (1 DOY) + (1 treelabel)</code>
Conclusion	modelG_4 was always (for each year) better than model_1 and better than other repeated measures models tried.

Table 3- 2: Showing the steps taken to determine the best GLMM model for sap flux treatment comparisons. Table 3- 2

970 **Published document follows (25 pages)**

Biogeosciences, 22, 1557–1581, 2025
<https://doi.org/10.5194/bg-22-1557-2025>
 © Author(s) 2025. This work is distributed under
 the Creative Commons Attribution 4.0 License.



Water usage of old-growth oak at elevated CO₂ in the FACE (Free-Air CO₂ Enrichment) of climate change

Susan E. Quick^{1,2}, Giulio Curioni^{1,2}, Nicholas J. Harper², Stefan Krause^{1,2,3,4}, and A. Robert MacKenzie^{1,2}

¹School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, B15 2TT, UK

²Birmingham Institute of Forest Research, University of Birmingham, Birmingham, B15 2TT, UK

³Laboratoire d'écologie des hydrosystèmes naturels et anthropiques (LEHNA),

University Claude Bernard, Lyon1, Lyon, France

⁴Institute for Global Innovation, University of Birmingham, Birmingham, B15 2TT, UK

Correspondence: A. Robert MacKenzie (a.r.mackenzie@bham.ac.uk)

Received: 5 July 2023 – Discussion started: 19 July 2023

Revised: 12 November 2024 – Accepted: 15 January 2025 – Published: 25 March 2025

Abstract. Predicting how increased atmospheric CO₂ levels will affect water usage by whole, mature trees remains a challenge. The present study investigates diurnal (i.e. day-light) water usage of oaks within an old-growth forest during an experimental treatment season (April–October, inclusive). Over the years 2017–2021, inclusive (years 1–5 of the experiment), we collected individual tree data from 18 oaks (*Quercus robur* L.) within a large-scale manipulative experiment at the Birmingham Institute of Forest Research (BIFoR) Free-Air CO₂ Enrichment (FACE) temperate forest in central England, UK. Diurnal tree water usage per day (TWU, L d^{−1}) across the leaf-on seasons was derived from these data. Equal tree numbers were monitored in each treatment: FACE infrastructure arrays (+150 μmol mol^{−1}) of elevated CO₂ (eCO₂), FACE infrastructure control ambient CO₂ (aCO₂) arrays, and control “ghost” (no-treatment, no-infrastructure) arrays. TWU was linearly proportional to tree stem radius, R_b (~3.1 L d^{−1} mm^{−1}; 274 mm ≤ R_b ≤ 465 mm). R_b was also a very good proxy for projected canopy area, A_c (m²), which was linearly proportional to R_b (~617 m² m^{−1}). Applying the stem-to-canopy relation implied a mean July water usage of ~5 L d^{−1} m^{−2} of projected oak canopy in the BIFoR FACE forest. We normalised TWU by individual tree R_b to derive TWU_n (L d^{−1} mm^{−1}). We report whole-season treatment effects, differing year on year, alongside July-only results. In the 2019 and 2021 seasons, after correction for repeated measures, there was a 13 %–16 %, reduction in eCO₂ TWU_n compared to aCO₂ TWU_n, with a marginal 4 % reduction in 2020, but these model results were not statisti-

cally significant. Control trees exhibited a significant 27 % increase in aCO₂ TWU_n compared to ghost TWU_n in the whole season in 2019, with lesser, nonsignificant fixed effects in 2020 and 2021. Several factors may have contributed: the installation or operation of FACE infrastructure; array-specific differences in soil moisture, slope, or soil respiration; or the mix of subdominant tree species present. Our results showing normalised per-tree water savings under eCO₂ align with sap flow results from other FACE experiments and greatly extend the duration of observations for oak, elucidating seasonal patterns and interannual differences. Our tree-centred viewpoint complements leaf-level and ground-based measurements to extend our understanding of plant water usage in an old-growth oak forest.

1 Introduction

Long-term manipulation experiments test how environmental drivers, such as increased atmospheric carbon dioxide levels, affect plants and ecosystems. Plant hydraulics are adapted to expected ranges of environmental parameters, with larger plants exhibiting greater resilience to wider parameter variation due to their ability to maintain water and food reserves. To maintain transpiration demands, trees accommodate themselves to diel variation in solar radiation, respiration fluctuation, high temperatures, and seasonal soil water deficits. Short-term mechanisms include stomatal regulation; the use of reserves, as evidenced by stem diame-

ter variations (Sánchez-Costa et al., 2015); the use of stored plant water (Köcher et al., 2013); and the use of available water at variable soil depth (David et al., 2013; Flo et al., 2021; Gao and Tian, 2019; Nehemy et al., 2021). Development of resilient root structures (David et al., 2013; Flo et al., 2021) and minimisation of embolism mitigated by different xylem structures (Gao and Tian, 2019) may also occur. The ability of mature trees to withstand climate extremes may rely in part on using these buffering traits, which act to prevent permanent damage and maintain viability (Iqbal et al., 2021; Moene, 2014). This prompts the further question of how increasing atmospheric carbon dioxide levels will affect the hydraulic resilience of trees (Sect. 1.1 below).

The response of woody plants to drought varies considerably by species (Leuzinger et al., 2005; Vitasse et al., 2019), location (e.g. north versus south in Europe; Stagge et al., 2017), soil characteristics such as soil texture (Laverne et al., 2020), and combinations thereof (Fan et al., 2017; Salomón et al., 2022; Sulman et al., 2016; Venturas et al., 2017). For example, large oak trees, the focus of the current study, may maintain their transpiration rates (compared to beech) during mild water stress but remain vulnerable to embolism and root damage (Süßel and Brüggemann, 2021).

1.1 Future-forest atmospheric carbon dioxide and water usage

Primary producers may respond to elevated CO₂ (eCO₂) levels by assimilating and storing more carbon, which happens during photosynthesis for plants containing chlorophyll. Global carbon and water cycle models (Guerrieri et al., 2016; De Kauwe et al., 2013; Medlyn et al., 2015; Norby et al., 2016) predict that, at least until the middle of the 21st century, trees and plants could potentially photosynthesise more efficiently, which may induce increased carbon storage. This could be beneficial for individual tree productivity. Stomatal regulation determines the trade-offs between carbon assimilation and water loss and, for a given leaf area and stomatal density, determines the rate and quantity of water usage seen in the stems of woody plants. Water usage at the tree level is, therefore, a strongly integrative measure of the whole-plant response to environmental drivers (such as temperature and precipitation) and experimental treatments (such as eCO₂).

Untangling the canopy water exchange and soil moisture hydraulic recharge dynamics within forest Free-Air CO₂ Enrichment (FACE) experiments can be complex, but responses to eCO₂ manipulations (including stepwise increases; Drake et al., 2016) inform our understanding of plant responses to climate change scenarios. Specific studies concerning transpiration and water savings of eCO₂ responses (Ellsworth, 1999; Li et al., 2003) have already improved the model predictions (De Kauwe et al., 2013; Donohue et al., 2017; Warren et al., 2011a), and here we seek additional evidence that will enable further advances to the predictive model capacity.

Experimental research into ecohydrological responses of old-growth and long-established deciduous forests to changing atmospheric CO₂ levels has been limited. The Web-FACE study (Leuzinger and Körner, 2007) reported on old individuals of temperate species and found that eCO₂ reduced water usage in *Fagus sylvatica* L. (dominant) and *Carpinus betulus* L. (subdominant) by about 14 % but had no significant effect on the water usage of *Quercus petraea* (Matt.) Liebl., the other dominant species present. The six *Q. petraea* included in Leuzinger and Körner's (2007) study were monitored to give measurements of accumulated sap flux (normalised against peak values in each tree) over two 21 d periods. Changes in water usage by long-established oak trees at eCO₂, measured for longer periods (greater than a month) across the leaf-on season, have not previously been reported.

Previous forest FACE experiments that reported tree/stand water usage (De Kauwe et al., 2013; Warren et al., 2011a), apart from Web-FACE, all constituted younger deciduous and mixed plantations of less than 30 years of age (Schäfer et al., 2002; Tricker et al., 2009; Uddling et al., 2008; Wullschlegel and Norby, 2001; Wullschlegel et al., 2002). Some of these eCO₂ treatments are long-term (durations of over 10 years in some cases), but all are limited in their period of monitoring sap flow (Schäfer et al., 2002). There are further (2010 onwards) sap flux studies of deciduous oak from studies that do not manipulate CO₂ but offer helpful data for comparison, for example within Europe (Aszalós et al., 2017; Čermak et al., 1991; Hassler et al., 2018; Perkins et al., 2018; Schoppach et al., 2021; Süßel and Brüggemann, 2021; Wiedemann et al., 2016) and in North America (Fontes and Cavender-Bares, 2019). Robert et al. (2017) have reviewed the hydraulic characteristics of oak species from multiple studies, which help us to place our results in context. Within the UK maritime temperate climate, only a few ecohydrological studies (e.g. Herbst et al., 2007; Renner et al., 2016) have previously considered the sap flow responses to water availability and drought for mixed-forest *Quercus* species.

The FACE method was developed to eliminate chamber and/or infrastructure influences (see, e.g. Pinter et al., 2000; Miglietta et al., 2001) and provides good control over CO₂ elevation levels (e.g. Hart et al., 2020). Nevertheless, few studies have explored the effects of the experimental infrastructure (e.g. on the resulting microclimate; LeCain et al., 2015). Disturbance of the vegetation was kept to a minimum while constructing the Birmingham Institute of Forest Research (BIFoR) FACE (Hart et al., 2020), but some clearing of ground flora and removal of coppice stems did occur, plausibly reducing competition and making more soil water available to the oaks. Other possible effects of infrastructure on water availability are discussed in Sect. 3.5.2.

1.2 Improving global vegetation models and questions of scale

Global vegetation models have been developed based on leaf-level plant knowledge alongside that of soil–tree–atmosphere exchange (e.g. Medlyn et al., 2015). These models have predicted reduced canopy conductance, G_s , and increased runoff in future climate scenarios, but an important gap has been identified between estimated and observed water fluxes (De Kauwe et al., 2013).

At the forest scale, studies of the effects of drought (2018–2019) on forested landscapes have shown that plant growth, stem shrinkage (Dietrich et al., 2018), and branch mortality may be affected for several years, especially for deciduous species in mature or old-growth forests (Salomón et al., 2022). At this forest scale (Keenan et al., 2013; Renner et al., 2016), there is also a more complex impact on the ecosystem and atmospheric demands as planetary-scale CO₂ levels increase, affecting boundary layer feedbacks.

In contrast to forest- and leaf-scale studies, canopy transpiration estimates from stem xylem sap flux (Granier et al., 2000; Poyatos et al., 2016; Wullschlegel and Norby, 2001; Wullschlegel et al., 2002) reflect how the whole canopy transpires rather than concentrating on individual leaf stomatal conductance to water. The present study is tree focused, bridging model-informing data gaps that were previously identified (De Kauwe et al., 2013; Medlyn et al., 2015). Tree-scale studies have provided essential data for calibration and validation of tree–water models (De Kauwe et al., 2013; Wang et al., 2016), identified key parameters driving responses to expected water shortages (Aranda et al., 2012), and compared species differences in mature tree responses to ambient $a\text{CO}_2$ (Catovsky et al., 2002) or $e\text{CO}_2$ (Catoni et al., 2015; Tor-ngern et al., 2015). Xu and Trugman (2021) have updated the previous empirical parametric and mechanistic model approaches to global vegetation modelling, reinforcing the need to use measured tree parameters (such as sapwood area) to improve model predictions.

Here we focus on whole-tree species characteristics and link these parameters to diurnal (i.e. daylight) tree water usage per day (TWU, L d^{-1}) from marginally intrusive stem xylem sap measurements. These measurements provide highly time-resolved data of plant water usage for several years with minimal maintenance. Heat-based measurement techniques (Forster, 2017; Granier et al., 1996; Green and Clothier, 1988) have been used over the past 40 years for measurements of plant xylem hydraulic function (Landsberg et al., 2017; Steppe and Lemeur, 2007), with automated data capture enabling increasingly realistic models of whole-tree xylem function.

1.3 Objectives, research questions, and hypotheses

This study provides new data from a field-based FACE environmental manipulation experiment to characterise seasonal

and inter-year patterns of daily water usage by old-growth oak trees under $e\text{CO}_2$. We test for significant differences between treatments within these water usage distributions and patterns and take into account the effect of FACE infrastructure. The paper relates diurnal tree water usage per day (TWU, L d^{-1}) to measurable tree traits (stem radius, R_b (mm), and canopy area, A_c (m^2)) and examines variation in TWU with environmental drivers and soil moisture. It also examines the limitations of water usage measurement by compensation heat pulse (HPC) sap transducers.

The following specific research questions and associated hypotheses are considered.

1. Is there a measurable difference in TWU distribution under $e\text{CO}_2$ compared to (infrastructure-containing) ambient CO₂ control ($a\text{CO}_2$) across the seasonal cycle?

Hypothesis 1 – a detectable $e\text{CO}_2$ treatment effect is present, reducing TWU for $e\text{CO}_2$ trees.

2. Does the presence of FACE infrastructure measurably affect TWU?

Hypothesis 2 – TWU is greater in the presence of FACE infrastructure.

2 Materials and methods

2.1 BIFoR FACE

At the BIFoR FACE experiment in central England, UK (Hart et al., 2020), we investigate soil–plant–atmosphere flows and fluxes of energy and water (Philip, 1966). We monitor soil–xylem–stomatal responses to $a\text{CO}_2$ and $e\text{CO}_2$ levels in a mixed-deciduous temperate forest of approximately 180-year-old trees. The $e\text{CO}_2$ treatment represents conditions expected by the middle of the 21st century (see the shared socioeconomic pathways (SSPs) in IPCC, 2021). This long-term experiment presents a rare opportunity to gain new insight into the complexity of water usage of old-growth forest trees in a changed atmospheric composition. BIFoR FACE is unique amongst free-air experiments in its ability to study the ecohydrology of these pedunculate oaks (*Quercus robur* L., subsequently abbreviated as oak) under $e\text{CO}_2$.

The BIFoR FACE facility is in Staffordshire (52.801°N, 2.301°W), England, UK (Fig. 1). The forest is a circa 1840 plantation of oak with a *Corylus avellana* (hazel) copice. Naturally propagated *Acer pseudoplatanus* (sycamore), *Crataegus monogyna* (hawthorn), *Ilex aquifolium* (holly), and smaller numbers of woody plants of other orders (e.g. *Ulmus* (elm), *Fraxinus* (ash)) of varying ages up to circa 100 years old are also present. Some subdominant trees, e.g. sycamore, hawthorn, and elm, impinge on the high, closed canopy. Each experimental array, ~30 m in diameter, was selected to contain ~six live oak trees. There are nine experimental arrays: three with infrastructure injecting $e\text{CO}_2$

(+150 parts per million by volume (ppmv)), three infrastructure controls injecting αCO_2 (~ 410 to $430\ \mu\text{mol mol}^{-1}$ for 2017–2021, inclusive), and three “ghost” (no-treatment, no-infrastructure) controls. Data were collected for all three FACE facility treatments. Here we concentrate on oak, the dominant species, with sap flow monitoring restricted to 2 oaks in each of the nine experimental arrays, totalling 18 trees.

The climate at the site is temperate maritime, with a pre-experimental mean annual temperature of 9°C and mean annual precipitation of 690 mm.

We compare individual tree sap flux and TWU responses under the three treatments across the leaf-on seasons for these early experimental FACE years, looking at within-year and inter-year relationships. We also describe daily, monthly, and seasonal changes to sap flux and TWU for mature oak over 5 years and discuss how these results, from our tree-centred viewpoint, will improve our understanding of future-forest water dynamics of old-growth forest, contributing to the development of more realistic ecohydrological vegetation, soil, and landscape models.

Typical experimental arrays showing target oak trees are shown in Fig. 1. Parameter symbols used in this paper are covered in Appendix A, Table A1. The term “sap flow” is used generally when referencing heat transducer methods to measure the water movement through sapwood. The use of the terms “sap velocity” and “sap flux” is defined in Tables A1 and A2. These usages (from Lemeur et al., 2009; Tranzfö Manual, 1998) may differ from other authors’ usage (e.g. Poyatos et al., 2021).

2.2 Measurements overview

Our experiment is a triple-replicated (array) between-group (treatment type) design within which we selected our sample of oak tree individuals for continuous monitoring. There are three manipulation types, from which we select two individual trees in each array (a total of sample six trees per treatment). The individuals were not changed during the continuous experiment, nor were the measurement devices removed.

We report data from five treatment seasons, July 2017 to the end of October 2021. We focus on diurnal (i.e. day-light) responses within our experimental treatment season of April–October, inclusive. Sap flux and TWU datasets for the 18 oaks are calculated from half-hourly tree stem sap flow measurements derived from HPC transducers. TWU data is accumulated from sap flux and then analysed monthly within the treatment season across each year. For all xylem-sap-flux-monitored oak trees, tree identification, treatment type, array number, and stem circumference and average R_b at the probe set insertion point are shown in the Supplement (Table S1).

Figure S1 shows the key measurement points related to tree hydraulics in this project. This sap flow study is supported by other core environmental (detailed below) and soil

data available at our FACE experimental site (MacKenzie et al., 2021). Table S2 shows instrumentation types and related parameters used for analysis within this paper, which are described below.

We experienced early leaf-on herbivory attacks on oaks by European winter moth larvae, especially in 2018 and 2019 (Roberts et al., 2022), which decreased leaf area by 20 %–30 % and affected the timing of canopy closure. A longer dry period occurred in the meteorological summer of 2018 (Rabai et al., 2023), with wide variation in summer monthly precipitation across the study years. From 2016 to 2021 (treatment years), these 6-year averages (MAP 750 mm, MAT 10.6°C) measured at or near the site were higher (+9 % and +18 %, respectively) than the previous reported means (see above). Precipitation is further discussed below, with additional detail in the Supplement (Table S14, Figs. S10, S14, S15, and S16).

2.3 Seasonal definitions

We define a plant hydraulic year as being from the start of the dormant season (1 November) to the end of senescence (31 October). The 7 months of CO₂ treatment per year (with 6 months of leaf-on photosynthesis) do not easily divide into standardised meteorological seasons (spring, summer), so we define our months of interest, including non-treatment months, as shown in Table 1. The table includes 2 months, March to April, of pre-leaf growth when oak sap starts to rise.

Season length, between first leaf and full senescence/the first bare tree, is relatively constant at 8 months for the years studied, although the start and end vary year on year (Table 1). Canopy greenness, recorded as part of the PhenoCam network (https://phenocam.nau.edu/webcam/roi/millhaft/DB_1000/, last access: 10 July 2023), shows a very rapid decline towards wintertime values from the beginning of November. We have not collected phenology data specific to our target trees to capture any variability amongst individuals (see Sass-Klaassen et al., 2011).

2.4 FACE and meteorological measurements

Local precipitation (from a mixture of sources including meteorological towers; see MacKenzie et al., 2021) was recorded. Treatment levels of $e\text{CO}_2$, the diurnal CO₂ treatment period, top of canopy air temperature (T_a ; $^\circ\text{C}$), and total solar radiation (TG; W m^{-2}) (see Figs. S9 and S11) were available from the FACE control system (Hart et al., 2020; MacKenzie et al., 2021). Data were averaged across the six infrastructure arrays for TG and T_a , as the ghost arrays have no FACE measurements. Set point levels of ambient CO₂ were used to control treatment application in the $e\text{CO}_2$ arrays. There are also increases in the ambient levels of CO₂ present across the years of this study of about $3.5\ \text{ppmv yr}^{-1}$. The enrichment level is unique to BIFoR

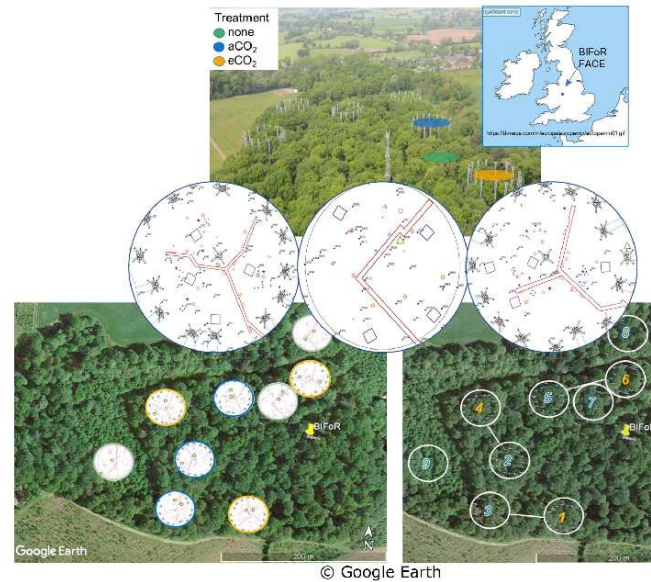


Figure 1. BIFoR FACE research woods showing three treatment types and nine arrays. Top left – aerial view, adapted (with permission) from Ch. 7, Fig. 7 of Bradwell (2022). Top right – location of BIFoR FACE within the British Isles; the map is cropped from <https://d-maps.com/m/europa/europemin/europemin01.gif> (last access: 7 November 2022). Middle – examples of three arrays, one per treatment, showing small brown rings within arrays to indicate the oak trees monitored for xylem sap flow. Bottom left – oak positions in all arrays. Bottom right – infrastructure treatment arrays are paired for similar soil conditions.

FACE at $+150 \mu\text{mol mol}^{-1}$, tracking the increasing ambient levels of CO₂ present across the years of this study (~ 410 to $430 \mu\text{mol mol}^{-1}$ for 2017–2022; Fig. S12) and altering the relative percentage change in $e\text{CO}_2 : a\text{CO}_2$ year on year by about 1 %, from 36 % (beginning of 2018) down to 35 % (end of 2021) (Fig. S12).

Water inputs of throughfall precipitation under the oak canopy (within 2 to 3 m of an oak stem and situated near a soil moisture monitoring position) were measured in all arrays, with all transducers positioned under an oak canopy near the stem. Pre-treatment (2015–2017 for all arrays) and on-site soil and throughfall data were used to characterise the site. Supplementary (2018 onwards) throughfall and/or soil monitoring sites were added (see MacKenzie et al., 2021). These data were captured by the same CR1000 data logger as the sap flow data. Given the frequency of rainy days during the treatment season, we have not segregated our analyses into rainy and dry days.

2.5 Tree selection

Variation between individuals in treatment plots can arise from their precise location within an array or from inherent (biological) variation between individuals, as well as from different treatments (Chave, 2013). This individual-tree experiment design aims to minimise atypical variation. Accordingly, the following criteria were used to select trees for sap flow monitoring:

- canopy cover completely within the array ($e\text{CO}_2$ and $a\text{CO}_2$ arrays);
- central location within the plot near the logger and adjacent to access facilities at height ($e\text{CO}_2$ and $a\text{CO}_2$ arrays for sampling and porometry access);
- straight stem, preferably with little epicormic growth;
- no large dead branches within the canopy that might affect the comparative biomass of the tree;
- unlikely to experience seasonal standing or stream water at the base.

Table 1. Definition of treatment season periods and dates for oak phenology at BIFoR FACE according to Nature's Calendar criteria for the years 2017–2021, inclusive (canopy closure data were not recorded). Dates within parentheses during dormant periods are not exact due to limited site visits by authors. First tint is also recorded in the year 2016 on 4 October.

Calendar months	FACE treatment season label	Note	Oak phenology at BIFoR FACE				
			2017	2018	2019	2020	2021
Mar–Apr (eCO ₂ starts in Apr)	Budburst and first leaf	March is pre-treatment. First leaf dates for oak shown.	6 Apr ^a	25 Apr ^a	29 Mar ^a	No data ^a (ca. 6 Apr)	27 Apr ^a
May–Jun	Early leaf-on	Includes canopy closure early leaf-on of oak.	–	–	–	–	–
Jul–Aug	Middle leaf-on		–	–	–	–	–
Sep–Oct (eCO ₂ until the end of October)	Late leaf-on	Includes start of senescence, i.e. first tint.	6 Sep	12 Sep	1 Oct	15 Sep	28 Sep
Nov–Feb	Dormant	All remaining non-treatment months.	–	(after 21 Nov) ^b	26 Nov ^b	(after 3 Nov) ^b	7 Dec ^b
		Assumed leaf-fall season.	6 Sep 2017 to 25 Apr 2018	12 Sep 2018 to 29 Mar 2019	1 Oct 2019 to ca. 6 Apr 2020	15 Sep 2020 to 27 Apr 2021	28 Sep 2021 to 24 Apr 2022

The terms budburst, first leaf, first tint, and bare tree are used as defined by Nature's Calendar. ^a On-site first leaf data (not obtained in 2020 due to the Covid-19 pandemic; 6 April 2020 was noted as budburst; unverified first leaf is recorded as 24 April 2020). Note that separate records of leaf-fall season are recorded for LAI calculation purposes, as Nature's Calendar data do not discriminate first leaf fall by leaf colour. ^b First bare-tree date recorded. The Nature's Calendar link is <https://naturescalendar.woodlandtrust.org.uk/> (last access: 29 January 2022). PhenoCam data are additionally available for all years (https://phenocam.nau.edu/webcam/roi/millhaft/DB_1000/, last access: 10 July 2023).

Target oak trees conforming to the above were chosen for monitoring to suit physical limitations of the transducer-to-logger constraints (e.g. cabling lengths including extensions to the same).

2.6 Tree characteristics

All oak trees were of a similar height (~25 m). Stem circumference (metres) at the insertion height of probes was measured at installation (from 2017 onwards) and in subsequent winters (January 2020–February 2022). The range and mean-per-treatment values in 2022 are tabulated (Tables S1 and S3). We note that tree size will affect TWU (Bütikofer et al., 2020; Laverigne et al., 2020; Verstraeten et al., 2008).

Canopy spread of all target oak trees was measured, using a clinometer and a laser distance device to identify canopy extent (canopy radius) at the four cardinal compass points (Hemery et al., 2005). We assume that the two-dimensional canopy area, A_c (m²), derived from the mean canopy diameter plus stem diameter is correlated with canopy transpiration. Canopies were first measured around installation dates (2017–2018) and then repeated for all oaks in early 2022. On the second occasion in 2022, we measured the asymmetry of each tree stem across the probe set cardinal directions (east–

west) and at right angles to this (north–south) to check the mean R_b value for sap flux calculations.

2.7 Xylem sap flux

Details of the xylem sap flux measurement method and associated calculations are provided in Appendix A, Table A2, and Appendix C. Each target oak tree had two probe sets, facing east and west (Fig. S2). Appendix B discusses the lower detection limit that the time-out characteristic places on this set of HPC data and on the consequent results. The validity of high value extrema is considered within our analysis.

To determine the whole-tree sap flux, two tree characteristics were used: (a) tree stem circumference at the insertion point (to determine the stem radius) and (b) bark thickness. From these data, we derived the tree stem cambium radius at the insertion point (R , m) and subsequently the heartwood radius (H , m) from the sensor spacing. H could not be determined from 10 cm cores, as these were not taken for all sap trees monitored.

The xylem sap flow installations in target trees commenced in January 2017. All ghost oak trees provided data from August 2017, and commissioning of all 18 oak trees was completed by autumn 2018. All oak sap flow instal-

lations were successful, and a total of 12 259 d of individual tree data (770 667 diurnal sap flux measurements across all months) were processed for the 2017–2021 TWU analysis. Data gaps in the earliest installations affected 4 of the 36 probe sets installed in four trees from August 2017 until September 2019.

With respect to the quality assurance of raw HPC data, commissioning and failure data were recorded for each probe set. This enabled a combination of data file amendment (especially for the earliest installations on separate loggers) and post-capture filtering to eliminate periods of invalid data for each probe set.

Example diel sap flux patterns for the ghost arrays in August 2019 (before filtering to eliminate nocturnal data) are shown in Fig. S3a, with east- and west-facing probe sets in each column. The sap flux data still show the minimum threshold levels (which vary by tree size) determined by the post-heat-pulse sampling period. It is noticeable that there is often a circumferential imbalance in xylem sap flux in the east (left-hand column of Fig. S3a) and west (right-hand column of Fig. S3a) probe set position data, which reflect the asymmetry in growth ring width around the stem typical in these old oak trees. The blank panels represent faulty probes (in two ghost trees), which were corrected by autumn 2019.

To compare individual tree responses across the leaf-on seasons, we filter the half-tree sap flux parameters using the solar azimuth and solar radiation parameters captured from the FACE control instrumentation (solar azimuth $> -6^\circ$ and solar radiation $> 0 \text{ W m}^{-2}$) to give just daylight (diurnal) data. Where both probe sets in a tree are providing good data, a mean whole-tree sap flux is derived and incorporated into the TWU (Fig. S3b). We had sufficient whole-tree data to exclude single-probe-set results.

There are day-to-day differences in TWU between trees in our study (e.g. Fig. S3b), even though all of them experience very similar environmental conditions, and this pattern is replicated across the three treatment types. The TWU data reported here compare well to results from other studies (Table S4 in David et al., 2013; Sánchez-Pérez et al., 2008; Tatarinov et al., 2005; Baldocchi et al., 2001).

2.8 Data processing, visualisation, and analysis

Manually collected data were preprocessed as .csv files for import to R (R version 4.4.1, 2024). Raw data from data loggers were processed, visualised, and consequently analysed using R and R Studio (Posit Team, 2024) on Windows 10 x64 (build 1909). Most figures in the Results section were created using the R package *ggplot* (Wickham, 2016). Other standard packages (e.g. *lubridate*) are listed in the R scripts accompanying the data (see “Data availability” section below). Regressions between R_b and water usage and between R_b and A_c were calculated using the *lm* function. Box and whisker plots to visualise seasonal and monthly differences in sap flux and water usage between trees and treatments were generated

in *ggplot*, where standard Tukey (McGill et al., 1978) percentiles (median + interquartile range), whiskers ($1.5 \cdot \text{IQR}$ from each hinge, where IQR is the interquartile range), and points for outliers are used. LOESS (locally estimated scatterplot smoothing) was used for exploratory analysis of the time series data (e.g. Fig. S13), an approach that does not rely on specific assumptions about the distributions from which observations are drawn. Levene tests (Levene, 1960) were carried out using *leveneTest* from the *car* library. Analysis of variance (ANOVA) models were used to test hypotheses with the functions *anova* and *summary*. The *autoplot* function from the *ggfortify* library was used to test the assumptions of normality of the ANOVA residuals. Nonparametric Wilcoxon rank-sum tests were used to check the ANOVA results, using *wilcox.test*. Generalised linear mixed-effect models (GLMMs) (using the function *glmer* from *lme4*) were selected to assess the random effects of the longitudinal (time-based) and individual (tree) repeated measures and estimate corrected fixed effects of treatments. The *summary* and *tab_plot* functions (from the *sjPlot* package) were used to extract the results.

3 Results

This section is organised as follows. We first report general characteristics of the distribution of sap flux (Sect. 3.1) and then develop relationships between TWU; R_b ; and projected canopy area, A_c (Sect. 3.2). Subsequently, we discuss the characteristics of TWU_n ($\text{L d}^{-1} \text{ mm}^{-1}$, Sect. 3.3), which we use to test for treatment effects in an analysis of variance (Sect. 3.4). We provide a qualitative discussion of the effects of seasonal weather in the Supplement Appendix.

3.1 Sap flux within the season and between years

Diurnal stem sap flux responses to canopy photosynthetic demand typically exhibit increased sap flux from dawn to around midday (UTC – local solar time at the site), with an approximately symmetrical decrease until dusk (Fig. S3). Figure 2 shows the diurnal sap flux (i.e. Q_E and/or Q_W data derived from each east- and west-facing probe set installed per tree) in each of three ghost array oaks (selected for the smallest, largest, and medium-sized stem; see Tables S1 and S3) for the treatment seasons from 2018 to 2021. The partial year 2017 is not shown. We have retained Q_E and Q_W to show more of the short-term variability rather than averaging to Q_T , as single-probe-set results demonstrate circumferential imbalance (Fig. S3a), which can change with the year of operation (Fig. S4).

Interquartile ranges are generally larger in the middle of the growing season for all sizes of example trees and collapse towards the minimum detectable value for each tree size at either end of the growing season. The minimum detectable sap flux using the present method is tree-size dependent (Ap-

lations were successful, and a total of 12 259 d of individual tree data (770 667 diurnal sap flux measurements across all months) were processed for the 2017–2021 TWU analysis. Data gaps in the earliest installations affected 4 of the 36 probe sets installed in four trees from August 2017 until September 2019.

With respect to the quality assurance of raw HPC data, commissioning and failure data were recorded for each probe set. This enabled a combination of data file amendment (especially for the earliest installations on separate loggers) and post-capture filtering to eliminate periods of invalid data for each probe set.

Example diel sap flux patterns for the ghost arrays in August 2019 (before filtering to eliminate nocturnal data) are shown in Fig. S3a, with east- and west-facing probe sets in each column. The sap flux data still show the minimum threshold levels (which vary by tree size) determined by the post-heat-pulse sampling period. It is noticeable that there is often a circumferential imbalance in xylem sap flux in the east (left-hand column of Fig. S3a) and west (right-hand column of Fig. S3a) probe set position data, which reflect the asymmetry in growth ring width around the stem typical in these old oak trees. The blank panels represent faulty probes (in two ghost trees), which were corrected by autumn 2019.

To compare individual tree responses across the leaf-on seasons, we filter the half-tree sap flux parameters using the solar azimuth and solar radiation parameters captured from the FACE control instrumentation (solar azimuth $> -6^\circ$ and solar radiation $> 0 \text{ W m}^{-2}$) to give just daylight (diurnal) data. Where both probe sets in a tree are providing good data, a mean whole-tree sap flux is derived and incorporated into the TWU (Fig. S3b). We had sufficient whole-tree data to exclude single-probe-set results.

There are day-to-day differences in TWU between trees in our study (e.g. Fig. S3b), even though all of them experience very similar environmental conditions, and this pattern is replicated across the three treatment types. The TWU data reported here compare well to results from other studies (Table S4 in David et al., 2013; Sánchez-Pérez et al., 2008; Tatarinov et al., 2005; Baldocchi et al., 2001).

2.8 Data processing, visualisation, and analysis

Manually collected data were preprocessed as .csv files for import to R (R version 4.4.1, 2024). Raw data from data loggers were processed, visualised, and consequently analysed using R and R Studio (Posit Team, 2024) on Windows 10 x64 (build 1909). Most figures in the Results section were created using the R package *ggplot* (Wickham, 2016). Other standard packages (e.g. *lubridate*) are listed in the R scripts accompanying the data (see “Data availability” section below). Regressions between R_b and water usage and between R_b and A_c were calculated using the *lm* function. Box and whisker plots to visualise seasonal and monthly differences in sap flux and water usage between trees and treatments were generated

in *ggplot*, where standard Tukey (McGill et al., 1978) percentiles (median + interquartile range), whiskers ($1.5 \cdot \text{IQR}$ from each hinge, where IQR is the interquartile range), and points for outliers are used. LOESS (locally estimated scatterplot smoothing) was used for exploratory analysis of the time series data (e.g. Fig. S13), an approach that does not rely on specific assumptions about the distributions from which observations are drawn. Levene tests (Levene, 1960) were carried out using *leveneTest* from the *car* library. Analysis of variance (ANOVA) models were used to test hypotheses with the functions *anova* and *summary*. The *autoplot* function from the *ggfortify* library was used to test the assumptions of normality of the ANOVA residuals. Nonparametric Wilcoxon rank-sum tests were used to check the ANOVA results, using *wilcox.test*. Generalised linear mixed-effect models (GLMMs) (using the function *glmer* from *lme4*) were selected to assess the random effects of the longitudinal (time-based) and individual (tree) repeated measures and estimate corrected fixed effects of treatments. The *summary* and *tab_plot* functions (from the *sjPlot* package) were used to extract the results.

3 Results

This section is organised as follows. We first report general characteristics of the distribution of sap flux (Sect. 3.1) and then develop relationships between TWU; R_b ; and projected canopy area, A_c (Sect. 3.2). Subsequently, we discuss the characteristics of TWU_n ($\text{L d}^{-1} \text{ mm}^{-1}$, Sect. 3.3), which we use to test for treatment effects in an analysis of variance (Sect. 3.4). We provide a qualitative discussion of the effects of seasonal weather in the Supplement Appendix.

3.1 Sap flux within the season and between years

Diurnal stem sap flux responses to canopy photosynthetic demand typically exhibit increased sap flux from dawn to around midday (UTC – local solar time at the site), with an approximately symmetrical decrease until dusk (Fig. S3). Figure 2 shows the diurnal sap flux (i.e. Q_E and/or Q_W data derived from each east- and west-facing probe set installed per tree) in each of three ghost array oaks (selected for the smallest, largest, and medium-sized stem; see Tables S1 and S3) for the treatment seasons from 2018 to 2021. The partial year 2017 is not shown. We have retained Q_E and Q_W to show more of the short-term variability rather than averaging to Q_T , as single-probe-set results demonstrate circumferential imbalance (Fig. S3a), which can change with the year of operation (Fig. S4).

Interquartile ranges are generally larger in the middle of the growing season for all sizes of example trees and collapse towards the minimum detectable value for each tree size at either end of the growing season. The minimum detectable sap flux using the present method is tree-size dependent (Ap-

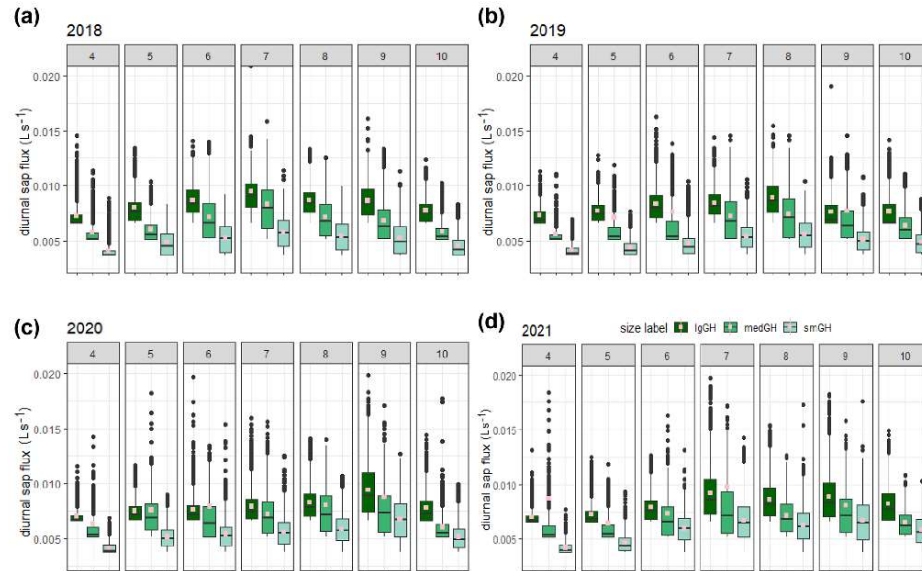


Figure 2. Comparison of diurnal sap flux measured by each probe set for three (small (smGH), medium (medGH), and large (lgGH)) ghost (no-infrastructure, no-control) trees in the years 2018–2021 (panels (a), (b), (c), and (d), respectively) across the treatment season, April–October (numbered subpanels within panels a–d). Monthly 95th percentile values are shown separately (Fig. S5 in the Supplement). The graphical display is cropped at a sap flux of 0.02 L s⁻¹. Mean values, calculated from the entire range of data, are shown as spots (pink).

pendix A, Table A2, stage 5): 0.0035 L s⁻¹ (3.5 mL s⁻¹) for the smallest tree in Fig. 2, 5.2 mL s⁻¹ for the medium-sized tree, and 6.5 mL s⁻¹ for the largest tree shown. The imbalance between the probe set data on the example trees (Fig. 2) can be up to $\pm 25\%$ (Fig. S4). This is greater in the earlier years, 2017 and 2018. This imbalance determines the spread of the IQR when normalised tree values are combined. All the large-tree-example sap flux values lie within a factor of 3 of the minimum detectable value (i.e. to 0.02 L s⁻¹) until June 2020 (more than 4 years after installation), after which larger extreme values are present.

The distributions of the three examples are clearly offset by tree stem size, suggesting that normalisation by size may be useful for inter-tree and array-to-array comparison (Sect. 3.3). For these example trees, mean monthly diurnal sap flux increased in the spring from first leaf (see phenology in Table 1 above) to achieve peak values in July in 2018 and 2021. In 2019 and 2020, increases in mean monthly diurnal sap flux were more gradual throughout the treatment season, reaching peak values in August (2019) and September (2020). There was then a faster decrease in sap flux until end of October (the end of the CO₂ treatment season) or later,

presumably due to leaf senescence and the shortening of day length.

It is evident that there are highly skewed distributions in Fig. 2. Figure S5 reports 95th percentile sap flux values for each probe set and month across 2017–2021, showing variability in the timing of the highest monthly 95th percentile sap flux of the half-tree data for each tree across the years of study. The 95th percentile sap flux is not synchronised for all probe sets on a monthly basis, indicating the dominant role of individual tree characteristics or position in determining sap flux extrema (Dragoni et al., 2009). Although minimised as much as practicable (see Sect. 2.6 above), such individual characteristics cannot be completely eliminated and may relate to major changes in branch structure (e.g. from wind damage or mortality) affecting canopy photosynthetic controls. They may also depend on the aspect of the tree and competition for root water (proximity to other trees), indicating seasonal influences on leaf, branch, and root growth.

3.2 Diurnal TWU variation between trees

3.2.1 TWU as a function of stem radius

Example relationships between R_b (mm) and monthly mean TWU ($\overline{\text{TWU}}$, L d^{-1}) for all trees with both probe sets working were first analysed for the 2019 summer months. July was used for comparison, as typically it exhibits maximum TWU. The hypothesis that TWU is a function of R_b was tested by a simple regression model. The best fit model for the combined 2019 data across all treatments was a simple linear fit; quadratic fits were tested and rejected. During July 2019 due to two probe sets malfunctioning, ghost tree TWU results did not include trees as large as the largest in the infrastructure arrays, as explained in the Methods above. Data from 6, 13, 17, and 17 trees were used for the years 2018, 2019, 2020, and 2021, respectively.

Data for July of the years 2017–2021 are shown in Fig. 3, illustrating the differences by year (Table 2) and treatment. The shorter regression for the ghost array trees (Fig. 3a and b) has a smaller slope than infrastructure ($e\text{CO}_2$ and $a\text{CO}_2$) array trees that exhibit similar slopes. Table 2 lists July model slopes for the years 2018–2021 for all treatments combined. The slopes are within +20 % and –30 %, giving a mean slope of $3.1 \pm 0.4 \text{ L d}^{-1} \text{ mm}^{-1}$ for $274 \text{ mm} \leq R_b \leq 465 \text{ mm}$, although the steepest slope (July 2018) and the shallowest slope (July 2020) differ by more than their combined standard errors and so may represent different relationships. The intercept of the linear regression is not physically meaningful, as we are only considering a relationship for trees of R_b between 0.25 and 0.5 m. The results confirm the recent study by Schoppach et al. (2021) (and supporting reference Hassler et al., 2018) with respect to the relationship between diameter at breast height (DBH; $\sim 1.3 \text{ m}$ above the ground surface) and the water usage of oak.

The slopes of the three treatments for July data in all years were also compared to determine the differences due to treatment (Table S5). There is a 10 % difference between infrastructure treatment tree slopes (Table S5 and Fig. 3d; $a\text{CO}_2$ slope = 3.86 , $\text{SE} = 1.25 \text{ L d}^{-1} \text{ mm}^{-1}$, $e\text{CO}_2$ slope = 3.55 , $\text{SE} = 0.31 \text{ L d}^{-1} \text{ mm}^{-1}$), which is not statistically significant given the standard error of the slopes. Overall, for infrastructure treatment trees ($e\text{CO}_2$ and $a\text{CO}_2$; Fig. 3d), the slope is greater than for no-infrastructure ghost array trees (slope = 1.2 , $\text{SE} = 0.47 \text{ L d}^{-1} \text{ mm}^{-1}$) (Fig. 3b), and the magnitude of $\overline{\text{TWU}}$ for all infrastructure trees is greater for a given size.

3.2.2 Factors affecting $\overline{\text{TWU}}$ as a function of R_b

The relationship with $\overline{\text{TWU}}$ varies on a year-to-year basis between 2.2 and $3.7 \text{ L d}^{-1} \text{ mm}^{-1}$ of R_b . This is due, in years of lower values, to relatively larger decreases in $\overline{\text{TWU}}$ of large trees compared to that of the smaller trees in the sample. Oaks respond subdaily to solar radiation reduction events

(Fig. S9) during cloud cover, suggesting that the year-to-year differences in slope (Table 2) are affected primarily by environmental factors (Wehr et al., 2017). In July 2020, the wettest year for mid-leaf (see Table S14 and Figs. S14 and S16), we are not able to distinguish whether the smaller slope arises from (a) smaller-tree $\overline{\text{TWU}}$ being enhanced due to the truncation effect (Appendix B), (b) larger-tree $\overline{\text{TWU}}$ being suppressed under poor light levels relatively more than the smaller trees, or (c) a combination of these two factors. Nonetheless, the inter-year variation in the regression is likely due to monthly weather variation, such as different numbers of days of full sunlight or more days of rain (Table S14 and Fig. S16).

3.2.3 Canopy area, A_c , as a function of stem radius, R_b

Canopy area, A_c (m^2), measured in the year of installation (first) and in early 2022 (last), correlates closely with R_b (Fig. 4 and Table 3, also Table S6 for data concerning repeat measures for each tree). On average, A_c is linearly proportional to R_b ($\sim 617 \pm 108 \text{ m}^2 \text{ m}^{-1}$; $0.261 \text{ m} \leq R_b \leq 0.473 \text{ m}$). There are changes in A_c (some positive and some negative) between the first and last measurements.

The three treatments do not show statistically significant differences in A_c per unit tree radius; $e\text{CO}_2$ has the greatest A_c per R_b , but the other fits, although smaller in the mean slope, fitted much worse to the linear model, resulting in much larger standard errors of the mean slope (Table 3, second column).

The measurements of A_c taken here are useful to assess water usage per unit of projected area of plant canopy but are insufficiently precise to quantify treatment effects. Changes in A_c , presumably due to a combination of measurement uncertainties and other influences, such as branch growth or loss during severe wind events, do not impact the significance of the overall A_c versus R_b relationship (Table 3, all points).

We could use either R_b or A_c to remove the tree size dependence of sap flux (Fig. 2) and hence TWU, exemplified in Fig. 3 above. Using the overall regressions in Tables 2 and 3, along with measurement error, the average July diurnal water usage is $5 \pm 0.3 \text{ L d}^{-1} \text{ m}^{-2}$ (A_c). We use R_b (Fig. 5) below as the more convenient normalising factor (as it can be measured manually more easily and accurately by forest practitioners).

3.3 Yearly and seasonal variation in TWU

Box and whisker plots show TWU (Fig. 5a, b, and c) for the years 2019–2021 and treatments across the treatment season (April–October). In comparison, TWU normalised by individual-tree bark stem radius R_b , which we will call TWU_n ($\text{L d}^{-1} \text{ mm}^{-1}$), is shown for the same years in Fig. 5d, e, and f. The years 2017 and 2018 are omitted because they have a smaller number of data points (Fig. S6)

Table 2. Linear regression model parameters for July mean TWU ($\overline{\text{TWU}}$, L d⁻¹) versus the stem radius at insertion point (R_b , mm). August 2019 is also shown. The final row shows model statistics for all months of July in the years 2017–2021 for 55 trees. The table does not discriminate TWU with respect to treatment. See Appendix A, Table A1 for statistical abbreviations.

	Slope (litres per day per millimetre)	SE	R^2	Adj. R^2	Intercept (litres)	t	df	p
July 2018	3.716	0.742	0.86	0.83	-721	5.011	5	$p < 0.01$
July 2019	3.268	0.442	0.82	0.81	-621	7.399	12	$p < 0.001$
July 2020	2.233	0.511	0.54	0.52	-286	4.370	16	$p < 0.001$
July 2021	2.967	0.654	0.58	0.55	-476	4.537	16	$p < 0.001$
August 2019	2.913	0.310	0.88	0.87	-552	9.391	12	$p < 0.001$
July 2017 –2021	3.100	0.422	0.50	0.49	-545	7.340	54	$p < 0.001$

Table 3. Oak tree canopy area A_c (m²) versus stem radius (m) at the insertion point. Data from 18 trees for two (first, last) A_c measurements are shown (fourth row) and modelled by treatment (rows one to three). The first readings were taken soon after installation; the last readings were done in early 2022.

	Slope (m ² m ⁻¹)	SE	R^2	Adj. R^2	Intercept (m ²)	t	Df	p
2017–2022 canopy area $a\text{CO}_2$	391	195.1			30.50	2.00	10	0.073
2017–2022 canopy area $e\text{CO}_2$	698	30.44			-120.7	7.72	10	$p < 0.001$
2017–2022 canopy area ghost	560	263.0			-58.16	2.14	10	0.059
2017–2022 all points	617	108.3	0.49	0.47	-75.54	5.69	34	$p < 0.001$

and are not fully representative of the tree size range across treatments.

For the years 2019–2021 (Fig. 5a, b, c), mean, median, and 75th percentile TWUs (L d⁻¹) increase steadily with day length and solar radiation (Fig. S11) from around budburst (April/May) to a broad summer maximum (June, July, August) and then decline with day length to full-leaf senescence (October–November). Similar patterns are exhibited during 2017 and 2018 (Fig. S6).

In comparison, TWU_n exhibits lower within-month variability, indicated by smaller interquartile ranges, although the basic relationship between treatments remains (Fig. 5d, e and f). There is close correspondence between TWU and TWU_n inter-year patterns for all three treatments across the leaf-on seasons. The starting levels in April (lowest in 2019) and the peak month (July or August) of median TWU_n vary year on year, likely resulting from differing throughfall and soil moisture retention within the previous 12 months (Supplement Appendix S-A). Figure S7 shows the monthly sap flux and TWU_n 95th percentiles for all trees, illustrating the variation in high value extrema.

3.4 Statistical testing of hypotheses

Using summary statistics (a traditional method for repeated-measure analysis) for each individual (e.g. the mean of TWU_n ($\overline{\text{TWU}}_n$)) on a monthly and an annual-treatment-season basis, with ANOVA and equivalent nonparametric tests, inter-year and seasonal trends were identified. Initially, variance of tree water usage was tested using normalised data (TWU_n) per tree grouped by treatment type. The ANOVA results, before consideration of residuals, are outlined in the Supplement (Fig. S8, Tables S7 and S8). Mean values for TWU_n ($\overline{\text{TWU}}_n$) in the treatment season and in July for each of the years from 2019 to 2021 were calculated and compared. Levene's test was applied, showing the heterogeneity of variance of TWU_n data for each model using both the median and the mean. The results are reported in Table S9 for the infrastructure groups ($e\text{CO}_2$ and $a\text{CO}_2$) and Table S10 for the control groups ($a\text{CO}_2$ and ghost). Additional analyses were undertaken: ANOVA using the natural logarithm transform and the nonparametric Wilcoxon ranked-sum test. Both produced very similar results to the initial ANOVAs, and corrections were considered for application to reflect the repeat use of $a\text{CO}_2$ data, but these do not enable a full as-

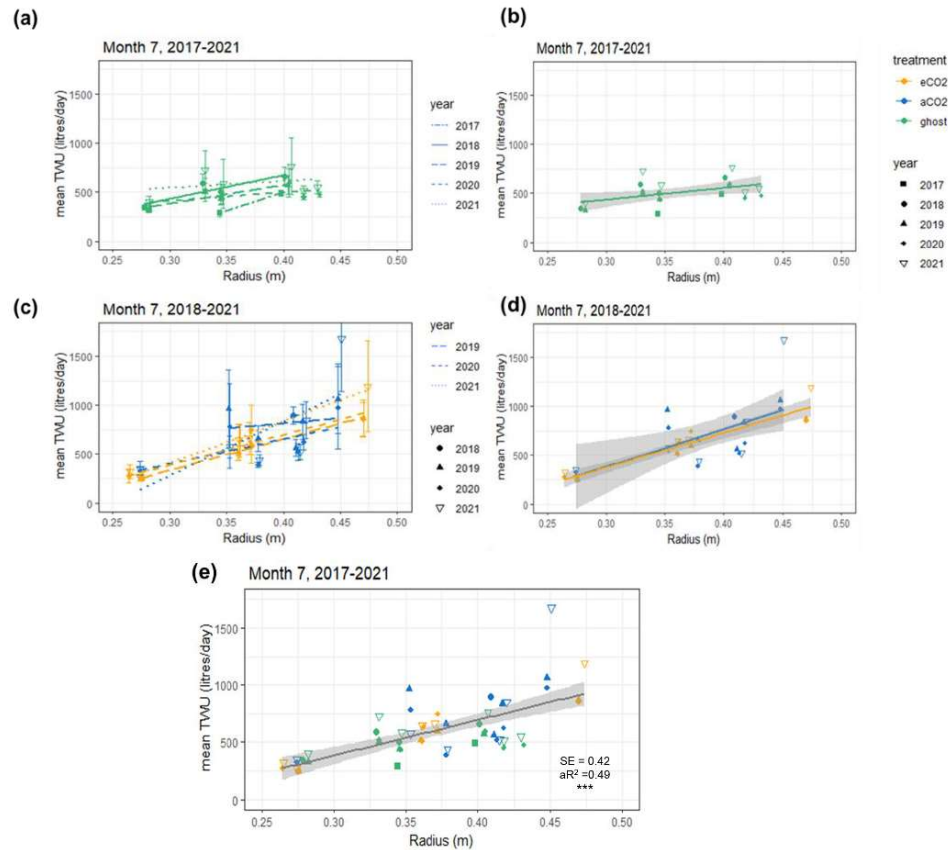


Figure 3. Mean July TWU ($\overline{\text{TWU}} \text{ L d}^{-1}$) versus stem radius R_b (m) at measurement height is shown for the three treatment types in the years 2017–2021. Panels (a) and (b) show ghost (no-infrastructure, no-treatment) trees in all years. Panels (c) and (d) show infrastructure arrays for treatment ($e\text{CO}_2$) and infrastructure control ($a\text{CO}_2$) for the years 2018–2021. Panels (b) and (d) show treatment regression lines for all years combined. Panel (e) shows points for all years with a single regression line. R_b is linearly proportional to TWU (mean slope $3.1 \pm 0.4 \text{ L d}^{-1} \text{ mm}^{-1}$). The error bars in panels (a) and (c) show SD. Panel (e) additionally shows significance (***) $p < 0.001$, SE, and the adjusted R^2 (aR^2) values.

assessment of the repeated measures concerning the trees and time.

3.4.1 Models for repeated-measure nonlinear data

TWU_n data for each whole season of interest were next modelled using GLMMs for each season of interest (2019, 2020, 2021). The intention was to develop a simple and parsimonious model sufficient to test the hypotheses outlined in

Sect. 1. Before modelling, some tree TWU_n data were removed from each season data model where more than half of the days were missing from the season (see Table S11).

To test hypothesis 1, infrastructure tree TWU_n data ($e\text{CO}_2$ and $a\text{CO}_2$) for each CO₂ season of interest (2019, 2020, 2021) were analysed using gamma GLMM models with a log link. A random-intercept model structure was adopted, incorporating random components for each tree (tree label) and for time (DOY): $\text{TWU}_n \sim \text{CO}_2 + (1 \mid \text{DOY}) + (1 \mid \text{tree})$

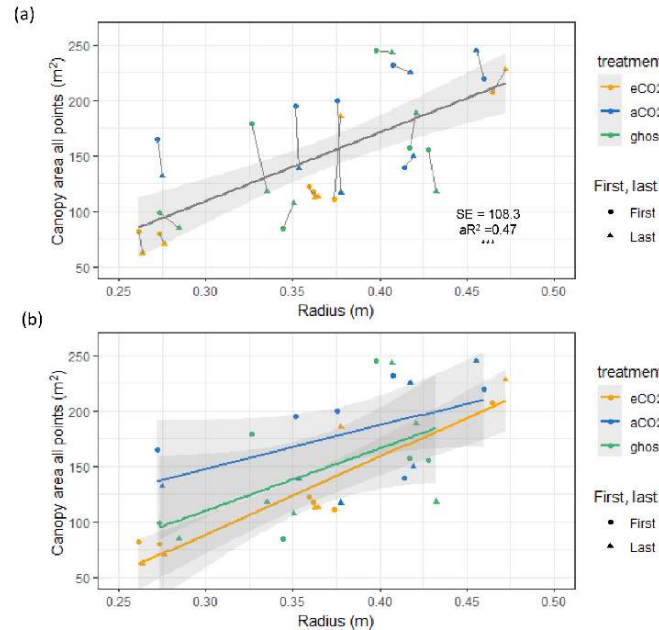


Figure 4. Canopy area A_c (m²) variation with stem radius R_b (m) for target oak trees measured in the dormant season on two occasions per tree. Significance values, SE, adjusted R^2 (aR^2), and slopes are shown in Table 3. The first measurement year is 2017–2018. The last measurement year is 2022. Panel (a) shows a linear model for all trees monitored, showing the significance (***) signifies $p < 0.001$), SE, and aR^2 values. A_c is linearly proportional to R_b ($\sim 617 \pm 108 \text{ m}^2 \text{ m}^{-1}$; $0.261 \text{ m} \leq R_b \leq 0.473 \text{ m}$). A line joins the first and last measurements. Panel (b) shows linear model relationships by treatment. Error bars show SD.

label). We tried other model structures, i.e. just the array and a nested array–tree as random elements, but using the Akaike information criterion (AIC) and Bayesian information criterion (BIC) from the *anova* function in R for model comparison, we selected the above as the best model. Figure 6 and the associated Table 4 show the results of these repeated-measure models for $e\text{CO}_2$. Complete model statistics are tabulated in Table S12 in the Supplement.

To test the hypothesis 2 control group, tree data ($a\text{CO}_2$ and ghost) were analysed for infrastructure (inf) effects using the same the treatment seasons (2019, 2020, 2021) and similar random-intercept models: $\text{TWU}_n \sim \text{inf} + (1 \mid \text{DOY}) + (1 \mid \text{tree label})$. Figure 6 and the associated Table 5 show the results of these repeated-measure models for infrastructure. Complete model statistics are tabulated in Table S13 in the Supplement.

4 Discussion

Mean normalised water usage, $\overline{\text{TWU}}_n$, ANOVAs are suitable for initial testing of treatment hypotheses, as they use accumulated daily data and mean values across longer periods (months, years). These analyses align with techniques previously used for FACE experiments, extending those from previous $e\text{CO}_2$ studies of oak (e.g. Leuzinger and Körner, 2007) by being over of a longer duration of treatment (numbers of years and numbers of treatment days per year) and a greater size range and sample of trees. A more robust approach to account for random repeated-measure effects (individual and time) uses generalised linear mixed-effect model (GLMM) analysis. Findings from ANOVA and GLMM are discussed below versus each of the paired treatment comparisons undertaken.

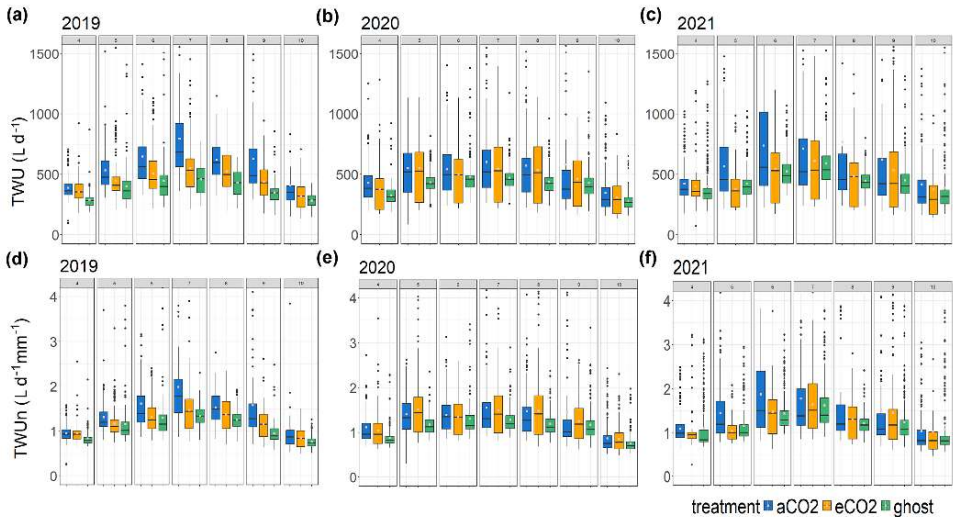


Figure 5. Treatment comparison of TWU. For the years 2019–2021, the TWU data are shown for the three treatment types. Tree data are combined for each treatment month from April to October. The distributions are shown as box and whisker plots showing the median and interquartile range (IQR, 25th percentile to 75th percentile), with whiskers calculated as $1.5 \times \text{IQR}$ from the hinge and points for outliers. Mean values, calculated from the entire range of data, are shown as spots (pink). Panels (a), (b), and (c) show TWU (L d⁻¹) for the years 2019, 2020, and 2021. Panels (d), (e), and (f) show TWUn (L d⁻¹ mm⁻¹), i.e. TWU normalised by the stem radius (mm) at the stem probe insertion height.

Table 4. Hypothesis 1 – CO₂ effects. GLMM random-intercept model outputs for (eCO₂ : aCO₂) TWUn for each year from 2019 to 2021, inclusive. The estimate, SE, 95 % CI, and significance are shown. Significance values Pr(>|z|) are taken from *summary* outputs. All other results (including *p*) are from *tab_model* outputs in the original data units for TWUn (L d⁻¹ mm⁻¹). Bold typeface indicates a *p* value < 0.1.

Parameter	Estimate (original units)	SE (original units)	95 % CI (original units)	Significance Pr(> z) (<i>p</i>)	Year
Intercept	1.31	1.111	1.07–1.62	0.00944** (0.010)	2019
CO2Y	0.87	1.149	0.66–1.14	0.319	
Intercept	1.23	1.131	0.97–1.57	0.0927. (0.093)	2020
CO2Y	0.96	1.182	0.69–1.34	0.826	
Intercept	1.34	1.183	0.96–1.86	0.0845. (0.085)	2021
CO2Y	0.84	1.261	0.53–1.32	0.450	

Significance categories are *** < 0.001, ** < 0.01, * < 0.05, and “/” < 0.1. The 1 is included to show no significance category (between 0.1 and 1), and the zero is the boundary point for *** (between 0 and 0.001).

Table 5. Hypothesis 2 – infrastructure effects. Random-intercept GLMM outputs for (α CO₂ : ghost) TWU_n for each year from 2019 to 2021, inclusive. The estimate, SE, 95 % CI, and significance are shown. Significance values $\Pr(>|z|)$ are taken from summary outputs. All other results (including p) are from *tab_model* outputs in the original data units for TWU_n (L d⁻¹ mm⁻¹). Bold typeface indicates a p value < 0.1.

Parameter	Estimate	SE (original units)	95 % CI (original units)	Significance (original units)	Year Pr(> z) (p)
Intercept	1.05	1.100	0.87–1.27	0.5879	2019
infY	1.27	1.121	1.01–1.58	0.0389* (0.039)	
Intercept	1.06	1.113	0.86–1.30	0.607	2020
infY	1.17	1.150	0.89–1.54	0.266	
Intercept	1.18	1.157	0.89–1.57	0.250	2021
infY	1.13	1.232	0.75–1.70	0.568	

Significance categories are *** < 0.001, ** < 0.01, * < 0.05, and “/” < 0.1. The 1 is included to show no significance category (between 0.1 and 1), and the zero is the boundary point for *** (between 0 and 0.001).

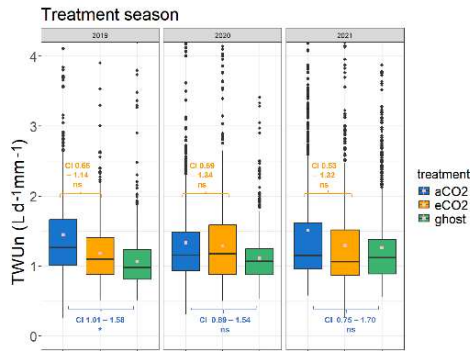


Figure 6. Treatment comparison of TWU_n (L d⁻¹ mm⁻¹) for the years 2019–2021. The April–October inclusive season data are combined for each year. The TWU_n y-axis scale is truncated for clarity, with small numbers of additional outliers. Maximum TWU_n values for each distribution each year (reading from left to right) are 8.0, 3.9, 4.2, 6.6, 4.1, 4.8, 6.6, 8.0, and 4.8 L d⁻¹ mm⁻¹. The whole-season mean values are shown in pink. The 95 % confidence intervals (CI) of the treatment effects from the GLMMs are indicated in orange (hypothesis 1, Table 4) for the e CO₂ : α CO₂ model and blue (hypothesis 2, Table 5) for α CO₂ : ghost model. Values for the treatment season TWU_n in each year are given. One-way ANOVA results are shown in Fig. S8a and b and Tables S7 and S8.

4.1 TWU_n differences under e CO₂

Table 4 reports reductions in e CO₂ TWU_n compared to α CO₂ TWU_n (for the ANOVA model results; see Fig. S8 and Table S7 in the Supplement). Across whole growing seasons, the estimated (converted to a percentage) fixed-effect reduction from the GLMM in 2019 is 13 %. In 2021 a 16 %

reduction is seen, whereas in 2020 only a 4 % reduction is shown. The model accounts for the individual and time-related repeated measures, but in all years these effects are low, supporting our original ANOVA model results. These models confirm our hypothesis 1, i.e. that there is likely to be reduced tree water usage over whole seasons. We also observe substantial interannual variability in fixed effects. This implies we cannot yet obtain a definitive prediction of the mean amount of TWU_n reduction per annum as a fixed effect of e CO₂. A greater number of years of results, given the changes in long-term trends of, for example, precipitation, would be necessary to detect any long-term trend. July-only results have not been adjusted for repeated measures and are less conclusive than the traditional ANOVA analyses (Table S7).

We are not aware of other whole-growing-season oak results with which to compare these TWU_n results. Each treatment TWU_n is a proxy for stand transpiration, so we next compare our FACE results to e CO₂ : control transpiration ratios from previous FACE experiments of oak and other deciduous species.

Short-duration midsummer FACE results have been reported for mature temperate broadleaves. Leuzinger and Körner (2007) were unable to statistically test species-specific differences in transpiration for adult *Q. petraea* (Matt.) Liebl. under e CO₂ in their Web-FACE experiment but found a 14 % reduction overall when results for *Q. petraea* were pooled with those of *Carpinus betulus* L., and *Fagus sylvatica* L. in the summers of 2004 and 2005. Their Web-FACE operated under a similar CO₂ elevation (ambient is not reported) to that used at BIFoR FACE. Our uncorrected July-only TWU_n results (reductions in every year but likely to be significant only for the uncorrected 26 % reduction in 2019) for *Q. robur* differ from their results for *Q. petraea* but strengthen their overall conclusions regarding adult decidu-

ous broadleaves and regarding the large interannual variability they also observe.

Both seasonal and summer results at Oak Ridge National Laboratory (ORNL) are reported by Warren et al. (2011a) for 11-, 16-, and 20-year-old *Liquidambar styraciflua* L. in the years 1999, 2004, and 2008 and in the early 2007 season by Warren et al. (2011b). Again, a similar FACE CO₂ elevation was used as at BIFoR FACE. ORNL ambient CO₂ levels were 380–400 $\mu\text{mol mol}^{-1}$, giving a $\sim 40\%$ elevation compared to our current $\sim 35\%$. Warren et al. (2011a, their Table III) report 10%–16% seasonal reductions in stand transpiration under $e\text{CO}_2$, which increased with the year of treatment. This may reflect a differing species response to $e\text{CO}_2$. For summer only, a 7%–16% reduction was reported, whilst Warren et al. (2011b, their Fig. 1) report $\sim 28\%$ reductions in the (non-drought) first half of a single growing season at the same ORNL site. This again reflects large interannual summer response variability.

The reductions in TWU_n in our study are consistent with other treatment effects seen at BIFoR FACE: diurnal results for photosynthetic enhancement ($23 \pm 4\%$ higher for $e\text{CO}_2$, Gardner et al., 2022, although not specifically targeted at the focal TWU_n trees), and fine root production (45% higher for $e\text{CO}_2$ in the first 2 years (Ziegler et al., 2023) covering the whole year rather than the season only). Synthesis of these treatment effects into quantitative budgets for water and carbon is outside the scope of the present work.

4.2 FACE infrastructure effect on TWU_n

The effect of FACE infrastructure on tree water usage has, to our knowledge, not been previously reported. TWU_n was higher for the infrastructure control $a\text{CO}_2$ trees compared to ghost (i.e. no-infrastructure) trees across the 3 treatment years analysed, 2019–2021. In the 2019, 2020, and 2021 seasons, the ANOVA models (Fig. S8, Table S8) found a consistently significant 37% to 20% increase in mean $a\text{CO}_2$ TWU_n compared with ghost TWU_n. In contrast, the GLMMs showed a significant fixed effect only in 2019 (Fig. 4, Table 5), estimated to be a 27% increase under infrastructure conditions. In 2020 and 2021, the models showed nonsignificant fixed effect increases of 17% and 13%. These tests confirm our hypothesis 2 that there is increased water usage within FACE infrastructure treatments but do not yet enable us to predict the amounts in any given year due to the environmental interannual variations.

The results of this study indicate the importance of infrastructure controls in forest FACE experiments. The greater $a\text{CO}_2$ TWU_n may be due to one or more of several factors: the effects of FACE infrastructure gas injection on air mixing and turbulence and hence changes in microclimate; differences in ground cover; or array-specific differences in soil moisture, slope, soil respiration, or the species of subdominant trees present. Further work is needed to fully explore these results.

Since the treatment effect for $e\text{CO}_2$ has the opposite sign to that for infrastructure (i.e. we see a reduction in $e\text{CO}_2$ TWU_n compared with $a\text{CO}_2$ TWU_n but an increase in $a\text{CO}_2$ TWU_n compared with ghost TWU_n), the infrastructure treatment effect cannot cause a pseudo- $e\text{CO}_2$ effect in the statistics, but it does further reduce any certainty about the absolute magnitude of the $e\text{CO}_2$ effect.

4.3 Capabilities, limitations, and usability of sap flux data from HPC probe sets

Although this experiment had a relatively small sample size (a total of 18 trees, with 6 per treatment), it was nevertheless a substantive experiment consisting of 12 259 d of individual tree data (770 667 diurnal sap flux measurements) in a unique experimental setting, making this dataset of high value for modellers of dynamic vegetation, water, and climate. We have defined a parameter TWU_n to enable consistent water usage comparisons between individual trees and hence treatments diurnally across both summer months and whole seasons. We believe that this method of processing HPC sap flux results, along with the extensive and continuous dataset for all no-infrastructure ghost control trees over more than 4 years, provides high confidence in this normalised data.

We can clearly demonstrate that the use of four thermocouple positions across the sapwood for each of our HPC probe sets has enabled us to successfully capture the position and size of point sap flux density (derived from sap velocity) and that this has given us a more reliable basis to explore the effects of tree size on both whole-probe-set sap flux and TWU. With respect to sap flux, single-probe sets are unlikely to provide representative results of TWU due to asymmetry in sapwood radial width around the circumference of the tree. The effects of the time-out value in the HPC measurement system have been discussed (Appendix B), and recommendations are made to ensure that any repeat experiments using this technique consider truncation effects for diurnal sap flux.

5 Conclusions

Water usage was calculated from stem sap flux for 18 oaks in an old-growth even-aged plantation during the first 5 years of an $e\text{CO}_2$ treatment. The oaks were distributed across the three treatment conditions: $e\text{CO}_2$, $a\text{CO}_2$, and ghost. Diurnal (i.e. daylight) responses that accumulated over days, months, and growing seasons (April–October, inclusive) were the focus. Within a given year, median, mean, and extreme (95th percentile) diurnal sap flux increased in the spring from first leaf to achieve peak daily values in summer months (July, August). We aggregated sap flux daily to derive water usage information for each tree, averaging results from two probe sets per tree to eliminate orientation imbalances.

Differences in tree water usage varied according to tree size. Tree characteristics, R_b and A_c , were measured and

correlated linearly with mean diurnal water usage, $\overline{TWU}$, for July, confirming a recent study (Schoppach et al., 2021). The linear relationship between A_c and $\overline{TWU}$ is less certain than that between R_b and $\overline{TWU}$ but can be used to convert tree-based transpiration to a stand scale (Granier et al., 2000) for comparison with dynamic vegetation and climate models.

Normalisation of TWU by R_b to give TWU_n enabled comparison of data combined from multiple trees across the treatments. A growing-season reduction in TWU_n under eCO_2 was detected; the signal was less clear for July-only data. Repeated measures due to individual trees and time affected the response but did not dominate the perceived effects when modelled. There was considerable interannual variability in the treatment effect for growing-season and July-only averages, likely related to environmental drivers, but which remains to be diagnosed or modelled fully.

Growing-season and July-only increases in TWU_n under aCO_2 compared to non-infrastructure controls (ghost trees) were detected consistently in ANOVA in all years, whilst the repeated-measure GLMMs estimated significant effects only in the 2019 season (and reduced the percentages by up to a third in all seasons in comparison to ANOVA). This shows that either the presence of infrastructure affects water usage or the ghost positions are not comparable to those of the infrastructure arrays due to array-specific differences in soil moisture, slope, soil respiration, or the presence of subdominant tree species.

Whilst the experiment produced reliable data across the 5 years, outlier incidence appears to be increasing, and re-installation of probe sets is recommended. To detect cavitation and embolism in situ as a possible cause of outlier data, separate (e.g. acoustic) monitoring would be required. Whilst much further work remains, this first set of tree water usage results strongly supports the conclusion that old-growth oak forests conserve water under eCO_2 at the whole-plant level.

Appendix A: Additional tables

Table A1. Table of parameter symbols and statistical abbreviations.

Symbol	Description	Units used in this publication (* not SI)
A_c	Canopy area (i.e. the area of ground covered by a plant canopy)	m ²
A_{sw}	Cross-sectional sapwood area	cm ² *
A_z	Annular ring cross-sectional area at thermocouple z	cm ² *
F_L	Volume fraction of the water element of the xylem woody matrix	Unitless
F_M	Volume fraction of the wood element of the xylem woody matrix	Unitless
G_s	Canopy stomatal conductance	mm s ⁻¹
H	Heartwood radius	m
J	Sap flux density	m s ⁻¹
J_z	Point sap flux density across the xylem sapwood area at the measurement point. The unit derivation is m ³ (water) m ⁻² (xylem sapwood area) s ⁻¹	m s ⁻¹
P	Precipitation	mm
P_r	Local precipitation (outside forest)	mm
P_{fs}	Throughfall estimate within the treatment season, April–October	%
P_{is}	Interception estimate within the treatment season, April–October	%
Q_p	Probe set volumetric sap flux (across sapwood)	L s ⁻¹
Q_T	Whole-tree sap flux	L s ⁻¹
R	Cambium radius	m
R_b	Stem radius	mm
r_z	Radius of the measurement point within the sap transducer ($z = 1$ to 4).	m
T_a	Temperature	°C
TG	Total solar radiation	W m ⁻²
TWU	Tree diurnal (dawn to dusk) water usage per day	L d ⁻¹
$\overline{TWU}$	Monthly mean TWU	L d ⁻¹
TWU_n	Tree diurnal (dawn to dusk) water usage per day normalised by the stem radius at the point of probe set insertion, R_b (mm)	L d ⁻¹ mm ⁻¹
$\overline{TWU_n}$	Monthly mean TWU_n	L d ⁻¹ mm ⁻¹
t_z	Time to heat balance point for one thermocouple pair position ($z = 1$ to 4) in the xylem sap compensation heat pulse (HPC) probe set data	s
V_s	Raw heat velocity (uncompensated)	mm s ⁻¹
V_c	Wound compensated heat velocity	m s ⁻¹
X_d	Vertical distance between the heater probe and upper (downstream) sap sensor probe	mm
X_u	Vertical distance between the heater probe and lower (upstream) sap sensor probe	mm
Statistical term abbreviations		
SE – standard error; adjusted R ² – adjusted coefficient of determination; t – Student's t test; df – degrees of freedom; p – significance; CI – confidence interval; marginal R ² – the variance explained by the fixed effects; and conditional R ² – the variance explained by the whole GLMM.		

Table A2. Calculations of stages 1 to 7 showing the flow of data processing to obtain TWU from time to heat balance t_z from all differential HPC probe set thermocouple radial positions.

Stage	Parameter	Relationship equation	Eq.	References
Stage 1	t_z Time to heat balance point	At each position $z = 1$ to 4.		Tranzflo manual
Stage 2	V_s Raw heat velocity (uncompensated)	At each position $z = 1$ to 4, $V_s = \left\{ \frac{(X_d + X_u)}{2t_z} \right\},$ for long probes $X_d = 20$, $X_u = 5$ in mm $V_s = 12.5/t_z$.	(A1)	Swanson (1962), Tranzflo manual
Stage 3	V_c Wound-compensated heat velocity	At each position $z = 1$ to 4, $V_c = a + bV + cV^2 + dV^3,$ where V is V_s in m s^{-1} . Empirical parameters a , b , c and d were chosen for the probe diameter of 2 mm.	(A2)	Green and Clothier (1988)
Stage 4	J_z Probe set (four-point) sap flux density for each radius transducer position	$J_z = \{ (0.505 F_M + F_L) V_{c,z} \}; z = 1 : 4,$ where J_z is the sap flux density at each position $z = 1$ to 4. Defining the conversion factor $c1$ as $c1 = (0.505 F_M + F_L)$ gives $J = c1 V_{c,z}$.	(A3) (A4) (A5)	Edwards and Warwick (1984), Marshall (1958)
Stage 5	Q_p Probe set volumetric sap flux across sapwood	For each probe set, $Q_p = \sum_{z=1}^4 A_z J_z; p = \text{E (east) or W (west)}.$ The area-weighted sum of sap flux density, where associated sapwood areas, A_z for $z = 1$ to 4 for long probes, is calculated from $R, r_z, r_{z+1}, \dots, r_{z+3}$ (radii) and H .	(A6)	Hatton et al. (1990), Tranzflo manual
Stage 6	Q_T Whole-tree sap flux	For each probe set at each sample time: $(Q_{p1} + Q_{p2})/2$ simplified model $Q_T = \frac{(Q_E + Q_W)}{2},$ where E and W indicate east-facing and west-facing probes.	(A7)	
Stage 7	TWU Tree diurnal water usage	$\text{TWU} = N \sum_{i=\text{dawn}}^{i=\text{dusk}} Q_{Ti},$ Where i is the 30 min sample time of Q_{Ti} and N is the conversion factor from per second (Q_T) to per diurnal day dawn to dusk.	(A8)	

Appendix B: Limitations of the time-out characteristic and outliers

There are known limitations in the ability of the HPC system to measure low and reverse sap velocities (Forster, 2017) and to some extent high sap velocities (Burgess et al., 2001). With respect to our setup, we optimised the high end of this limitation. The sap probe manufacturer Tranzflo has extensive experience in a wide range of deciduous trees. The probe sets used were custom-made for us to suit oak trees of this size, using sensor spacings suggested by Tranzflo. The limitations of the time-out characteristic and the effect this places on HPC data are recorded in several references (e.g. see Tranzflo, *Measurements of Sap Flow by the Heat-Pulse Method. An Instruction Manual for the HPV system*, 2016). The limitations impact our choice of data processing (e.g. diurnal versus diel) and feed through into the statistics we report. These limitations also introduce a truncation effect at lower heat velocities such that the distribution of the resulting raw and processed data is not symmetrical. We had limited options to extend the time-out period due to the multiple types of data that we needed to capture with our single logger–multiplexer arrangement in each array.

To normalise the data, given the above time-out effect, we select only those daylight periods where we can be confident that all four thermocouples are measuring and where they exhibit a shaped maximum point sap flux density value. We also focus on accumulation and percentile ranges rather than point time results. It is possible to use fewer positions for our calculations if, for example, one probe set position is giving a constant truncated value. These instances would need individual verification.

There were limited options to extend the time-out period for the combination of Tranzflo probe set system and Campbell Scientific logger–multiplexer used in this project. Based on our experience, extending the time out beyond double (i.e. beyond 660 s = 11 min) would be impractical for the current setup. Extension beyond, say, 7 min (420 s) would likely require a decrease in the sampling frequency to 1 s (currently set to 0.5 s) to ensure that sufficient memory is available during the differential calculation period. This decrease in sampling frequency is an inevitable compromise between capturing low heat velocities and offering sufficient data discrimination to capture the variation in heat velocity towards the maxima of the daily cycle.

Appendix C: Details of xylem sap flow measurements and calculations

In each research array, a data logger and multiplexer (CR1000 and AM25T, Campbell Scientific, Logan, Utah, USA) were used for year-round 24 h capture of raw data from sap flux HPC probe sets manufactured by Tranzflo (New Zealand) soil and throughfall measurement devices.

The logger was programmed for data capture using CR-BasicEditor under LoggerNet (versions up to 4.6.2), also by Campbell Scientific, Logan, Utah, USA. We tested our prototype installation setup in midsummer 2017 to determine whether we could capture the expected range of heat velocities and applied similar capture programs to all array loggers.

Each target oak tree had two probe sets, east and west facing, using long (7 cm four-sensor) probes. Each probe set was inserted at a stem height between 1.1 and 1.3 m and contained a central heat pulse probe and two measurement probes (with each long probe containing four thermocouples) upstream and downstream of the heater (Fig. S2). Transducers were positioned radially in the stem (to suit the ring-porous characteristics and bark thickness of these *Q. robur* trees). Each probe set was protected from natural heating by reflective insulation covers during the treatment season.

During monitoring, a heater pulse of 1.5–2.5 s in duration was applied half-hourly through a heater box (one per tree) to the heater probes. The pulse duration was dependent on the number of heaters pulsed simultaneously. A 2 s pulse was standard for the two oak trees per array (four long probe set) configuration. Each thermocouple pair in the upstream and downstream positions took up to 330 s (5.5 min) to reach a differential heat balance point, and this time determined the minimum detectable heat velocity for a time just within this time-out period. The thermocouple data logger sampling rate of 0.5 s determined the maximum detectable speed (minimum time to balance), which, given normal interference levels, was adequate for deriving the maximum heat velocity. A total of 16 differential thermocouple configurations were sampled per array in one 6 min time slot every 30 min, giving time-to-balance t_z data in seconds.

Data collection problems, due to logger grounding and sap probe disconnections at manufacture, caused data loss early in the project. Contact with sapwood was maintained for all oak trees from installation to March 2021, when 2 out of the 36 probe sets failed.

C1 Raw file processing

Logger data from the nine C1000 FACE research loggers were collated by array and transducer type (i.e. 7 cm probe set datasets for oaks only) using R and then combined into year files for further data processing.

C2 Xylem sap flux calculations

Following quality checks, each stage of calculation to produce wound-corrected sap velocity and sap flux density at each transducer position (four per probe set) was performed in stages (see Table A2). Table A2 lists the methodology and equations, along with the associated literature sources for each stage.

At stage 3 (Table A2), the Green and Clothier (1988) polynomial factors were used for wound compensation. For stage

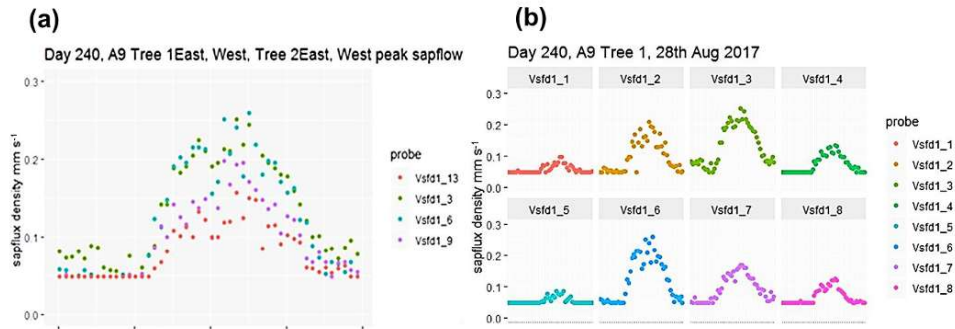


Figure C1. (a) Example (DOY 240) of the stage 4 output showing peak point sap flux density in two trees for a sunny day in August 2017. The tree 1 (vsfd1_3 and vsfd1_6) stem radius is larger than that of tree 2 (vsfd1_9 and vsfd1_13). (b) Example (DOY 240) of the stage 4 output showing changes to point sap flux density across the active xylem for east-facing (top) and west-facing (bottom) probe sets of one tree (tree 1) on the same day in August 2017. The left-hand probe position is nearest to the bark, and the right-hand probe position is nearest to the heartwood. Note that the peak value occurs at different sensor positions for the two probe sets.

4, the conversion factor c_1 was derived from measurement of wet and dry wood cores and micro-cores (Eqs. A4 and A5) to calculate xylem sap velocity from heat velocity in oak wood.

Short incremental wood cores (~ 10 cm long, 4 mm diameter) were taken from two oaks outside of the experimental arrays. Micro-cores were also taken near all 36 target oak probe set installation positions. These cores were used to determine wood hydraulic properties (Edwards and Warwick, 1984; Marshall, 1958) for sap velocity and flux calculations (see stage 4, Table A2, and the definitions in Table A1). In summer 2021, wood cores taken from some of the target oaks were further analysed to verify the active xylem radial width. The visibly active xylem (sapwood) is typically between 7 and 50 mm when viewed in wet cores. The uncertainty in heartwood boundary H (m), as described in Appendix B, could be resolved in future similar studies by taking short cores prior to installing instrumentation.

C2.1 Heat pulse to xylem sap flux calculations.

Figure C1 shows example positional (i.e. thermocouple-specific) point sap flux density data from four probe sets in two trees, illustrating the positioning of peak sap flux through the sapwood. Figure C1a pools results from both trees. Data from the thermocouple radial position giving the highest diurnal values (one thermocouple position for each probe set) are selected from the four-position data and shown across a 24 h period (Fig. C1a). The diurnal maxima from the larger tree 1 are larger than those for the smaller tree 2. Figure C1b pools probe set results from the larger tree for east-facing (top) and west-facing (bottom) probe sets. Note the increase in sap flux density towards the centre of the sapwood, decreasing again towards the heartwood (Fig. C1b).

The nocturnal/predawn response of the smaller tree in Fig. C1a (vsfd1_9 and vsfd1_13) and the less vigorous thermocouple positions in the larger tree in Fig. C1b (vsfd1_1, vsfd1_4, vsfd1_5, and vsfd1_8) have their minima determined by the previously mentioned time-out limit (i.e. t_z of 330 s). These minima do not affect the processing of diurnal values but influence the nocturnal value accuracy of the lowest point sap flux density (see Appendix B). The radial pattern of sap flux density increases in amplitude to a peak position within the probe set measurement zone and then decreases again towards the heartwood boundary as depth from the cambium increases (Fig. C1a and b), a characteristic of these ring-porous oak species. The radial amplitude patterns vary across seasons.

C2.2 Converting point xylem sap flux data to whole-tree water usage

An adapted simple integration method (Hatton et al., 1990) based on a weighted average approach was used, where the point sap flux density is weighted by the areas of the annular rings associated with each r_z . Hatton et al. (1990) consider their method, in comparison with alternatives (e.g. fitting a least-squares polynomial), a simpler and more accurate approach for the estimation of the volume flux.

Using cambium radius (R) data, estimated heartwood radius (H) (0.05 m smaller than the inner-sensor radial position), and transducer radius positions (r_z), point sap flux density from the four measurement points is converted to volumetric (half-tree) total sap flux for each probe set using the integration of the point sap fluxes over the active sapwood conducting area (Appendix A, Table A2, stage 5, and Appendix C).

Code availability. R code for the sap flux, TWU data analysis, and logger CS Basic programs can be requested from the correspondence author (A. Robert MacKenzie) or the first author (Susan E. Quick).

Data availability. All data used to carry out this study are available upon request via the correspondence author (A. Robert MacKenzie); this includes logged data, physical tree measurements, or ecological information, for example. Sap flux data are available at the UBIRA eData repository: <https://doi.org/10.25500/edata.bham.00000972> (Quick, 2023). Phenocam data are available at https://phenocam.nau.edu/webcam/roi/millhaft/DB_1000/ (BIFoR PhenoCam, 2023).

Supplement. The supplement related to this article is available online at <https://doi.org/10.5194/bg-22-1557-2025-supplement>.

Author contributions. SEQ designed and carried out the sap flow investigation and prepared the paper as part of the FACE programme, which was designed by ARMK. SEQ installed sap instrumentation; programmed the data loggers; curated, visualised, and analysed the sap data; and manually collected, visualised, and analysed the wood cores and physical tree data. NH reviewed the logger software, provided initial raw data visualisation, designed and reported all array CO₂ monitoring, and installed and managed the FACE data network and local server. GC and NH curated the raw FACE engineering data. GC curated and visualised the reference and on-site weather data, as well as all core soil data, supported by the FACE team. ARM and SK supervised the project. All co-authors discussed the results and contributed to the finalised paper. Conceptualisation – SEQ, ARMK, and SK. Data curation – SEQ, GC, and NH. Formal analysis – SEQ. Investigation, methodology and Project administration – SEQ. Resources – ARMK. Software – SEQ and NH. Supervision – ARMK and SK. Visualisation – SEQ, GC, and NH. Writing (original draft preparation) – SEQ. All authors contributed to the review and editing.

Competing interests. The contact author has declared that none of the authors has any competing interests.

Disclaimer. Publisher's note: Copernicus Publications remains neutral with regard to jurisdictional claims made in the text, published maps, institutional affiliations, or any other geographical representation in this paper. While Copernicus Publications makes every effort to include appropriate place names, the final responsibility lies with the authors.

Acknowledgements. All authors acknowledge support from the Birmingham Institute of Forest Research. The BIFoR FACE facility is a research infrastructure project supported by the JABBS Foundation and the University of Birmingham. A. Robert MacKenzie gratefully acknowledges support from NERC (grant nos. NE/S015833/1

and NE/S002189/1). The authors gratefully acknowledge the BIFoR FACE operations team's contribution to logger and instrumentation implementation, data visualisation, and data curation. Laboratories and workshops were provided at BIFoR FACE. Special thanks to Neil Loader (University of Swansea) for supporting the Trephor micro-corer usage, for wood core analysis, and for dendrochronological dating of trees. Thanks to Liz Hamilton and Ian Phillips for advice on repeated-measure GLMMs and statistical analysis; any inaccuracies in application or interpretation are those of the authors. We gratefully acknowledge the Woodland Trust and the Centre for Ecology and Hydrology for enabling use of site phenology data, which were collected in 2016–2022 for submission to Nature's Calendar by Susan E. Quick as a citizen scientist. Shawbury historical precipitation data were provided by the National Meteorological Library and Archive – Met Office, UK.

Financial support. This research has been supported by the Natural Environment Research Council (grant nos. NE/S015833/1 and NE/S002189/1).

Review statement. This paper was edited by Anja Rammig and reviewed by Benjamin Hesse and two anonymous referees.

References

- Aranda, I., Forner, A., Cuesta, B., and Valladares, F.: Species-specific water use by forest tree species: From the tree to the stand, *Agric. Water Manag.*, 114, 67–77, <https://doi.org/10.1016/J.AGWAT.2012.06.024>, 2012.
- Aszalós, R., Horváth, F., Máza, K., Ódor, P., Lengyel, A., Kovács, G., and Bölöni, J.: First signs of old-growth structure and composition of an oak forest after four decades of abandonment, *Biologia*, 72, 1264–1274, <https://doi.org/10.1515/biolog-2017-0139>, 2017.
- Baldocchi, D., Falge, E., Gu, L., Olson, R., Hollinger, D., Running, S., Anthoni, P., Bernhofer, C., Davis, K., Evans, R., Fuentes, J., Goldstein, A., Katul, G., Law, B., Lee, X., Malhi, Y., Meyers, T., Munger, J., Oechel, W., and Richardson, F.: FLUXNET: A New Tool to Study the Temporal and Spatial Variability of Ecosystem-Scale Carbon Dioxide, Water Vapor, and Energy Flux Densities, *Am. Meteorol. Soc.*, 82, [https://doi.org/10.1175/1520-0477\(2001\)082<2415:FANTTS>2.3.CO;2](https://doi.org/10.1175/1520-0477(2001)082<2415:FANTTS>2.3.CO;2), 2001.
- BIFoR PhenoCam: https://phenocam.nau.edu/webcam/roi/%0Amillhaft/DB_1000/, last access: 10 July 2023.
- Bradwell, J.: Norbury Park An Estate Tackling Climate Change, Second edn., Norbury Park, Staffordshire, UK, ISBN 10: 1527297349, 2022.
- Burgess, S. S. O., Adams, M. A., Turner, N. C., Beverly, C. R., Ong, C. K., Khan, A. A. H., and Bleby, T. M.: An improved heat pulse method to measure low and reverse rates of sap flow in woody plants, *Tree Physiol.*, 21, 589–598, 2001.
- Bütikofer, L., Anderson, K., Bebb, D. P., Bennie, J. J., Early, R. I., and Maclean, I. M. D.: The problem of scale in predicting biological responses to climate, *Glob. Change Biol.*, 26, 6657–6666, <https://doi.org/10.1111/gcb.15358>, 2020.

- Catoni, R., Gratani, L., Sartori, F., Varone, L., and Granata, M. U.: Carbon gain optimization in five broadleaf deciduous trees in response to light variation within the crown: Correlations among morphological, anatomical and physiological leaf traits, *Acta Bot. Croat.*, 74, 71–94, <https://doi.org/10.1515/botcro-2015-0010>, 2015.
- Catovsky, S., Holbrook, N. M., and Bazzaz, F. A.: Coupling whole-tree transpiration and canopy photosynthesis in coniferous and broad-leaved tree species, *Can. J. For. Res.*, 32, 295–309, 2002.
- Čermák, J., Kučera, J., and Štěpánková, M.: Water consumption of full-grown oak (*Quercus robur* L.) in a floodplain forest after the cessation of flooding, *Dev. Agric. Manag. For. Ecol.*, Volume 15, 397–417, <https://doi.org/10.1016/b978-0-444-98756-3.50034-4>, 1991.
- Chave, J.: The problem of pattern and scale in ecology: what have we learned in 20 years?, *Ecol. Lett.*, 16, 4–16, <https://doi.org/10.1111/ele.12048>, 2013.
- David, T. S., Pinto, C. A., Nadezhdina, N., Kurz-Besson, C., Henriques, M. O., Quilhó, T., Cermák, J., Chaves, M. M., Pereira, J. S., and David, J. S.: Root functioning, tree water use and hydraulic redistribution in *Quercus suber* trees: A modeling approach based on root sap flow, *For. Ecol. Manage.*, 307, 136–146, <https://doi.org/10.1016/j.foreco.2013.07.012>, 2013.
- Dietrich, L., Zweifel, R., and Kahmen, A.: Daily stem diameter variations can predict the canopy water status of mature temperate trees, *Tree Physiol.*, 38, 941–952, <https://doi.org/10.1093/treephys/tpy023>, 2018.
- Donohue, R. J., Roderick, M. L., McVicar, T. R., and Yang, Y.: A simple hypothesis of how leaf and canopy-level transpiration and assimilation respond to elevated CO₂ reveals distinct response patterns between disturbed and undisturbed vegetation, *J. Geophys. Res.-Biogeo.*, 122, 168–184, <https://doi.org/10.1002/2016JG003505>, 2017.
- Dragoni, D., Caylor, K. K., and Schmid, H. P.: Decoupling structural and environmental determinants of sap velocity: Part II. Observational application, *Agric. For. Meteorol.*, 149, 570–581, <https://doi.org/10.1016/j.agrformet.2008.10.010>, 2009.
- Drake, J. E., Macdonald, C. A., Tjoelker, M. G., Crous, K. Y., Gimeno, T. E., Singh, B. K., Reich, P. B., Anderson, I. C., and Ellsworth, D. S.: Short-term carbon cycling responses of a mature eucalypt woodland to gradual stepwise enrichment of atmospheric CO₂ concentration, *Glob. Change Biol.*, 22, 380–390, <https://doi.org/10.1111/gcb.13109>, 2016.
- Edwards, W. R. N. and Warwick, N. W. M.: Transpiration from a kiwifruit vine as estimated by the heat pulse technique and the penman-monteith equation, *New Zeal. J. Agric. Res.*, 27, 537–543, <https://doi.org/10.1080/00288233.1984.10418016>, 1984.
- Ellsworth, D. S.: CO₂ enrichment in a maturing pine forest: are CO₂ exchange and water status in the canopy affected?, *Plant. Cell Environ.*, 22, 461–472, <https://doi.org/10.1046/j.1365-3040.1999.00433.x>, 1999.
- Fan, Y., Miguez-Macho, G., Jobbágy, E. G., Jackson, R. B., and Otero-Casal, C.: Hydrologic regulation of plant rooting depth, *P. Natl. Acad. Sci. USA*, 114, 10572–10577, <https://doi.org/10.1073/pnas.1712381114>, 2017.
- Flo, V., Martínez-Vilalta, J., Mencuccini, M., Granda, V., Anderegg, W. R. L., and Poyatos, R.: Climate and functional traits jointly mediate tree water-use strategies, *New Phytol.*, 231, 617–630, <https://doi.org/10.1111/nph.17404>, 2021.
- Fontes, C. G. and Cavender-Bares, J.: Toward an integrated view of the ‘elephant’: unlocking the mysteries of water transport and xylem vulnerability in oaks, *Tree Physiol.*, 40, 1–4, <https://doi.org/10.1093/treephys/tpz116>, 2019.
- Forster, M.: How Reliable Are Heat Pulse Velocity Methods for Estimating Tree Transpiration?, *Forests*, 8, 350, <https://doi.org/10.3390/f8090350>, 2017.
- Gao, J. and Tian, K.: Stem and leaf traits as co-determinants of canopy water flux., *Plant Divers.*, 41, 258–265, 2019.
- Gardner, A., Ellsworth, D. S., Crous, K. Y., Pritchard, J., and MacKenzie, A. R.: Is photosynthetic enhancement sustained through three years of elevated CO₂ exposure in 175-year-old *Quercus robur*?, *Tree Physiol.*, 42, 130–144, <https://doi.org/10.1093/treephys/tpab090>, 2022.
- Granier, A., Biron, P., Bréda, N., Pontailler, J.-Y., and Saugier, B.: Transpiration of trees and forest stands: short and long-term monitoring using sapflow methods, *Glob. Change Biol.*, 2, 265–274, <https://doi.org/10.1111/j.1365-2486.1996.tb00078.x>, 1996.
- Granier, A., Loustau, D., and Bréda, N.: A generic model of forest canopy conductance dependent on climate, soil water availability and leaf area index, *Ann. For. Sci.*, 57, 755–765, <https://doi.org/10.1051/forest:2000158>, 2000.
- Green, S. R. and Clothier, B. E.: Water use of kiwifruit vines and apple trees by the heat-pulse technique, *J. Exp. Bot.*, 39, 115–123, <https://doi.org/10.1093/jxb/39.1.115>, 1988.
- Guerrieri, R., Lepine, L., Asbjornsen, H., Xiao, J., and Ollinger, S. V.: Evapotranspiration and water use efficiency in relation to climate and canopy nitrogen in U.S. forests, *J. Geophys. Res.-Biogeo.*, 121, 2610–2629, <https://doi.org/10.1002/2016JG003415>, 2016.
- Hart, K. M., Curioni, G., Blaen, P., Harper, N. J., Miles, P., Lewin, K. F., Nagy, J., Bannister, E. J., Cai, X. M., Thomas, R. M., Krause, S., Tausz, M., and MacKenzie, A. R.: Characteristics of free air carbon dioxide enrichment of a northern temperate mature forest, *Glob. Change Biol.*, 26, 1023–1037, <https://doi.org/10.1111/gcb.14786>, 2020.
- Hassler, S. K., Weiler, M., and Blume, T.: Tree-, stand- and site-specific controls on landscape-scale patterns of transpiration, *Hydrol. Earth Syst. Sci.*, 22, 13–30, <https://doi.org/10.5194/hess-22-13-2018>, 2018.
- Hatton, T. J., Catchpole, E. A., and Vertessy, R. A.: Integration of sapflow velocity to estimate plant water use, *Tree Physiol.*, 6, 201–209, <https://doi.org/10.1093/treephys/6.2.201>, 1990.
- Hemery, G. E., Savill, P. S., and Pryor, S. N.: Applications of the crown diameter–stem diameter relationship for different species of broadleaved trees, *For. Ecol. Manage.*, 215, 285–294, <https://doi.org/10.1016/j.foreco.2005.05.016>, 2005.
- Herbst, M., Roberts, J. M., Rosier, P. T. W., Taylor, M. E., and Gowing, D. J.: Edge effects and forest water use: A field study in a mixed deciduous woodland, *For. Ecol. Manage.*, 250, 176–186, <https://doi.org/10.1016/j.foreco.2007.05.013>, 2007.
- IPCC: Summary for Policymakers, *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S. L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M. I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J. B. R., Maycock, T.

- K., Waterfield, T., Yelekçi, O., Yu, R., and Zhou, B., 3–32, <https://doi.org/10.1017/9781009157896.001>, 2021.
- Iqbal, S., Zha, T., Jia, X., Hayat, M., Qian, D., Bourque, C. P.-A., Tian, Y., Bai, Y., Liu, P., Yang, R., and Khan, A.: Interannual variation in sap flow response in three xeric shrub species to periodic drought, *Agric. For. Meteorol.*, 297, 108276, <https://doi.org/10.1016/j.agrformet.2020.108276>, 2021.
- De Kauwe, M. G., Medlyn, B. E., Zaehle, S., Walker, A. P., Dietze, M. C., Hickler, T., Jain, A. K., Luo, Y., Parton, W. J., Prentice, I. C., Smith, B., Thornton, P. E., Wang, S., Wang, Y.-P., Wärlind, D., Weng, E., Crous, K. Y., Ellsworth, D. S., Hanson, P. J., Seok Kim, H., Warren, J. M., Oren, R., and Norby, R. J.: Forest water use and water use efficiency at elevated CO₂: a model-data intercomparison at two contrasting temperate forest FACE sites, *Glob. Change Biol.*, 19, 1759–1779, <https://doi.org/10.1111/gcb.12164>, 2013.
- Keenan, T. F., Hollinger, D. Y., Bohrer, G., Dragoni, D., Munger, J. W., Schmid, H. P., and Richardson, A. D.: Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise, *Nature*, 499, 324–327, <https://doi.org/10.1038/nature12291>, 2013.
- Köcher, P., Horna, V., and Leuschner, C.: Stem water storage in five coexisting temperate broad-leaved tree species: significance, temporal dynamics and dependence on tree functional traits, *Tree Physiol.*, 33, 817–832, <https://doi.org/10.1093/treephys/tpu055>, 2013.
- Landsberg, J., Waring, R., and Ryan, M.: Water relations in tree physiology: where to from here?, *Tree Physiol.*, 37, 18–32, <https://doi.org/10.1093/treephys/tpw102>, 2017.
- Lavergne, A., Sandoval, D., Hare, V. J., Graven, H., and Prentice, I. C.: Impacts of soil water stress on the acclimated stomatal limitation of photosynthesis: Insights from stable carbon isotope data, *Glob. Change Biol.*, 26, 7158–7172, <https://doi.org/10.1111/gcb.15364>, 2020.
- LeCain, D., Smith, D., Morgan, J., Kimball, B. A., Pendall, E., and Miglietta, F.: Microclimatic Performance of a Free-Air Warming and CO₂ Enrichment Experiment in Windy Wyoming, USA, *PLoS One*, 10, <https://doi.org/10.1371/journal.pone.0116834>, 2015.
- Leuzinger, S. and Körner, C.: Water savings in mature deciduous forest trees under elevated CO₂, *Glob. Change Biol.*, 13, 2498–2508, <https://doi.org/10.1111/j.1365-2486.2007.01467.x>, 2007.
- Leuzinger, S., Zotz, G., Asshoff, R., and Körner, C.: Responses of deciduous forest trees to severe drought in Central Europe, *Tree Physiol.*, 25, 641–650, <https://doi.org/10.1093/treephys/25.6.641>, 2005.
- Levene, H.: Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling, edited by: Olkin, I., Stanford University Press, ISBN 0804705968, 9780804705967, 278–292, 1960.
- Li, J.-H., Dugas, W. A., Hymus, G. J., Johnson, D. P., Hinkle, C. R., Drake, B. G., and Hungate, B. A.: Direct and indirect effects of elevated CO₂ on transpiration from *Quercus myrtifolia* in a scrub-oak ecosystem, *Glob. Change Biol.*, 9, 96–105, <https://doi.org/10.1046/j.1365-2486.2003.00557.x>, 2003.
- MacKenzie, A. R., Krause, S., Hart, K. M., Thomas, R. M., Blaen, P. J., Hamilton, R. L., Curioni, G., Quick, S. E., Kourmouli, A., Hannah, D. M., Comer-Warner, S. A., Brekenfeld, N., Ullah, S., and Press, M. C.: BIFoR FACE: Water–soil–vegetation–atmosphere data from a temperate deciduous forest catchment, including under elevated CO₂, *Hydrol. Process.*, 35, e14096, <https://doi.org/10.1002/hyp.14096>, 2021.
- Marshall, D. C.: Measurement of Sap Flow in Conifers by Heat Transport., *Plant Physiol.*, 33, 385–396, <https://doi.org/10.1104/pp.33.6.385>, 1958.
- McGill, R., Tukey, J. W., and Larsen, W. A.: Variations of Box Plots, *Am. Stat.*, 32, 12–16, <https://doi.org/10.2307/2683468>, 1978.
- Medlyn, B. E., Zaehle, S., De Kauwe, M. G., Walker, A. P., Dietze, M. C., Hanson, P. J., Hickler, T., Jain, A. K., Luo, Y., Parton, W., Prentice, I. C., Thornton, P. E., Wang, S., Wang, Y.-P., Weng, E., Iversen, C. M., McCarthy, H. R., Warren, J. M., Oren, R., and Norby, R. J.: Using ecosystem experiments to improve vegetation models, *Nat. Clim. Chang.*, 5, 528–534, 2015.
- Miglietta, F., Peressotti, A., Vaccari, F. P., Zaldei, A., DeAngelis, P., and Scarascia-Mugnozza, G.: Free-air CO₂ enrichment (FACE) of a poplar plantation: the POPFACE fumigation system, *New Phytol.*, 150, 465–476, <https://doi.org/10.1046/j.1469-8137.2001.00115.x>, 2001.
- Moene, A. F.: Vegetation: Transport Processes Inside and Outside of Plants, in: *Transport in the Atmosphere-Vegetation-Soil Continuum*, edited by: Moene, A. F. and Dam, J. C. van, Cambridge University Press, Cambridge, 200–251, <https://doi.org/10.1017/CBO9781139043137.007>, 2014.
- Nehemy, M. F., Benettin, P., Asadollahi, M., Pratt, D., Rinaldo, A., and McDonnell, J. J.: Tree water deficit and dynamic source water partitioning, *Hydrol. Process.*, 35, e14004, <https://doi.org/10.1002/hyp.14004>, 2021.
- Norby, R. J., De Kauwe, M. G., Domingues, T. F., Duursma, R. A., Ellsworth, D. S., Goll, D. S., Lapola, D. M., Luus, K. A., Mackenzie, A. R., Medlyn, B. E., Pavlick, R., Rammig, A., Smith, B., Thomas, R., Thonicke, K., Walker, A. P., Yang, X., and Zaehle, S.: Model-data synthesis for the next generation of forest free-air CO₂ enrichment (FACE) experiments, *New Phytol.*, 209, 17–28, <https://doi.org/10.1111/nph.13593>, 2016.
- Perkins, D., Uhl, E., Biber, P., Du Toit, B., Carraro, V., Rötzer, T., and Pretzsch, H.: Impact of Climate Trends and Drought Events on the Growth of Oaks (*Quercus robur* L. and *Quercus petraea* (Matt.) Liebl.) within and beyond Their Natural Range, *Forests*, 9, 108, <https://doi.org/10.3390/f9030108>, 2018.
- Philip, J. R.: Plant Water Relations: Some Physical Aspects, *Annu. Rev. Plant Physiol.*, 17, 245–268, <https://doi.org/10.1146/annurev.pp.17.060166.001333>, 1966.
- Pinter, P. J., Kimball, B. A., Wall, G. W., LaMorte, R. L., Hunsaker, D. J., Adamsen, F. J., Frumau, K. F. A., Vugts, H. F., Hendrey, G. R., Lewin, K. F., Nagy, J., Johnson, H. B., Wechsung, F., Leavitt, S. W., Thompson, T. L., Matthias, A. D., and Brooks, T. J.: Free-air CO₂ enrichment (FACE): blower effects on wheat canopy microclimate and plant development, *Agric. For. Meteorol.*, 103, 319–333, [https://doi.org/10.1016/S0168-1923\(00\)00150-7](https://doi.org/10.1016/S0168-1923(00)00150-7), 2000.
- Posit Team: RStudio: Integrated Development Environment for R, Software, PBC, Boston, MA, <http://www.posit.co/> (last access: 26 November 2024), 2024.
- Poyatos, R., Granda, V., Molowny-Horas, R., Mencuccini, M., Steppe, K., and Martínez-Vilalta, J.: SAPFLUXNET: towards a global database of sap flow measurements, *Tree Physiol.*, 36, 1449–1455, <https://doi.org/10.1093/treephys/tpw110>, 2016.
- Poyatos, R., Granda, V., Flo, V., Adams, M. A., Adorján, B., Aguadé, D., Aidar, M. P. M., Allen, S., Alvarado-Barrientos, M.

S. E. Quick et al.: Water usage of old-growth oak at elevated CO₂ in the FACE of climate change

1581

- water can limit forest carbon uptake and transpiration as severely as dry soil, *Geophys. Res. Lett.*, 43, 9686–9695, <https://doi.org/10.1002/2016GL069416>, 2016.
- Süßel, F. and Brüggemann, W.: Tree water relations of mature oaks in southwest Germany under extreme drought stress in summer 2018, *Plant Stress*, 1, 100010, <https://doi.org/10.1016/j.stress.2021.100010>, 2021.
- Swanson, R. H.: An instrument for detecting sap movement in woody plants, Rocky Mountain Forest and Range Experiment Station, <https://doi.org/10.5962/bhl.title.80872>, 1962.
- Tatarinov, F. a, Kučera, J., and Cienciala, E.: The analysis of physical background of tree sap flow measurement based on thermal methods, *Meas. Sci. Technol.*, 16, 1157–1169, <https://doi.org/10.1088/0957-0233/16/5/016>, 2005.
- Tor-ngern, P., Oren, R., Ward, E. J., Palmroth, S., McCarthy, H. R., and Domec, J.-C.: Increases in atmospheric CO₂ have little influence on transpiration of a temperate forest canopy, *New Phytol.*, 205, 518–525, <https://doi.org/10.1111/nph.13148>, 2015.
- Tranzflo: Measurements of Sap Flow by the Heat-Pulse Method. An Instruction Manual for the HPV system: https://www.tranzflo.co.nz/index_files/HPVMANUAL.PDF (last access: 12 October 2016), 1998.
- Tricker, P. J., Pecchiari, M., Bunn, S. M., Vaccari, F. P., Peressotti, A., Miglietta, F., and Taylor, G.: Water use of a bioenergy plantation increases in a future high CO₂ world, *Biomass and Bioenergy*, 33, 200–208, Elsevier, <https://doi.org/10.1016/j.biombioe.2008.05.009>, 2009.
- Uddling, J., Teclaw, R. M., Kubiske, M. E., Pregitzer, K. S., and Ellsworth, D. S.: Sap flux in pure aspen and mixed aspen–birch forests exposed to elevated concentrations of carbon dioxide and ozone, *Tree Physiol.*, 28, 1231–1243, <https://doi.org/10.1093/treephys/28.8.1231>, 2008.
- Venturas, M. D., Sperry, J. S., and Hacke, U. G.: Plant xylem hydraulics: What we understand, current research, and future challenges, *J. Integr. Plant Biol.*, 59, 356–389, <https://doi.org/10.1111/jipb.12534>, 2017.
- Verstraeten, W. W., Veroustraete, F., and Feyen, J.: Assessment of Evapotranspiration and Soil Moisture Content Across Different Scales of Observation, *Sensors*, 8, 70–117, <https://doi.org/10.3390/s8010070>, 2008.
- Vitasse, Y., Bottero, A., Cailleret, M., Bigler, C., Fonti, P., Gessler, A., Lévesque, M., Rohner, B., Weber, P., Rigling, A., and Wohlgemuth, T.: Contrasting resistance and resilience to extreme drought and late spring frost in five major European tree species, *Glob. Change Biol.*, 25, 3781–3792, <https://doi.org/10.1111/gcb.14803>, 2019.
- Wang, H., Guan, H., and Simmons, C. T.: Modeling the environmental controls on tree water use at different temporal scales, *Agric. For. Meteorol.*, 225, 24–35, <https://doi.org/10.1016/j.agrformet.2016.04.016>, 2016.
- Warren, J. M., Pötzelsberger, E., Wullschlegel, S. D., Thornton, P. E., Hasenauer, H., and Norby, R. J.: Ecohydrologic impact of reduced stomatal conductance in forests exposed to elevated CO₂, 4, 196–210, <https://doi.org/10.1002/eco.173>, 2011a.
- Warren, J. M., Norby, R. J., and Wullschlegel, S. D.: Elevated CO₂ enhances leaf senescence during extreme drought in a temperate forest, *Tree Physiol.*, 31, 117–130, <https://doi.org/10.1093/treephys/tpu002>, 2011b.
- Wehr, R., Commann, R., Munger, J. W., McManus, J. B., Nelson, D. D., Zahniser, M. S., Saleska, S. R., and Wofsy, S. C.: Dynamics of canopy stomatal conductance, transpiration, and evaporation in a temperate deciduous forest, validated by carbonyl sulfide uptake, *Biogeosciences*, 14, 389–401, <https://doi.org/10.5194/bg-14-389-2017>, 2017.
- Wickham, H.: *ggplot2: Elegant Graphics for Data Analysis*, Springer-Verlag New York, <https://ggplot2.tidyverse.org> (last access: 8 August 2024), 2016.
- Wiedemann, A., Marañón-Jiménez, S., Rebmann, C., Herbst, M., and Cuntz, M.: An empirical study of the wound effect on sap flux density measured with thermal dissipation probes, *Tree Physiol.*, 36, 1471–1484, 2016.
- Wullschlegel, S. D. and Norby, R. J.: Sap velocity and canopy transpiration in a sweetgum stand exposed to free-air CO₂ enrichment (FACE), *New Phytol.*, 150, 489–498, <https://doi.org/10.1046/j.1469-8137.2001.00094.x>, 2001.
- Wullschlegel, S. D., Gunderson, C. A., Hanson, P. J., Wilson, K. B., and Norby, R. J.: Sensitivity of stomatal and canopy conductance to elevated CO₂ concentration – interacting variables and perspectives of scale, *New Phytol.*, 153, 485–496, <https://doi.org/10.1046/j.0028-646X.2001.00333.x>, 2002.
- Xu, X. and Trugman, A. T.: Trait-Based Modeling of Terrestrial Ecosystems: Advances and Challenges Under Global Change, *Curr. Clim. Change. Reports*, 7, 1–13, <https://doi.org/10.1007/s40641-020-00168-6>, 2021.
- Ziegler, C., Kulawska, A., Kourmouli, A., Hamilton, L., Shi, Z., MacKenzie, A. R., Dyson, R. J., and Johnston, I. G.: Quantification and uncertainty of root growth stimulation by elevated CO₂ in a mature temperate deciduous forest, *Sci. Total Environ.*, 854, 158661, <https://doi.org/10.1016/j.scitotenv.2022.158661>, 2023.

<https://doi.org/10.5194/bg-22-1557-2025>

Biogeosciences, 22, 1557–1581, 2025

References Chapter 3.

Quick, S. E. et al. (2025) 'Water usage of old-growth oak at elevated CO₂ in the FACE (Free-Air CO₂ Enrichment) of climate change', Biogeosciences. Copernicus Publications Göttingen, Germany, 22(6), pp. 1557–1581. doi: 10.5194/bg-22-1557-1000 2025.

Note that references for Chapter 3 are repeated here in publication format.

Aranda, I., Forner, A., Cuesta, B., and Valladares, F.: Species-specific water use by forest tree species: From the tree to the stand, *Agric. Water Manag.*, 114, 67–77, <https://doi.org/10.1016/J.AGWAT.2012.06.024>, 2012.

1005 Aszalós, R., Horváth, F., Mázsza, K., Ódor, P., Lengyel, A., Kovács, G., and Bölöni, J.: First signs of old-growth structure and composition of an oak forest after four decades of abandonment, *Biologia*, 72, 1264–1274, <https://doi.org/10.1515/biolog-2017-0139>, 2017.

Baldocchi, D., Falge, E., Gu, L., Olson, R., Hollinger, D., Running, S., Anthoni, P., 1010 Bernhofer, C., Davis, K., Evans, R., Fuentes, J., Goldstein, A., Katul, G., Law, B., Lee, X., Malhi, Y., Meyers, T., Munger, J., Oechel, W., and Richardson, F.: FLUXNET: A New Tool to Study the Temporal and Spatial Variability of Ecosystem–Scale Carbon Dioxide, Water Vapor, and Energy Flux Densities, *Am. Meteorol. Soc.*, 82, [https://doi.org/10.1175/1520-0477\(2001\)082<2415:FANTTS>2.3.CO;2](https://doi.org/10.1175/1520-0477(2001)082<2415:FANTTS>2.3.CO;2), 2001.

1015 BIFoR PhenoCam: https://phenocam.nau.edu/webcam/roi/%0Amillhaft/DB_1000/, last access: 10 July 2023.

Bradwell, J.: *Norbury Park An Estate Tackling Climate Change*, Second edn., Norbury Park, Staffordshire, UK, ISBN 10:1527297349, 2022.

- Burgess, S. S. O., Adams, M. A., Turner, N. C., Beverly, C. R., Ong, C. K., Khan, A. A.
1020 H., and Bleby, T. M.: An improved heat pulse method to measure low and reverse rates
of sap flow in woody plants, *Tree Physiol.*, 21, 589–598, 2001.
- Bütikofer, L., Anderson, K., Bebbber, D. P., Bennie, J. J., Early, R. I., and Maclean, I.
M. D.: The problem of scale in predicting biological responses to climate, *Glob. Change
Biol.*, 26, 6657–6666, <https://doi.org/10.1111/gcb.15358>, 2020.
- 1025 Catoni, R., Gratani, L., Sartori, F., Varone, L., and Granata, M. U.: Carbon gain
optimization in five broadleaf deciduous trees in response to light variation within the
crown: Correlations among morphological, anatomical and physiological leaf traits,
Acta Bot. Croat., 74, 71–94, <https://doi.org/10.1515/botcro-2015-0010>, 2015.
- Catovsky, S., Holbrook, N. M., and Bazzaz, F. A.: Coupling wholetree transpiration and
1030 canopy photosynthesis in coniferous and broad-leaved tree species, *Can. J. For. Res.*,
32, 295–309, 2002.
- Čermák, J., Kučera J., Štěpánková M.: Water consumption of full-grown oak (*Quereus
robur* L.) in a floodplain forest after the cessation of flooding, *Dev. Agric. Manag. For.
Ecol.*, Volume 15, 397–417, <https://doi.org/10.1016/b978-0-444-98756-3.50034-4>,
1035 1991.
- Chave, J.: The problem of pattern and scale in ecology: what have we learned in 20
years?, *Ecol. Lett.*, 16, 4–16, <https://doi.org/10.1111/ele.12048>, 2013.
- David, T. S., Pinto, C. A., Nadezhdina, N., Kurz-Besson, C., Henriques, M. O., Quilhó,
T., Cermak, J., Chaves, M. M., Pereira, J. S., and David, J. S.: Root functioning, tree
1040 water use and hydraulic redistribution in *Quercus suber* trees: A modeling approach

based on root sap flow, *For. Ecol. Manage.*, 307, 136–146,
<https://doi.org/10.1016/j.foreco.2013.07.012>, 2013.

Dietrich, L., Zweifel, R., and Kahmen, A.: Daily stem diameter variations can predict the canopy water status of mature temperate trees, *Tree Physiol.*, 38, 941–952,
1045 <https://doi.org/10.1093/treephys/tpy023>, 2018.

Donohue, R. J., Roderick, M. L., McVicar, T. R., and Yang, Y.: A simple hypothesis of how leaf and canopy-level transpiration and assimilation respond to elevated CO₂ reveals distinct response patterns between disturbed and undisturbed vegetation, *J. Geophys. Res.-Biogeo.*, 122, 168–184, <https://doi.org/10.1002/2016JG003505>, 2017.

1050 Dragoni, D., Caylor, K. K., and Schmid, H. P.: Decoupling structural and environmental determinants of sap velocity: Part II. Observational application, *Agric. For. Meteorol.*, 149, 570–581, <https://doi.org/10.1016/j.agrformet.2008.10.010>, 2009.

Drake, J. E., Macdonald, C. A., Tjoelker, M. G., Crous, K. Y., Gimeno, T. E., Singh, B. K., Reich, P. B., Anderson, I. C., and Ellsworth, D. S.: Short-term carbon cycling
1055 responses of a mature eucalypt woodland to gradual stepwise enrichment of atmospheric CO₂ concentration, *Glob. Change Biol.*, 22, 380–390, <https://doi.org/10.1111/gcb.13109>, 2016.

Edwards, W. R. N. and Warwick, N. W. M.: Transpiration from a kiwifruit vine as estimated by the heat pulse technique and the penman-monteith equation, *New Zeal. J. Agric. Res.*, 27, 537– 543, <https://doi.org/10.1080/00288233.1984.10418016>, 1984.
1060

Ellsworth, D. S.: CO₂ enrichment in a maturing pine forest: are CO₂ exchange and water status in the canopy affected?, *Plant Cell Environ.*, 22, 461–472, <https://doi.org/10.1046/j.1365-3040.1999.00433.x>, 1999.

- Fan, Y., Miguez-Macho, G., Jobbágy, E. G., Jackson, R. B., and Otero-Casal, C.:
1065 Hydrologic regulation of plant rooting depth, *P. Natl. Acad. Sci. USA*, 114, 10572–
10577, <https://doi.org/10.1073/pnas.1712381114>, 2017.
- Flo, V., Martínez-Vilalta, J., Mencuccini, M., Granda, V., Anderegg, W. R. L., and
Poyatos, R.: Climate and functional traits jointly mediate tree water-use strategies,
New Phytol., 231, 617–630, <https://doi.org/10.1111/nph.17404>, 2021.
- 1070 Fontes, C. G. and Cavender-Bares, J.: Toward an integrated view of the ‘elephant’:
unlocking the mysteries of water transport and xylem vulnerability in oaks, *Tree
Physiol.*, 40, 1–4, <https://doi.org/10.1093/treephys/tpz116>, 2019.
- Forster, M.: How Reliable Are Heat Pulse Velocity Methods for Estimating Tree
Transpiration?, *Forests*, 8, 350, <https://doi.org/10.3390/f8090350>, 2017.
- 1075 Gao, J. and Tian, K.: Stem and leaf traits as co-determinants of canopy water flux.,
Plant Divers., 41, 258–265, 2019.
- Gardner, A., Ellsworth, D. S., Crous, K. Y., Pritchard, J., and MacKenzie, A. R.: Is
photosynthetic enhancement sustained through three years of elevated CO₂ exposure
in 175-year-old *Quercus robur*?, *Tree Physiol.*, 42, 130–144,
1080 <https://doi.org/10.1093/treephys/tpab090>, 2022.
- Granier, A., Biron, P., Bréda, N., Pontailleur, J.-Y., and Saugier, B.: Transpiration of
trees and forest stands: short and long-term monitoring using sapflow methods, *Glob.
Change Biol.*, 2, 265–274, <https://doi.org/10.1111/j.1365-2486.1996.tb00078.x>, 1996.
- Granier, A., Loustau, D., and Bréda, N.: A generic model of forest canopy conductance
1085 dependent on climate, soil water availability and leaf area index, *Ann. For. Sci.*, 57,

755–765, <https://doi.org/10.1051/forest:2000158>, 2000.

Green, S. R. and Clothier, B. E.: Water use of kiwifruit vines and apple trees by the heat-pulse technique, *J. Exp. Bot.*, 39, 115– 123, <https://doi.org/10.1093/jxb/39.1.115>, 1988.

1090 Guerrieri, R., Lepine, L., Asbjornsen, H., Xiao, J., and Ollinger, S. V.: Evapotranspiration and water use efficiency in relation to climate and canopy nitrogen in U.S. forests, *J. Geophys. Res.-Biogeo.*, 121, 2610–2629, <https://doi.org/10.1002/2016JG003415>, 2016.

Hart, K. M., Curioni, G., Blaen, P., Harper, N. J., Miles, P., Lewin, K. F., Nagy, J.,
1095 Bannister, E. J., Cai, X. M., Thomas, R. M., Krause, S., Tausz, M., and MacKenzie, A. R.: Characteristics of free air carbon dioxide enrichment of a northern temperate mature forest, *Glob. Change Biol.*, 26, 1023–1037, <https://doi.org/10.1111/gcb.14786>, 2020.

Hassler, S. K., Weiler, M., and Blume, T.: Tree-, stand- and sitespecific controls on
1100 landscape-scale patterns of transpiration, *Hydrol. Earth Syst. Sci.*, 22, 13–30, <https://doi.org/10.5194/hess-22-13-2018>, 2018.

Hatton, T. J., Catchpole, E. A., and Vertessy, R. A.: Integration of sapflow velocity to estimate plant water use, *Tree Physiol.*, 6, 201–209, <https://doi.org/10.1093/treephys/6.2.201>, 1990.

1105 Hemery, G. E., Savill, P. S., and Pryor, S. N.: Applications of the crown diameter–stem diameter relationship for different species of broadleaved trees, *For. Ecol. Manage.*, 215, 285–294, <https://doi.org/10.1016/j.foreco.2005.05.016>, 2005.

- Herbst, M., Roberts, J. M., Rosier, P. T.W., Taylor, M. E., and Gowing, D. J.: Edge effects and forest water use: A field study in a mixed deciduous woodland, *For. Ecol. Manage.*, 250, 176–186, <https://doi.org/10.1016/j.foreco.2007.05.013>, 2007.
- 1110 IPCC: Summary for Policymakers., *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S. L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L.,
- 1115 Gomis, M. I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J. B. R., Maycock, T. K., Waterfield, T., Yelekçi, O., Yu, R., and Zhou, B., 3–32, <https://doi.org/10.1017/9781009157896.001>, 2021.
- Iqbal, S., Zha, T., Jia, X., Hayat, M., Qian, D., Bourque, C. P.- A., Tian, Y., Bai, Y., Liu, P., Yang, R., and Khan, A.: Interannual variation in sap flow response in three xeric shrub species to periodic drought, *Agric. For. Meteorol.*, 297, 108276, <https://doi.org/10.1016/j.agrformet.2020.108276>, 2021.
- 1120 De Kauwe, M. G., Medlyn, B. E., Zaehle, S., Walker, A. P., Dietze, M. C., Hickler, T., Jain, A. K., Luo, Y., Parton, W. J., Prentice, I. C., Smith, B., Thornton, P. E., Wang, S., Wang, Y.-P., Wårlind, D., Weng, E., Crous, K. Y., Ellsworth, D. S., Hanson, P. J., Seok
- 1125 Kim, H.-, Warren, J. M., Oren, R., and Norby, R. J.: Forest water use and water use efficiency at elevated CO₂: a model-data intercomparison at two contrasting temperate forest FACE sites, *Glob. Change Biol.*, 19, 1759–1779, <https://doi.org/10.1111/gcb.12164>, 2013.
- Keenan, T. F., Hollinger, D. Y., Bohrer, G., Dragoni, D., Munger, J. W., Schmid, H. P.,
- 1130 and Richardson, A. D.: Increase in forest water-use efficiency as atmospheric carbon

dioxide concentrations rise, *Nature*, 499, 324–327, <https://doi.org/10.1038/nature12291>, 2013.

Köcher, P., Horna, V., and Leuschner, C.: Stem water storage in five coexisting temperate broad-leaved tree species: significance, temporal dynamics and
1135 dependence on tree functional traits, *Tree Physiol.*, 33, 817–832, <https://doi.org/10.1093/treephys/tpt055>, 2013.

Landsberg, J., Waring, R., and Ryan, M.: Water relations in tree physiology: where to from here?, *Tree Physiol.*, 37, 18–32, <https://doi.org/10.1093/treephys/tpw102>, 2017.

Lavergne, A., Sandoval, D., Hare, V. J., Graven, H., and Prentice, I. C.: Impacts of soil
1140 water stress on the acclimated stomatal limitation of photosynthesis: Insights from stable carbon isotope data, *Glob. Change Biol.*, 26, 7158–7172, <https://doi.org/10.1111/gcb.15364>, 2020.

LeCain, D., Smith, D., Morgan, J., Kimball, B. A., Pendall, E., and Miglietta, F.: Microclimatic Performance of a Free-Air Warming and CO₂ Enrichment Experiment in
1145 Windy Wyoming, USA, *PLoS One*, 10, <https://doi.org/10.1371/journal.pone.0116834>, 2015.

Leuzinger, S. and Körner, C.: Water savings in mature deciduous forest trees under elevated CO₂, *Glob. Change Biol.*, 13, 2498– 2508, <https://doi.org/10.1111/j.1365-2486.2007.01467.x>, 2007.

1150 Leuzinger, S., Zotz, G., Asshoff, R., and Körner, C.: Responses of deciduous forest trees to severe drought in Central Europe, *Tree Physiol.*, 25, 641–650, <https://doi.org/10.1093/treephys/25.6.641>, 2005.

- Levene, H.: Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling, edited by: Olkin, I., Stanford University Press, ISBN 0804705968, 1155 9780804705967, 278–292, 1960.
- Li, J.-H., Dugas, W. A., Hymus, G. J., Johnson, D. P., Hinkle, C. R., Drake, B. G., and Hungate, B. A.: Direct and indirect effects of elevated CO₂ on transpiration from *Quercus myrtifolia* in a scrub-oak ecosystem, *Glob. Change Biol.*, 9, 96–105, <https://doi.org/10.1046/j.1365-2486.2003.00557.x>, 2003.
- 1160 MacKenzie, A. R., Krause, S., Hart, K. M., Thomas, R. M., Blaen, P. J., Hamilton, R. L., Curioni, G., Quick, S. E., Kourmouli, A., Hannah, D. M., Comer-Warner, S. A., Brekenfeld, N., Ullah, S., and Press, M. C.: BIFoR FACE: Water–soil–vegetation–atmosphere data from a temperate deciduous forest catchment, including under elevated CO₂, *Hydrol. Process.*, 35, e14096, <https://doi.org/10.1002/hyp.14096>, 2021.
- 1165 Marshall, D. C.: Measurement of Sap Flow in Conifers by Heat Transport., *Plant Physiol.*, 33, 385–396, <https://doi.org/10.1104/pp.33.6.385>, 1958.
- McGill, R., Tukey, J.W., and Larsen, W. A.: Variations of Box Plots, *Am. Stat.*, 32, 12–16, <https://doi.org/10.2307/2683468>, 1978.
- Medlyn, B. E., Zaehle, S., De Kauwe, M. G., Walker, A. P., Dietze, M. C., Hanson, P. J., Hickler, T., Jain, A. K., Luo, Y., Parton, W., Prentice, I. C., Thornton, P. E., Wang, S., Wang, Y.-P., Weng, E., Iversen, C. M., McCarthy, H. R., Warren, J. M., Oren, R., and Norby, R. J.: Using ecosystem experiments to improve vegetation models, *Nat. Clim. Chang.*, 5, 528–534, 2015.
- Miglietta, F., Peressotti, A., Vaccari, F. P., Zaldei, A., DeAngelis, P., and Scarascia-

- 1175 Mugnozza, G.: Free-air CO₂ enrichment (FACE) of a poplar plantation: the POPFACE fumigation system, *New Phytol.*, 150, 465–476, <https://doi.org/10.1046/j.1469-8137.2001.00115.x>, 2001.
- Moene, A. F.: Vegetation: Transport Processes Inside and Outside of Plants, in: *Transport in the Atmosphere-Vegetation- Soil Continuum*, edited by: Moene, A. F. and
- 1180 Dam, J. C. van, Cambridge University Press, Cambridge, 200–251, <https://doi.org/10.1017/CBO9781139043137.007>, 2014.
- Nehemy, M. F., Benettin, P., Asadollahi, M., Pratt, D., Rinaldo, A., and McDonnell, J. J.: Tree water deficit and dynamic source water partitioning, *Hydrol. Process.*, 35, e14004, <https://doi.org/10.1002/hyp.14004>, 2021.
- 1185 Norby, R. J., De Kauwe, M. G., Domingues, T. F., Duursma, R. A., Ellsworth, D. S., Goll, D. S., Lapola, D. M., Luus, K. A., Mackenzie, A. R., Medlyn, B. E., Pavlick, R., Rammig, A., Smith, B., Thomas, R., Thonicke, K., Walker, A. P., Yang, X., and Zaehle, S.: Model-data synthesis for the next generation of forest free-air CO₂ enrichment (FACE) experiments, *New Phytol.*, 209, 17–28, <https://doi.org/10.1111/nph.13593>,
- 1190 2016.
- Perkins, D., Uhl, E., Biber, P., Du Toit, B., Carraro, V., Rötzer, T., and Pretzsch, H.: Impact of Climate Trends and Drought Events on the Growth of Oaks (*Quercus robur* L. and *Quercus petraea* (Matt.) Liebl.) within and beyond Their Natural Range, *Forests*, 9, 108, <https://doi.org/10.3390/f9030108>, 2018.
- 1195 Philip, J. R.: Plant Water Relations: Some Physical Aspects, *Annu. Rev. Plant Physiol.*, 17, 245–268, <https://doi.org/10.1146/annurev.pp.17.060166.001333>, 1966.

- Pinter, P. J., Kimball, B. A., Wall, G. W., LaMorte, R. L., Hunsaker, D. J., Adamsen, F. J., Frumau, K. F. A., Vugts, H. F., Hendrey, G. R., Lewin, K. F., Nagy, J., Johnson, H. B., Wechsung, F., Leavitt, S. W., Thompson, T. L., Matthias, A. D., and Brooks, T. J.:
 1200 Free-air CO₂ enrichment (FACE): blower effects on wheat canopy microclimate and plant development, *Agric. For. Meteorol.*, 103, 319–333, [https://doi.org/10.1016/S0168-1923\(00\)00150-7](https://doi.org/10.1016/S0168-1923(00)00150-7), 2000.
- Posit Team: RStudio: Integrated Development Environment for R, Software, PBC, Boston, MA, <http://www.posit.co/> (last access: 26 November 2024), 2024.
- 1205 Poyatos, R., Granda, V., Molowny-Horas, R., Mencuccini, M., Steppe, K., and Martínez-Vilalta, J.: SAPFLUXNET: towards a global database of sap flow measurements, *Tree Physiol.*, 36, 1449–1455, <https://doi.org/10.1093/treephys/tpw110>, 2016.
- Poyatos, R., Granda, V., Flo, V., Adams, M. A., Adorján, B., Aguadé, D., Aidar, M. P.
 1210 M., Allen, S., Alvarado-Barrientos, M. S., Anderson-Teixeira, K. J., Aparecido, L. M., Arain, M. A., Aranda, I., Asbjornsen, H., Baxter, R., Beamesderfer, E., Berry, Z. C., Berveiller, D., Blakely, B., Boggs, J., Bohrer, G., Bolstad, P. V., Bonal, D., Bracho, R., Brito, P., Brodeur, J., Casanoves, F., Chave, J., Chen, H., Cisneros, C., Clark, K., Cremonese, E., Dang, H., David, J. S., David, T. S., Delpierre, N., Desai, A. R., Do, F.
 1215 C., Dohnal, M., Domec, J.-C., Dzikiti, S., Edgar, C., Eichstaedt, R., El-Madany, T. S., Elbers, J., Eller, C. B., Euskirchen, E. S., Ewers, B., Fonti, P., Forner, A., Forrester, D. I., Freitas, H. C., Galvagno, M., Garcia-Tejera, O., Ghimire, C. P., Gimeno, T. E., Grace, J., Granier, A., Griebel, A., Guangyu, Y., Gush, M. B., Hanson, P. J., Hasselquist, N. J., Heinrich, I., Hernandez- Santana, V., Herrmann, V., Hölttä, T.,

- 1220 Holwerda, F., Irvine, J., Isarangkool Na Ayutthaya, S., Jarvis, P. G., Jochheim, H., Joly, C. A., Kaplick, J., Kim, H. S., Klemedtsson, L., Kropp, H., Lagergren, F., Lane, P., Lang, P., Lapenas, A., Lechuga, V., Lee, M., Leuschner, C., Limousin, J.-M., Linares, J. C., Linderson, M.- L., Lindroth, A., Llorens, P., López-Bernal, Á., Loranty, M. M., Lüttschwager, D., Macinnis-Ng, C., Maréchaux, I., Martin, T. A., Matheny, A.,
- 1225 McDowell, N., McMahon, S., Meir, P., Mészáros, I., Migliavacca, M., Mitchell, P., Mölder, M., Montagnani, L., Moore, G. W., Nakada, R., Niu, F., Nolan, R. H., Norby, R., Novick, K., Oberhuber, W., Obojes, N., Oishi, A. C., Oliveira, R. S., Oren, R., Ourcival, J.-M., Paljakka, T., Perez-Priego, O., Peri, P. L., Peters, R. L., Pfautsch, S., Pockman, W. T., Preisler, Y., Rascher, K., Robinson, G., Rocha, H., Rocheteau, A.,
- 1230 Röhl, A., Rosado, B. H. P., Rowland, L., Rubtsov, A. V., Sabaté, S., Salmon, Y., Salomón, R. L., Sánchez-Costa, E., Schäfer, K. V. R., Schuldt, B., Shashkin, A., Stahl, C., Stojanović, M., Suárez, J. C., Sun, G., Szatniewska, J., Tatarinov, F., Tesař, M., Thomas, F. M., Tor-ngern, P., Urban, J., Valladares, F., van der Tol, C., van Meerveld, I., Varlagin, A., Voigt, H., Warren, J., Werner, C., Werner, W., Wieser, G., Wingate, L.,
- 1235 Wullschleger, S., Yi, K., Zweifel, R., Steppe, K., Mencuccini, M., and Martínez-Vilalta, J.: Global transpiration data from sap flow measurements: the SAPFLUXNET database, *Earth Syst. Sci. Data*, 13, 2607–2649, <https://doi.org/10.5194/essd-13-2607-2021>, 2021.
- Quick, S. E.: Research data supporting the publication “Water usage of old-growth oak at elevated CO₂ in the FACE of climate change”, University of Birmingham [data set], <https://doi.org/10.25500/edata.bham.00000972>, 2023. Rabbai, A., Wendt, D. E., Curioni, G., Quick, S. E., MacKenzie, A. R., Hannah, D. M., Kettridge, N., Ullah, S.,
- 1240

- Hart, K. M., and Krause, S.: Soil moisture and temperature dynamics in juvenile and mature forest as a result of tree growth, hydrometeorological forcings, and drought, 1245 Hydrol. Process., 37, e14919, <https://doi.org/10.1002/hyp.14919>, 2023.
- Renner, M., Hassler, S. K., Blume, T., Weiler, M., Hildebrandt, A., Guderle, M., Schymanski, S. J., and Kleidon, A.: Dominant controls of transpiration along a hillslope transect inferred from ecohydrological measurements and thermodynamic limits, 1250 Hydrol. Earth Syst. Sci., 20, 2063–2083, <https://doi.org/10.5194/hess-20-2063-2016>, 2016.
- Robert, E. M. R., Mencuccini, M., and Martínez-Vilalta, J.: The Anatomy and Functioning of the Xylem in Oaks BT – Oaks Physiological Ecology. Exploring the Functional Diversity of Genus *Quercus* L., edited by: Gil-Pelegrín, E., Peguero-Pina, J. J., and Sancho-Knapik, D., Springer International Publishing, Cham, 261–302, 1255 https://doi.org/10.1007/978-3-319-69099-5_8, 2017.
- Roberts, A. J., Crowley, L. M., Sadler, J. P., Nguyen, T. T. T., Gardner, A. M., Hayward, S. A. L., and Metcalfe, D. B.: Effects of Elevated Atmospheric CO₂ Concentration on Insect Herbivory and Nutrient Fluxes in a Mature Temperate Forest, Forests, 13 , <https://doi.org/10.3390/f13070998>, 2022.
- 1260 Salomón, R. L., Peters, R. L., Zweifel, R., Sass-Klaassen, U. G.W., Stegehuis, A. I., Smiljanic, M., Poyatos, R., Babst, F., Cienciala, E., Fonti, P., Lerink, B. J. W., Lindner, M., Martinez-Vilalta, J., Mencuccini, M., Nabuurs, G.-J., van der Maaten, E., von Arx, G., Bär, A., Akhmetzyanov, L., Balanzategui, D., Bellan, M., Bendix, J., Berveiller, D., Blaženec, M., Čada, V., Carraro, V., Cecchini, S., Chan, T., Conedera, M., Delpierre, 1265 N., Delzon, S., Ditmarová, Dolezal, J., Dufrêne, E., Edvardsson, J., Ehekircher, S.,

Forner, A., Frouz, J., Ganthaler, A., Gryc, V., Güney, A., Heinrich, I., Hentschel, R., Janda, P., Ježík, M., Kahle, H.-P., Knüsel, S., Krejza, J., Kuberski, Ł., Kučera, J., Lebourgeois, F., Mikoláš, M., Matula, R., Mayr, S., Oberhuber, W., Obojes, N., Osborne, B., Paljakka, T., Plichta, R., Rabbel, I., Rathgeber, C. B. K., Salmon, Y.,
1270 Saunders, M., Scharnweber, T., Sitková, Z., Stangler, D. F., Stereńczak, K., Stojanović, M., Střelcová, K., Světlík, J., Svoboda, M., Tobin, B., Trotsiuk, V., Urban, J., Valladares, F., Vavřík, H., Vejpustková, M., Walthert, L., Wilmking, M., Zin, E., Zou, J., and Steppe, K.: The 2018 European heatwave led to stem dehydration but not to consistent growth reductions in forests, *Nat. Commun.*, 13, 28,
1275 <https://doi.org/10.1038/s41467-021-27579-9>, 2022.

Sánchez-Costa, E., Poyatos, R., and Sabaté, S.: Contrasting growth and water use strategies in four co-occurring Mediterranean tree species revealed by concurrent measurements of sap flow and stem diameter variations, *Agric. For. Meteorol.*, 207, 24–37, <https://doi.org/10.1016/j.agrformet.2015.03.012>, 2015.

1280 Sánchez-Pérez, J. M., Lucot, E., Bariac, T., and Trémoières, M.: Water uptake by trees in a riparian hardwood forest (Rhine floodplain, France), *Hydrol. Process.*, 22, 366–375, <https://doi.org/10.1002/hyp.6604>, 2008.

Sass-Klaassen, U., Sabajo, C. R., and den Ouden, J.: Vessel formation in relation to leaf phenology in pedunculate oak and European ash, *Dendrochronologia*, 29, 171–
1285 175, <https://doi.org/10.1016/j.dendro.2011.01.002>, 2011.

Schäfer, K. V. R., Oren, R., Lai, C.-T. C.-T., and Katul, G. G.: Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO₂ concentration, *Glob. Change Biol.*, 8, 895–911, 2002.

- Schoppach, R., Chun, K. P., He, Q., Fabiani, G., and Klaus, J.: Species-specific control
1290 of DBH and landscape characteristics on tree-to-tree variability of sap velocity, *Agric. For. Meteorol.*, 307, 108533, <https://doi.org/10.1016/j.agrformet.2021.108533>, 2021.
- Stagge, J. H., Kingston, D. G., Tallaksen, L. M., and Hannah, D. M.: Observed drought indices show increasing divergence across Europe, *Sci. Rep.*, 7, 14045, <https://doi.org/10.1038/s41598-017-14283-2>, 2017.
- 1295 Steppe, K. and Lemeur, R.: Effects of ring-porous and diffuseporous stem wood anatomy on the hydraulic parameters used in a water flow and storage model, *Tree Physiol.*, 27, 43–52, <https://doi.org/10.1093/treephys/27.1.43>, 2007.
- Sulman, B. N., Roman, D. T., Yi, K., Wang, L., Phillips, R. P., and Novick, K. A.: High atmospheric demand for water can limit forest carbon uptake and transpiration as
1300 severely as dry soil, *Geophys. Res. Lett.*, 43, 9686–9695, <https://doi.org/10.1002/2016GL069416>, 2016.
- Süßel, F. and Brüggemann, W.: Tree water relations of mature oaks in southwest Germany under extreme drought stress in summer 2018, *Plant Stress*, 1, 100010, <https://doi.org/10.1016/j.stress.2021.100010>, 2021.
- 1305 Swanson, R. H.: An instrument for detecting sap movement in woody plants, *Rocky Mountain Forest and Range Experiment Station*, <https://doi.org/10.5962/bhl.title.80872>, 1962.
- Tatarinov, F. a, Kučera, J., and Cienciala, E.: The analysis of physical background of tree sap flow measurement based on thermal methods, *Meas. Sci. Technol.*, 16, 1157–
1310 1169, <https://doi.org/10.1088/0957-0233/16/5/016>, 2005.

Tor-ngern, P., Oren, R., Ward, E. J., Palmroth, S., McCarthy, H. R., and Domec, J.-C.: Increases in atmospheric CO₂ have little influence on transpiration of a temperate forest canopy, *New Phytol.*, 205, 518–525, <https://doi.org/10.1111/nph.13148>, 2015.

Tranzflo: Measurements of Sap Flow by the Heat-Pulse Method. An Instruction Manual
1315 for the HPV system: https://www.tranzflo.co.nz/index_files/HPVMANUAL.PDF (last access: 12 October 2016), 1998.

Tricker, P. J., Pecchiari, M., Bunn, S. M., Vaccari, F. P., Peressotti, A., Miglietta, F., and Taylor, G.: Water use of a bioenergy plantation increases in a future high CO₂ world, *Biomass and Bioenergy*, 33, 200–208, Elsevier,
1320 <https://doi.org/10.1016/j.biombioe.2008.05.009>, 2009.

Uddling, J., Teclaw, R. M., Kubiske, M. E., Pregitzer, K. S., and Ellsworth, D. S.: Sap flux in pure aspen and mixed aspen–birch forests exposed to elevated concentrations of carbon dioxide and ozone, *Tree Physiol.*, 28, 1231–1243, <https://doi.org/10.1093/treephys/28.8.1231>, 2008.

1325 Venturas, M. D., Sperry, J. S., and Hacke, U. G.: Plant xylem hydraulics: What we understand, current research, and future challenges, *J. Integr. Plant Biol.*, 59, 356–389, <https://doi.org/10.1111/jipb.12534>, 2017.

Verstraeten, W. W., Veroustraete, F., and Feyen, J.: Assessment of Evapotranspiration and Soil Moisture Content Across Different Scales of Observation,
1330 *Sensors*, 8, 70–117, <https://doi.org/10.3390/s8010070>, 2008.

Vitasse, Y., Bottero, A., Cailleret, M., Bigler, C., Fonti, P., Gessler, A., Lévesque, M., Rohner, B., Weber, P., Rigling, A., and Wohlgemuth, T.: Contrasting resistance and resilience to extreme drought and late spring frost in five major European tree species,

Glob. Change Biol., 25, 3781–3792, <https://doi.org/10.1111/gcb.14803>, 2019.

- 1335 Wang, H., Guan, H., and Simmons, C. T.: Modeling the environmental controls on tree water use at different temporal scales, *Agric. For. Meteorol.*, 225, 24–35, <https://doi.org/10.1016/j.agrformet.2016.04.016>, 2016.

- Warren, J. M., Pötzelsberger, E., Wullschleger, S. D., Thornton, P. E., Hasenauer, H., and Norby, R. J.: Ecohydrologic impact of reduced stomatal conductance in forests exposed to elevated CO₂, 4, 196–210, <https://doi.org/10.1002/eco.173>, 2011a.

- 1340 Warren, J. M., Norby, R. J., and Wullschleger, S. D.: Elevated CO₂ enhances leaf senescence during extreme drought in a temperate forest, *Tree Physiol.*, 31, 117–130, <https://doi.org/10.1093/treephys/tpr002>, 2011b.

- Wehr, R., Commane, R., Munger, J.W., McManus, J. B., Nelson, D. D., Zahniser, M. S., Saleska, S. R., and Wofsy, S. C.: Dynamics of canopy stomatal conductance, transpiration, and evaporation in a temperate deciduous forest, validated by carbonyl sulfide uptake, *Biogeosciences*, 14, 389–401, <https://doi.org/10.5194/bg-14-389-2017>, 2017.

- Wickham, H.: *ggplot2: Elegant Graphics for Data Analysis.*, Springer-Verlag New York, 1350 <https://ggplot2.tidyverse.org> (last access: 8 August 2024), 2016.

Wiedemann, A., Marañón-Jiménez, S., Rebmann, C., Herbst, M., and Cuntz, M.: An empirical study of the wound effect on sap flux density measured with thermal dissipation probes, *Tree Physiol.*, 36, 1471–1484, 2016.

- Wullschleger, S. D. and Norby, R. J.: Sap velocity and canopy transpiration in a sweetgum stand exposed to freeair CO₂ enrichment (FACE), *New Phytol.*, 150, 489–1355

498, <https://doi.org/10.1046/j.1469-8137.2001.00094.x>, 2001.

Wullschleger, S. D., Gunderson, C. A., Hanson, P. J., Wilson, K. B., and Norby, R. J.: Sensitivity of stomatal and canopy conductance to elevated CO₂ concentration – interacting variables and perspectives of scale, *New Phytol.*, 153, 485–496,
1360 <https://doi.org/10.1046/j.0028-646X.2001.00333.x>, 2002.

Xu, X. and Trugman, A. T.: Trait-Based Modeling of Terrestrial Ecosystems: Advances and Challenges Under Global Change, *Curr. Clim. Chang. Reports*, 7, 1–13, <https://doi.org/10.1007/s40641-020-00168-6>, 2021.

Ziegler, C., Kulawska, A., Kourmouli, A., Hamilton, L., Shi, Z., MacKenzie, A. R.,
1365 Dyson, R. J., and Johnston, I. G.: Quantification and uncertainty of root growth stimulation by elevated CO₂ in a mature temperate deciduous forest, *Sci. Total Environ.*, 854, 158661, <https://doi.org/10.1016/j.scitotenv.2022.158661>, 2023

Chapter 4 : Soil-plant flow comparisons.

- 1370 This chapter contains a draft article formatted with a view to future publication. Reference to Supporting information or Supplement refers to material in Appendix 4 to this thesis.

Linking *Quercus robur* tree-water usage to soil-water dynamics within a forest FACE experiment.

1375 Susan Quick^{1 2}, Giulio Curioni^{1 2 5}, Furkan Tekinay^{1 2}, Robert Grzesik^{1 2}, A. Robert MacKenzie^{1 2}, Stefan Krause^{1 2 3 4}

¹School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, B15 2TT, UK

²Birmingham Institute of Forest Research, University of Birmingham, Birmingham, B15 2TT, UK

1380 ³Laboratoire d'écologie des hydrosystèmes naturels et anthropisés, University Claude Bernard, Lyon1, Lyon, France (LEHNA)

⁴Institute for Global Innovation, University of Birmingham, Birmingham, B15 2TT, UK

⁵Forest Research....

1385 *Correspondence to:* Susan Quick (sue_quick@yahoo.co.uk), Stefan Krause (s.krause@bham.ac.uk) official correspondence author

Abstract.

CO₂ levels are rising due in part to increased fossil fuel emissions since the industrial revolution. Defining how tree-water usage is affected by this changed atmosphere in the context of real-world soil-water conditions requires reliable experimental data. Using high-resolution data records from our temperate forest Free-Air CO₂ Enrichment (FACE) experiment, we tested the quality of these data between sensor types, considered relevant depths, overcoming the difficulty of in-situ calibration by normalising to give relative extractable water (REW). Analysis of key parameters associated with the soil, the trees and the environment aimed to elicit CO₂ effects on soil-water to plant-water dynamics. We compared daily averaged volumetric water content (VWC, %) and REW data from reliable Enviroscan probesets at selected depths between infrastructure treatment types, ambient CO₂ (aCO₂), elevated CO₂ (eCO₂) using no-infrastructure control (*Ghost*) data for back checking. During the leaf-on treatment seasons 2 to 5 of the experiment, eCO₂ five year REW down to 40 cm depth (REW_{5yr}) was found to be consistently higher than that of the ambient aCO₂ treatment, with these effects decreasing by depth, but there were too few locations of reliable data (four locations) to undertake meaningful statistical comparisons. Seven-

year normalisation of shallow Campbell Scientific sensor VWC data to REW (REW_{7yr})
1405 gave consistent inter-seasonal results. Over seasons 2 to 7 of the experiment, when
there were sufficient Δ giving data, we found that leaf-on season infrastructure data
 $eCO_2:aCO_2$ analysis showed significantly higher REW_{7yr} (+2.3%, F value 4.9 ,
 $p=0.027$) under eCO_2 . VWC and REW at shallow depths during drought decreased
similarly across treatments. These VWC and REW data might usefully contribute to
1410 quantification of soil-water-plant-water responses to eCO_2 within this FACE
experiment by use of more complex statistical or process models.

Keywords: FACE, oak, forest, tree-water, soil-water, REW

1 Introduction

Determination of soil-water constraints on vital tree functioning has been extensively
1415 studied at landscape (Seneviratne *et al.*, 2010) and catchment scale (Zhang, Dawes
and Walker, 2001; Zhang *et al.*, 2017). Individual trees share soil-water with adjacent
trees, shrubs and ground cover plants in a complex matrix according to rooting depth
and species traits. Soil-water acts as a storage mechanism for precipitation providing
a buffer to inter-annual and intra-annual anomalies. Climate change models can take
1420 advantage of soil-moisture measurements enabling prediction of land surface
feedback dynamics. Seneviratne *et al.*(2010) clearly identified the limitations to
progress of validating these models when ground observations are lacking.

At a patch scale, the monitoring of soil-water dynamics adjacent to mature trees has
been used to inform tree-soil-water relations within forest-based manipulation
1425 experiments (Barataud *et al.*, 1995; Bréda *et al.*, 1995; Reich *et al.*, 2018; Hanson and
Walker, 2020) and other treed landscapes (e.g. scrubland, (Iqbal *et al.*, 2021)).

Tree-water usage studies are pertinent to prediction of large trees' response to drought
(Buckley, 2019; Bachofen *et al.*, 2024) and to increased atmospheric CO₂ (e.g.
(Schäfer *et al.*, 2002; Wullschleger, Tschaplinski and Norby, 2002; Leuzinger and
1430 Körner, 2007; Warren, Norby and Wullschleger, 2011; Quick *et al.*, 2025) expected
under climate change scenarios (IPCC, 2023). Despite careful analysis of experimental
results from in-field studies, conclusions are mainly location specific (e.g. (Renninger
et al., 2014)), or at large scale (Verstraeten, Veroustraete and Feyen, 2008) and lack
microclimate data (De Frenne *et al.*, 2021), so do not facilitate generalisation to
1435 different forest environments. Problems arise in predicting soil-water dynamics under

different localised soil structures, for differing maturity and species of trees, landscape profiles (e.g. slope, altitude) and environmental drivers (e.g. precipitation, air temperature, humidity) even within a single forest patch and specific climatic zone (Bréda *et al.*, 1995, 2006). The heterogeneity of soil-water characteristics therefore
1440 makes soil-plant-water interactions harder to disentangle, despite development of a wide range of measurements techniques and analytic methods (e.g. see (Verstraeten, Veroustraete and Feyen, 2008)). Despite this a number of studies have predicted resilience to drought by *Quercus* species (e.g. (Bréda *et al.*, 2006; Baert *et al.*, 2015; Vitasse *et al.*, 2019)), but few studies have considered elevated CO₂ (eCO₂) effects or
1445 the complexity of multiple stressors (Pedersen, 1999).

Large tree drought vulnerability is still not fully understood (Stagge *et al.*, 2017) and the combination of drought and eCO₂ is very difficult to predict. The effects of drought often last a number of years, especially for deciduous forest trees (Salomón *et al.*, 2022) and may result in mortality due to drought xylem damage (Anderegg *et al.*,
1450 2015). The effects of drought on oak growth might also persist for several years (Perkins *et al.*, 2018), or else multiple pre-drought stressors may contribute to tree death (Pedersen, 1999). On a season basis, e.g. during drought years, it is expected that water transport cost along the soil–plant–atmosphere continuum (i.e. β , the ratio of cost factors for carboxylation and transpiration) might increase as soil-water
1455 depletion moves towards drought conditions (Lavergne *et al.*, 2020). This might be linked both to soil properties and tree species-specific hydraulic traits, characterised by through-season changes to soil-leaf-water potential, or use of water from differing depths of soil (Sánchez-Pérez *et al.*, 2008; Robinson, Slater and Schäfer, 2012; Nehemy *et al.*, 2021; Bachofen *et al.*, 2023, 2024).

1460 Soil type and root characteristics may strongly influence the way in which water is
stored and how nutrients are delivered to the tree (Köcher, Horna and Leuschner,
2013; Landsberg, Waring and Ryan, 2017). Use of stored water can also contribute to
nutrient transport acting to mitigate twig, branch, leaf or root mortality (Orlowski *et al.*,
2023). Ultimately, without water, a tree will die, so if, as predicted, eCO₂ results in
1465 some water saving in some species, this might affect mortality which this medium
length experiment cannot yet predict.

Complex mechanistic models of water flux tend to be divided according to the
disciplinary focus (Orlowski *et al.*, 2023), but to better understand these complex
systems a transdisciplinary approach is needed. Complex models are beyond the
1470 scope of this study. Models performing particular functions, e.g. for agricultural
irrigation, can rely on less detailed data once trained (e.g. exponential filter (EF) and
principle of maximum entropy (POME) models compared by (Mishra *et al.*, 2020)) and
embrace the known variability of infiltration and evapotranspiration (ET) influence on
soil-moisture dynamics at shallow depths (< 100 cm) but these are not yet tested on
1475 treed landscapes. Simple models of plant-water-soil interactions (e.g. (Stevenson *et al.*
et al., 2023; Birkel *et al.*, 2024)) have been used to evaluate the effectiveness of limited
parameter monitoring on plant performance and can test empirical data. By suggesting
a model based on Stevenson *et al.*, (2023) but pertinent to our own measurement data,
we consider the practicality of using limited daily data, (e.g. from soil-moisture/ soil-
1480 temperature sensors not calibrated in-situ for soil types), alongside tree stem xylem
sap transducer and environmental data.

One of the standard parameters derived for considering tree-soil-water relations is
relative extractable water (REW, dimensionless), also called beta, fractional

extractable water in root zone, or soil-water index (Black, 1979; Bréda *et al.*, 1995;
1485 Schmidt, 2007; Oishi *et al.*, 2013; Steger *et al.*, 2024). [Note, this differs slightly from
the REW used at a landscape scale, see Vicca *et al.*, (2012)]. Differences in soil
characteristics, which could affect the volumetric water content (VWC, %) measurements and water retention parameters of the soil, can be reconciled by using
REW as an effective normalisation. Parametric soil-water variation under differing
1490 above-ground treatment conditions in trees can then be effectively compared despite
heterogeneous soil conditions.

The limitations to using VWC monitoring to derive water release characteristics (if soil
water retention curves are not also available (see Vicca *et al.*, (2012)) are also
exhibited when attempting to measure matric water potential (Whalley, Ober and
1495 Jenkins, 2013; Oishi *et al.*, 2014). To gain a full picture of the water extracted by the
plant it is also necessary to study the root density characteristics (Bréda *et al.*, 1995;
Sánchez-Pérez *et al.*, 2008; Bader, Hiltbrunner and Körner, 2009) and to understand
any waterlogging. In a mixed forest environment the root density and morphology
characteristic estimation of deeper root profiles (e.g. below 50cm) is a labour intensive
1500 semi-destructive sampling activity. Whole tree analysis can only be able to be
undertaken on an occasional opportunistic basis (e.g. when a tree is uprooted by
storm) which did not occur during this period (2017-2022) of our Free-Air CO₂
Enrichment (FACE) experiment.

1.1 Research scope and aims

1505 The main aim was to fill some known knowledge gaps in the field of plant-water soil-
water relations for temperate forests experiencing changes to atmospheric CO₂. From
these results we aim to improve predictions of future high- CO₂ effects on temperate

forests, especially in respect of plant-hydraulics. A secondary purpose was to explore methods of VWC data normalisation to REW enabling sound statistical and process
1510 modelling to be pursued here and in the future.

We expected organic top soil layers and deeper mineral soils to respond differently to atmospheric (hydrometeorological) forcings in different CO₂ treatments so considered daily VWC and/ or REW soil-water dynamics selected from the top 1m depth profiles to determine drying and rewetting responses. Concerning fine roots, we considered
1515 results from soil-sample analysis of fine root type and density (Handy *et al.*, no date), to facilitate better understanding of the relative use of water across fine root depths.

Finally, we suggest how simple models of relative soil-water usage by mature trees under current and future eCO₂ levels might, in future, complement concurrent ongoing studies of soil-water-respiration, tree-root-water and nutrient uptake dynamics at our
1520 experimental site.

The study considers the following research questions, hypotheses and explorations:

- Q1: What are the implications for oak soil-water usage and below-ground biomass (e.g. fine-root growth) of eCO₂?

Hypothesis 1: Elevated CO₂ in comparison with aCO₂ control, will result in reduced
1525 extraction of soil-water by trees (i.e. higher REW) at depths of intensive fine root density.

- Q2: Does soil-water availability differently influence oak tree-water usage dynamics in different CO₂ treatments?

Hypothesis 2: During dry (non-rain) days, soil-moisture reduction at shallow soil depths
1530 doesn't vary between eCO_2 treatment and aCO_2 control but under eCO_2 soil-water in
deeper (>20cm) depths may not be utilised due to lower tree-water demands.

2 Methods

2.1 Site Overview

Birmingham Institute of Forest Research (BIFoR) FACE, an experimental site in
1535 Staffordshire (52.801° N, 2.301° W), England, UK, is within a 19 ha oak (*Quercus robur*
L.) dominated research forest planted mid-C19th (Hart *et al.*, 2020). CO₂ levels,
predicted by circa 2050, have resulted mainly from emissions of fossil fuel gases since
the industrial revolution. Triplicate arrays of the three treatments, i.e. no-infrastructure
ambient air (natural/ *Ghost*); ambient-air infrastructure FACE (*aCO₂*) and ambient plus
1540 150 ppmv CO₂ (*eCO₂*) formed the future-forest experimental platform. CO₂ treatment
season (also referred to as leaf-on season) was April to October embracing both bud
burst and green-leaf periods (May to October).

Catchment geology is composed of red Permo-Triassic sandstone overlain by
superficial deposits of glacial till up to 10 m thick, with organic topsoil between 0.15
1545 and 0.6 m thick (Blaen *et al.*, 2017). The soil is dystic cambisol of sandy-clay texture.
The forest has slope ranging from a highest point situated in the east of the forest, at
approximately +112 m a.s.l. dropping to a minimum of +90 m a.s.l. at a small stream
(Blaen *et al.*, 2017). Areas of experimental interest are situated at +108 ± 2.7 m a.s.l.
(Hart *et al.*, 2020). Water flows to the stream are present, both overground and through
1550 sub-surface flow (Blaen *et al.*, 2017).

The climate is moderately wet with a pre-experimental mean annual temperature of 9
°C and mean annual precipitation of 690 mm (Norby *et al.*, 2016). From 2016 to 2021,
the last year of oak sap flux monitoring, (treatment years 0 to 5 for this study) the mean
annual precipitation was circa 750 mm +/- 16%, and the mean annual temperature was

1555 10.6 °C +/- 10% measured on or near to site. These were both (+9% and +18% respectively) higher than the pre-experimental reported means.

2.2 Measurement methods

2.2.1 Data collection and analysis.

This research was carried out within the BIFoR FACE experiment using soil data to
 1560 visualise and analyse the comparative treatment effects. Tree-water data (Quick *et al.*, 2025) and root data were also used where available. Soil and tree measurements were restricted to BIFoR research arrays (see Fig. 4-1). Across seasons one to seven (2017 to 2023) of the FACE experiment we collected concurrent measurements of soil-moisture (VWC, %) at multiple depths down to 1 metre and soil temperature (T_{ss} or T_d ,
 1565 °C) to 60 cm depth, at positions adjacent to mature dominant oak (*Quercus robur* L) (Quick, 2025) Appendix 2-A) throughout the year.

Some limited on-site environmental and soil measurements commenced pre-treatment (2015-2017) for infrastructure and *Ghost* arrays (see (Kourmouli, 2022)) but were not sufficiently comprehensive to serve as baseline references. Soil-water inputs of
 1570 throughfall precipitation under the oak canopy (P_{fs} , mm) were measured in all arrays. These point throughfall measurements, when averaged, gave an indication of interception across the whole woodland (see (Quick *et al.*, 2025)). Supplementary soil monitoring sites (2018 onwards, and timeline in Fig. S1) and Met towers providing weather data were added (MacKenzie *et al.*, 2021) during this study. On completion
 1575 15 soil-moisture monitoring points (~ 2 - 3 metres from an oak stem), including nine points for profile soil-moisture plus nine adjacent profile soil-temperature points, were present. 12 additional infrastructure soil-water profile monitoring points were added in 2022.

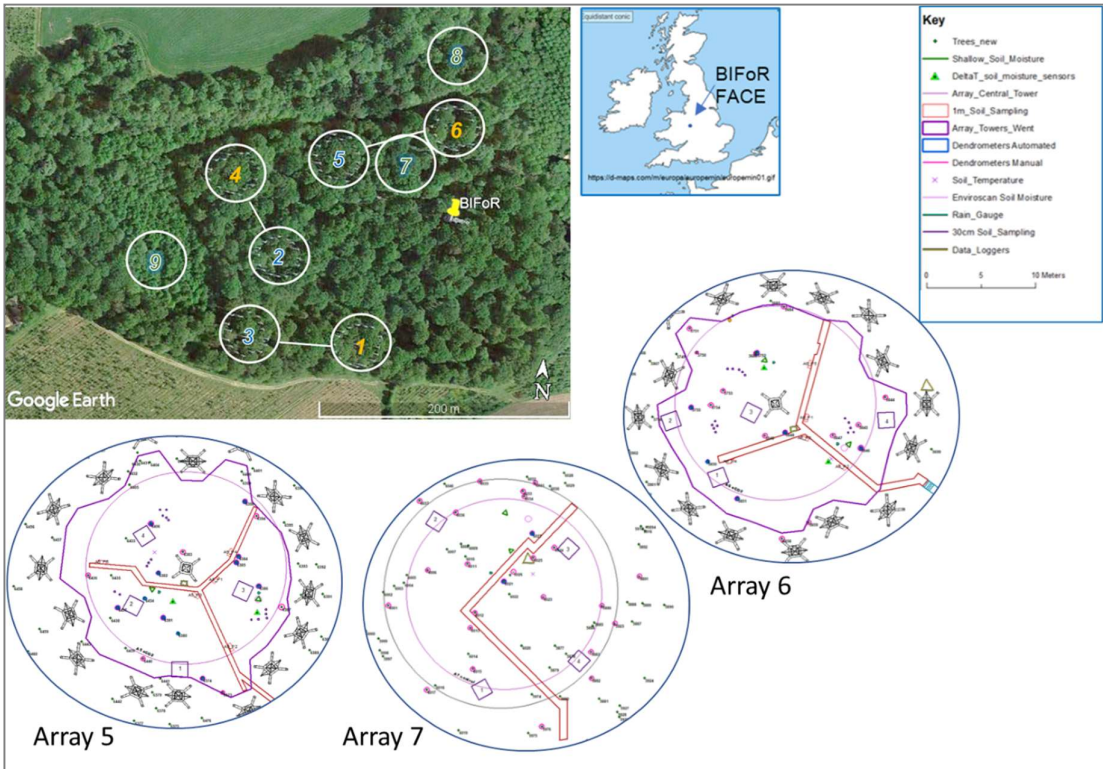


Figure 4- 1: BIFoR FACE Site details showing position of all Arrays and example soil-water and tree-water monitoring points in a three example treatment arrays: Array 5 (eCO₂), Array 6 (eCO₂), Array 7 (Ghost).

18 oak trees adjacent to soil-moisture monitoring points were monitored for sap flux data and daily daylight water usage derived from stem xylem sap transducer data (Quick *et al.*, 2025) during leaf-on seasons three to five. Compensated heat pulse (HPC) probesets manufactured by Tranzflo (New Zealand) were used to measure sap flux (Quick *et al.*, 2025). Long probesets (7cm length with 4 radial thermocouple transducers per sensor probe) were utilised for all oak monitoring starting in 2017 but only active comprehensively in all treatments from 2019.

Table 4-1 shows the types of parameters monitored (excluding sap probesets) and types of sensors used in arrays and at four Met towers (MacKenzie *et al.*, 2021). In addition to Met tower instrumentation, air temperature (T_c , °C) and CO₂ levels (ppmv) were monitored through the FACE instrumentation (Hart *et al.*, 2020; MacKenzie *et al.*, 2021; Quick *et al.*, 2025) in each infrastructure (i.e. aCO₂ or eCO₂) array. An average

of these FACE data was taken and used within the sap flux and whole tree-water use calculation as stated in Quick et al. (2025).

Description	Make	Model	Position	Parameter	Symbol	Where	Units	Logger rate
Shallow soil-moisture. Depth, d = 12 cm, water content reflectometry (WCR)	Campbell Scientific (CS) [Logan, USA]	CS655 ±3% v/v for typical soils	Inserted from ground level	Volumetric water content (VWC) + Surface soil-temperature	θ_{12} T_{ss}	Various all Arrays	% °C	At least every 30 mins
Soil-moisture profile sensors (2016 onwards – all nine arrays)	Enviroscan-Sentek	RT6	Adjacent to oak	VWC (depths d = 10, 20, 40, 60, 100cm)	θ_d	A 1-9 (not all active)	%	At least every 30 mins
Soil-moisture profile sensors (2022 onwards infrastructure arrays)	Delta-T	WET-150	Adjacent to oak & Campbell sensor position	Volumetric water content (depths d = 20, 50, 100cm)	θ_{DT}	A1-6	%	At least every 10 mins
Soil-temperature profile sensors	Campbell Scientific [Logan, USA]	T107	Adjacent to oak	Soil-temperature (depths d=10, 20, 40, 60 cm)	T_d	A 1-9	°C	30 mins (Av. per sampling period)
Tipping bucket gauges	Campbell Scientific	ARG-100	Ground level	throughfall precipitation - accumulative	P_{fs}	all Arrays	mm	30 mins
Met Tower environmental	Vaisala	HMP155RH + DTR503	1.4 m (bottom) above ground	Relative Humidity/ Temperature	RH_{bot} And $T_{a_{bot}}$	4 x Met Towers (mean)	% and °C	30 minutes
	Texas Electronics (Dallas; Texas)	TR-525M	2 m above ground	Precipitation - accumulative	P	4 x Met Towers (mean)	mm	15 minutes

Table 4- 1: Instrumentation types and sampling details for soil-water and environmental variables used in this study.

Separate soil and root analyses were undertaken as described in Kourmouli, (2021) (re year 2) and (Ziegler et al., 2023) (re year 3). Hamilton et al., (2025, unpublished) and (Handy et al., no date) provided further data to quantify soil type and fine root

1600 characteristics respectively. Forest-wide precipitation (P , mm) data were derived from a mixture of sources pre-2019 and Met towers from 2019 (see MacKenzie et al., 2021).

2.3 Data checks and analysis methods.

2.3.1 Soil-moisture quality check methods

Soil-moisture 30-minute data quality check methods were applied as described in the
1605 Supplementary Information (SI). Table S1 summarises the quality thresholds. Soil-moisture sensors had not been calibrated in-situ for soil type. The CS, Enviroscan and Delta-T sensors used the standard factory calibrations as we only required comparative VWC values.

This perched water-table site caused difficulties for analysis of deeper depth (>50cm)
1610 soil-moisture parameters due to intermittent saturation of deeper (60-100 cm) soil layers. For treatment comparison we concentrated our analysis on depths (0-50 cm) which were not intermittently saturated. Specific cross-sensor quality checks on both CS shallow and Enviroscan profile soil-moisture data were undertaken for this study due to uncertainty about data consistency. We checked the quality of the VWC and
1615 REW Enviroscan sensor profile (>10cm depth) data against additional Delta-T profile sensors, installed in 2021, for the 2022 season. REW shallow data from CS655 and Enviroscan sensors were also compared.

2.3.2 Calculation of REW

Two aspects of soil-water dynamics were considered, VWC and REW. Differences in
1620 daily soil-water and temperature dynamics under the three experimental conditions were analysed throughout the year, but with a focus on effects during treatment season. Daily Met tower precipitation (P , mm) data were used to filter rain-free days.

During rain-free days ($P < 2\text{mm}$), the values for field capacity (VWC maximum, $\theta_{max}, \%$) across the preceding autumn and winter (no-leaf season) were obtained. Permanent
 1625 wilting point values (VWC minima, $\theta_{min}, \%$) were similarly derived from VWC minima within the summer treatment season. REW was calculated at each soil-water monitoring point (differentiated by position, sensor type and depth) where we were confident that the sensor signal quality was consistent through the period of interest.

$$REW = \left\{ \frac{(\theta - \theta_{min})}{(\theta_{max} - \theta_{min})} \right\} \quad (1)$$

1630 Where θ is mean daily VWC, θ_{min} is daily VWC minimum within the leaf-on season, θ_{max} , is daily VWC maximum on low-precipitation days across the preceding autumn and winter (no-leaf season). Seasonal VWC maxima and minima were derived for each plant-hydraulic year (1st November to 31st October, leaf-on season). Note this differs from the hydrologic or water year. REW (Equation 1) was calculated across plant-
 1635 hydraulic years 2018-2023, firstly on an annual basis for θ_{max} and θ_{min} to give REW_{yr} . We then used a seven-year (2017-2023) basis to give REW (REW_{7yr} , no units) for shallow CS data and a five-year (2018-2022) basis to give REW (REW_{5yr} , no units) for the Enviroscan profile sensor.

2.3.3 Shallow soil-water VWC versus REW

1640 We considered the shallow soil data (10-12cm) from the two different transducer sources (CS655 and Enviroscan) available during the key study period, from end of season one (November 2017) to end of season five of treatment (October 2021), to correspond with the comprehensive sap flux monitoring campaign (2018 to 2021).

Figure 4-2 (a), (b), (c) illustrates the comparison of all daily shallow VWC, REW_{yr} and
 1645 precipitation (Method 1) across the key study years (2018 to 2021). We noted that

Ghost arrays had highest levels of soil-moisture VWC and *eCO2* arrays have lowest across the study period.

Figure S2 shows all sources of shallow $\theta_{max} - \theta_{min}$ also called available soil water (ASW, %) (Fares and Alva, 2000). Hydraulic year specific values of θ_{max} and θ_{min} for CS sensors were compared (Table S2). Comparing values of θ_{max} , θ_{min} and ASW between shallow sensor data from the two sources (CS and Enviroscan) by hydraulic years 2017 – 2022 (Table S3) showed synchronised inter-year responsiveness. Field capacity and permanent wilt point values compared favourably with previously standard values (Teepe, Dilling and Beese, 2003; Saxton and Rawls, 2006).

At 10cm depth REW_{yr} data is consistent for CS655 and Enviroscan sensors' data (see correlation, Fig. 4-3). REW_{yr} conversion brings together the treatment types, minimising the standard deviation of the data in each case but exhibits heterogeneity across the different tree-soil-water monitoring positions.

Next, using overall θ_{max} field capacity, θ_{max7yr} , and overall θ_{min} representing wilt point, θ_{min7yr} , for the study period (hydraulic years 2017-2023) we formed an overall ASW (ASW_{7yr}) (Fig.S3(a)). We then compared the range (mean and 95%-iles) across the 7 years of the study 2018-2023 of REW_{7yrCS} and REW_{7yrENV} relating to the two types of shallow soil-moisture CS sensor cluster or Enviroscan probeset (Fig.S4). The REW seven-year transformation (Method 2). enabled better comparison of sequential seasons. We used just the shallow CS VWC data (10 cm) to form REW_{7yr} for statistical analysis over the six hydraulic years 2018-2023. We retained REW_{7yr} values above one to demonstrate the soil-water characteristics during rainy days and the effects of drought on subsequent years (Method 2, Fig. 4-2 (d), (e), (f)), but values >1 were not

retained for statistical analysis. Inclusion of the 2022 and 2023 seasons increased our
1670 confidence in the normalisation process. A separate normalisation process was
consequently used to analyse the Enviroscan sensor profile data due to some sensor
unreliability.

2.3.4 Shallow soil moisture drought

Comparing hydraulic years visually (Fig. 4-2) we can see that the VWC and REW_{7yr} in
1675 spring 2019 indicated a failure of soil-moisture levels to recover during the preceding
autumn and winter (i.e. a lower θ_{max} in that hydraulic year). It was noticeable that θ_{max}
values were at a minimum (mean value 24.3%) in hydraulic year 2019 following a
longer drought in hydraulic year 2018, and that θ_{min} values were at a minimum in
hydraulic years 2018 (mean value 4.7%) and 2022 (mean value 5.1%) (Table S2), the
1680 latter experiencing both arboricultural drought and high air temperatures.

We define a treatment season drought as a sustained period (>7 days) when there are
low precipitation levels (< 2 mm) and the shallow VWC remains below 15%, an
effective soil water deficit. This drought analysis concentrated on two aspects: changes
in the slope of VWC at differing starting VWC levels to test our definition; the legacy
1685 effect of drought on REW in subsequent leaf-on seasons.

Dynamics of soil-water decrease during dry periods were quantified at each shallow
soil-monitoring cluster using daily average VWC at start and finish of drought period
(Eq.2). Relative rates of VWC decrease were calculated providing a proxy for the

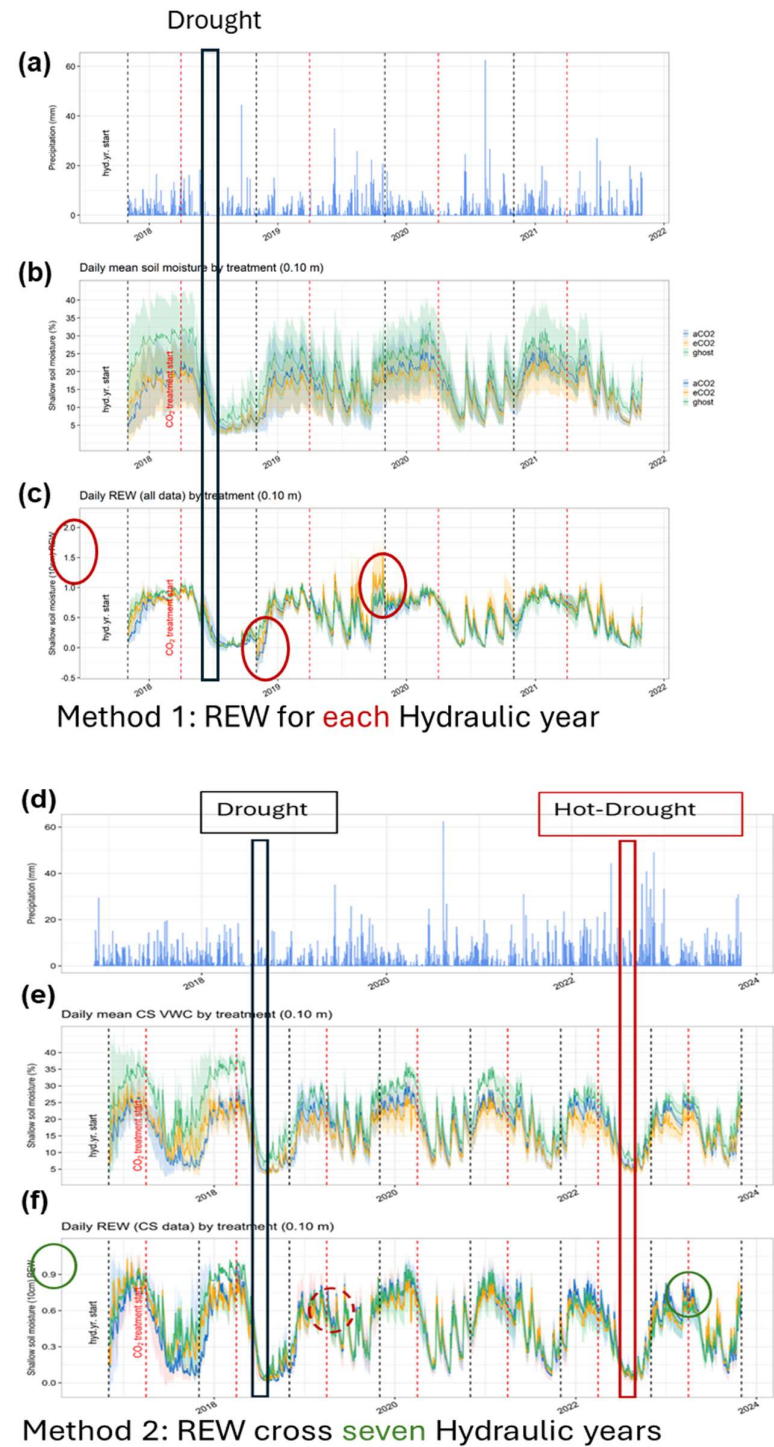
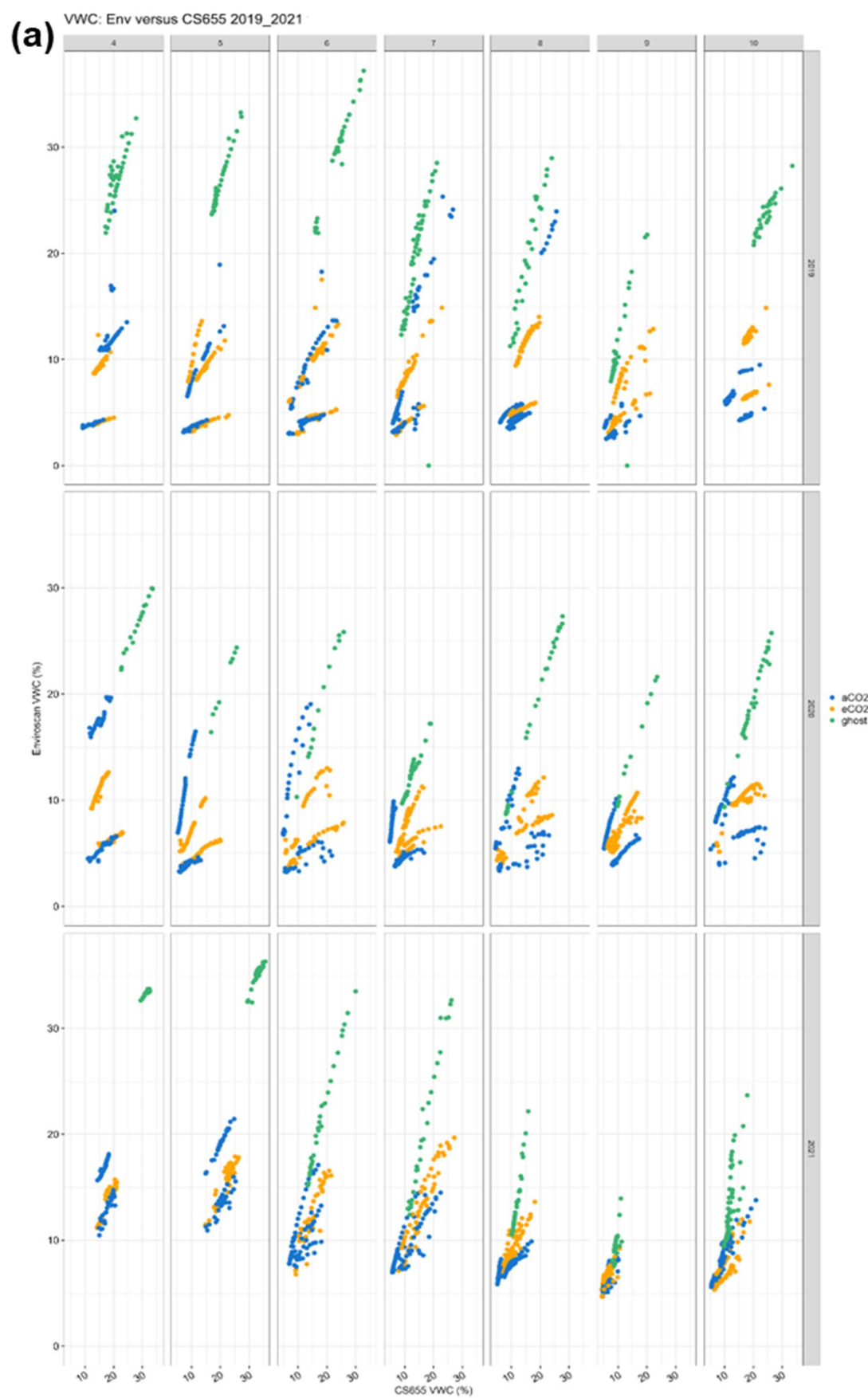
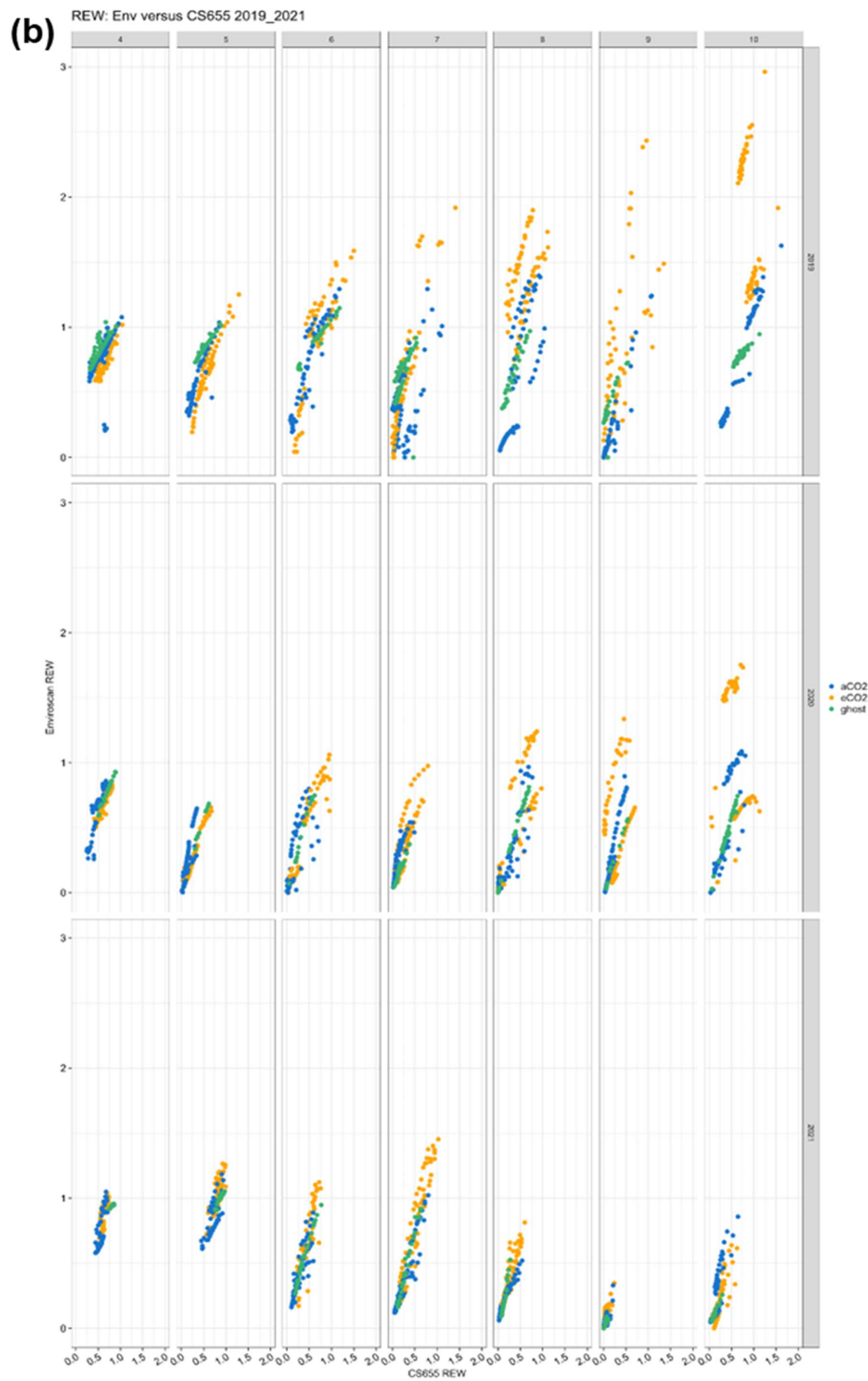


Figure 4- 2: Daily precipitation, daily average VWC with ribbon +/-sd by treatment and daily average REW with ribbon +/- sd by treatment, for all shallow soil-moisture data (10-12 cm depth). Top (a), (b), (c) show key period 2018-2021 hydraulic years using REW_y , where each hydraulic year is calculated separately for field capacity and wilt point.. Bottom (d), (e), (f) shows complete hydraulic year range 2017-2023 used for REW_{7yr} , where an overall seven-year field capacity and wilt point are extracted. Black box indicates a longer drought period in 2018. Red box indicates a longer hot drought period in 2022. Hydraulic year start (Black dotted lines) 2017-2023 are shown. Red dotted lines indicate start of treatment season in each year.





1700 **Figure 4- 3: (previous two pages) To show correlation between CS655 and Enviroscan shallow VWC, (a) and REW data (b) using hydological year theta ma and theta min. (a) for VWC and (b) for REW. Seasons 3 to 5 are shown (2019-2021).**

combined effects of infiltration, soil-water absorption/ soil rehydration, tree water usage and soil-surface evaporation. The main soil-water use during no rain periods was assumed to be by oak trees at these positions whilst recognising that subdominant
1705 species coexisted within this site which might affect the VWC response.

$$\Delta\theta = \frac{(\theta_1 - \theta_2)}{n} \quad (2)$$

Where θ_1 is mean daily VWC at start of low (< 2 mm d-1) precipitation period and θ_2 is mean daily VWC at last low precipitation day, n is number of days with continuously low precipitation.

1710 **2.3.5 Soil-water profile VWC and REW.**

Changes to VWC and REW dynamics through profile depths were analysed. Due to data anomalies, gaps and knowledge of fine root density, only depths < 50 cm were selected for further analysis of plant-water effects under treatment considerations.

Enviroscan profile sensors suffered some failures across the study years (itemised in
1715 Table S4). VWC for years 2018-2019 (Fig. S5) and years 2022-2023 (Fig.S7) are shown as examples. VWC and REW_{yr} daily data (averaged by treatment) to a depth of 0.4 m across 2019 are shown in Fig. 4-4(a). There is good correspondence across the treatments once the REW_{yr} normalisation has been completed. We had noted that transitions to saturation were unsynchronised at different locations for lower depths
1720 (0.6 and 1 m) (Fig. S5) so decided not to transform to REW at these depths for purposes of treatment analysis.

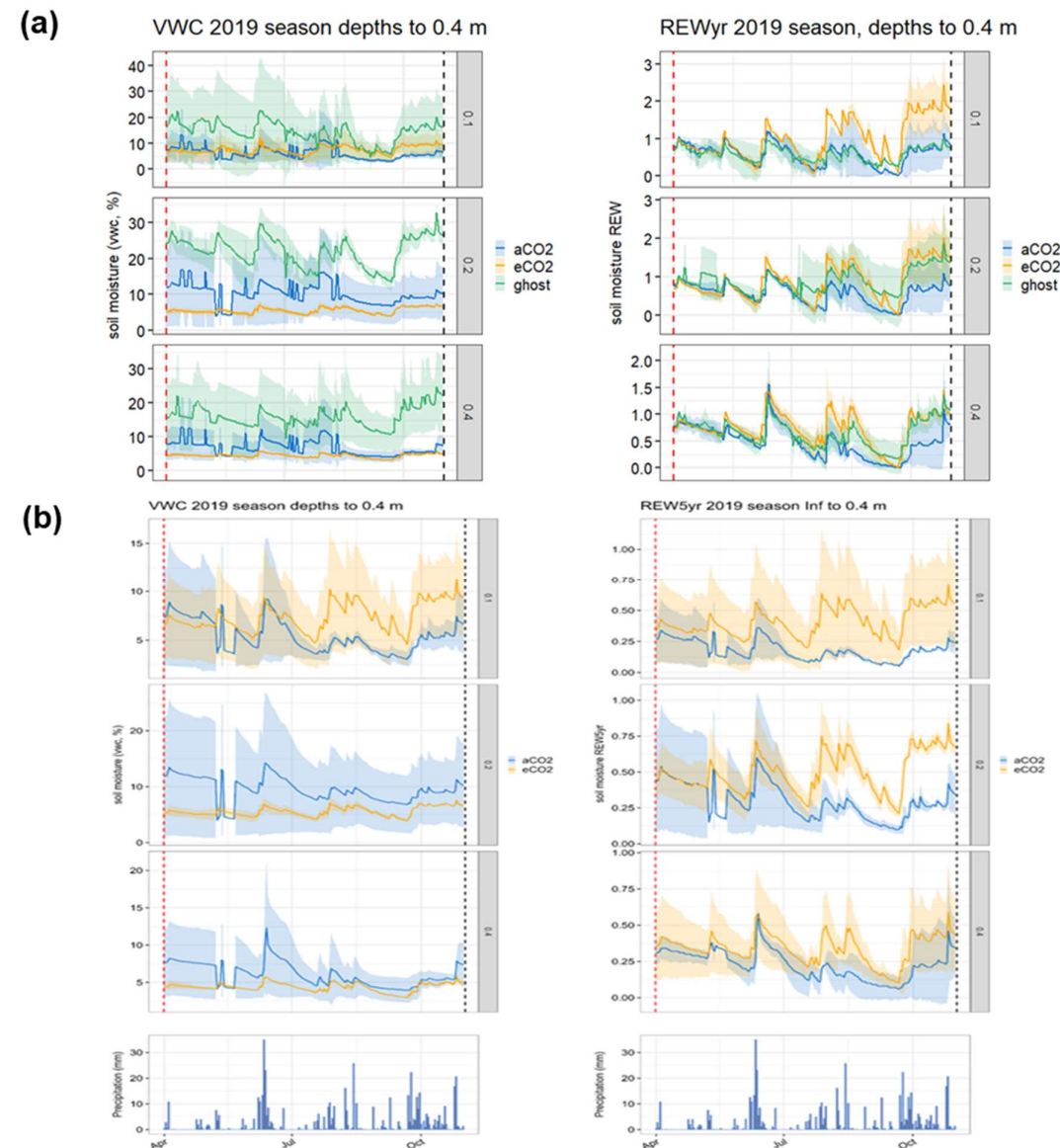


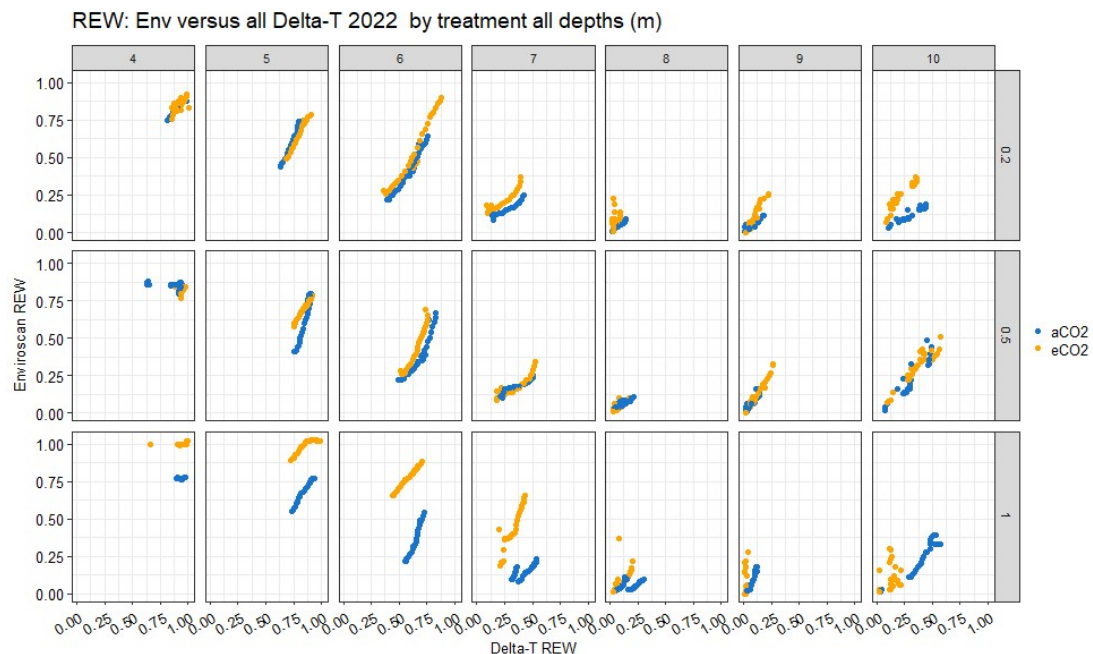
Figure 4- 4: Enviroscan profile for 2019 to 0.4 m depth (i.e. 10cm, 20 cm, 40 cm depth). Data is shown as mean daily value by treatment with +/- std deviation. Graphics are shown against daily precipitation (below). Comparison of VWC with REW in fine root depths in 2019 season. (a) selected infrastructure array positions giving reliable data (A1, A3, A4, A5) are visualised alongside all Ghost array data with VWC (left) and REW_{yr} (right). Averaged data include all rain period data (i.e. REW>1). (b) selected infrastructure array positions giving reliable data (A1, A3, A4, A5) are visualised with VWC (left) and REW_{5yr} (right). Averaged data exclude rain periods giving REW>1.

High frequency Delta-T (commissioned Spring 2022) sensor VWC data were obtained for season 6, 2022 and visualised against such active Enviroscan sensors (some of which had been recalibrated in 2021) as could provide sufficient infrastructure array continuous data (Fig. S6). We note the heterogeneity of VWC values even between

the two independent positions within each array (Delta-T). We also note that low values of VWC were exhibited within array 5 data for both types of sensors.

1735 We formed the REW_{yr} for the 2022 treatment season data for both Delta-T and Enviroscan sensors types. At 50 cm depth we took the average of the daily REW_{yr} data for depths 40 cm and 60 cm from the Enviroscan sensors. The correlative graphics are shown in Fig. 4-5. The two-per array Delta-T sensor positions show heterogeneity even with the REW_{yr} transformation. There is good (mostly linear) correlation between the

1740 two sensor types even at 1 m depth, though variation in leaf senescence timing between trees skewed the results during September and October. We note that REW_{yr} in 2022 consistently declines from unity in all depths across the treatment season months 4 to 8 (April to August) and starts to increase again from September.



1745 **Figure 4- 5: Correlation of daily REW_{yr} data from Enviroscan and Delta-T type profile sensors across treatment season months (labels at top) for each coincident nearest tree position during season 6 (2022). Enviroscan 40cm and 60cm depth data were used to derive 50cm data (depth labels on right).**

For statistical analysis we selected only four infrastructure array Enviroscan sensors which provided consistent shallow root-depth data across five consecutive seasons, 2018-2022. Table 4-2 shows infrastructure positions selected (with all positions shown in Table S4) alongside ghost array sensor status. These good profile positions' VWC data were transformed to REW_{5yr} (hydraulic years (2018-2022) (example transformation Fig. 4-4(b)).

	Treatment	2018	2019	2020	2021	2022
A1	eCO ₂	OK	OK	OK	OK	OK
A3	aCO ₂	OK	OK	OK	OK	OK
A4	eCO ₂	OK	OK	OK	OK	OK
A5	aCO ₂	OK	OK	OK	OK	OK
A7	Ghost	OK	OK	OK	OK	F
A8	Ghost	OK	OK	F	F	OK
A9	Ghost	F	OK	OK	OK	OK

Table 4- 2: Enviroscan infrastructure positions utilised for analysis and all Ghost positions showing failures in particular treatment seasons. OK indicates working sensor, F indicates a failure of one or more depth positions (N.B. A7 probeset had no data from 1 m depth throughout).

2.3.6 Sap flux and daily daylight tree-water usage

Within-season (April to October) sap flux data for daylight hours only (as HPC probesets cannot measure negative or very low xylem flow and daylight photosynthesis is known to dominate tree water usage) were used in this project for soil-water to tree water-usage comparisons. Oak trees' normalised daily daylight tree-water-usage (TWU_n , litres d⁻¹ per tree) dynamics under the three experimental CO₂ conditions (Quick et al., 2025) were considered in relation to daily soil-water (REW_{5yr}) dynamics down to 40cm depth.

2.3.7 Data processing, visualization and analysis software

Arrays graphics (Fig. 4-1) used ARCGIS FACE data. A datalogger in each research array (CR1000+AM25T, Campbell Scientific, Logan, Utah, USA) captured raw data from sap probesets (Quick et al., 2025), soil sensors and throughfall rain gauges (Quick

et al., 2025). The loggers were programmed for data capture in sequential mode due to the sap transducer multiplexer requirements, using CRBasicEditor under LoggerNet (versions to 4.6.2) also by Campbell Scientific, Logan, Utah, USA. Other data loggers (e.g. for Delta-T sensors, FACE control and Met towers) were programmed and managed by the BIFoR FACE operations team. All data were pre-processed as .csv files for import. All analysis was in 'R' (R Core Team, 2024) using RStudio (Posit Team, 2024) with full lists of packages provided in scripts. Results figures were created using R package *ggplot* (Wickham, 2016). Box and whisker plots, to visualise seasonal and monthly differences in VWC and REW through the depth profile and by treatment used standard Tukey (McGill, Tukey and Larsen, 1978) percentiles (median + interquartile range) whiskers ($1.5 * \text{IQR}$ from each hinge, where IQR is the inter-quartile range) plus points for outliers are used. Initial statistical treatment comparisons for shallow VWC and REW used one-way ANOVA for larger datasets. Tests for homogeneity of variance and normality (Levene, Shapiro-Wilks) were used.

3 Results

3.1 Fine-root depth profile soil-moisture dynamics

1785 We had noted from qualitative visualisations (Figs. 4-4, S5, S6) that VWC and REW in organic top soil layers respond differently to atmospheric (hydrometeorological) forcings to deeper mineral soils. Our results concentrate on the profile depths used by fine roots at < 50 cm depth.

3.1.1 Depth comparisons across hydraulic years and treatment seasons.

1790 Considering Fig. 4-6, showing profile REW_{5yr} range for depths < 50cm across the five years of reliable data (2018-2022), we can see that there are different trends specific to the hydraulic year in each treatment array with increasing depth. Firstly, the highest mean values of REW_{5yr} are exhibited in year 2021 and lowest means in 2018, at all depths. This suggests a legacy effect from the 2018 drought summer. To fully explore
1795 these phenomena with respect to eCO_2 treatment we next focus on results from the treatment seasons.

Figure 4-7 shows Visual comparison of Enviroscan infrastructure treatments fine root depth data by box and whisker plots during treatment seasons 2019-2021 for probes giving reliable data (A1, A3, A4, A5). Through the fine-root profile during 2019 to 2021
1800 seasons qualitative analysis suggested that these probesets there were consistently higher REW exhibited across the leaf-on season at 10cm depth, but more variable effects between seasons. For example, a gradual decline in REW levels occurred in both 2019 and 2021 at 20 cm depths, with 2020 exhibiting month by month individual changes possibly due to intermittent summer rain. For all three years the greatest
1805 differences in REW prior to senescence are seen during mid-summer (June, July and

August) at 20 cm depth. At 40 cm depth REW treatment comparisons are not as great as in shallower depths and seem to be unique to the year.

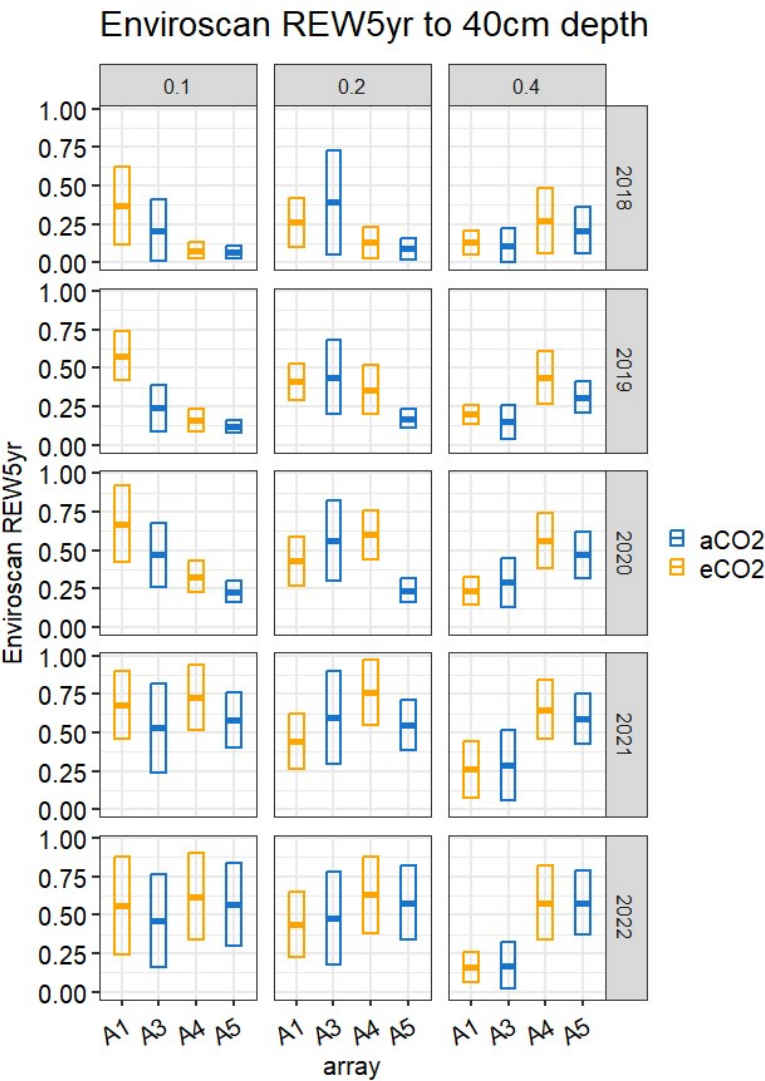


Figure 4- 6: To show range of REW_{5yr} (mean and 95%-iles) across the 5 hydraulic years of the study 2018-2022 when sufficient sensors were working reliably. Horizontal facet shows depths in metres and vertical facet shows hydraulic years. Transducer position are within selected arrays (x-axis labels). Values of REW_{5yr} > 1 have been omitted.

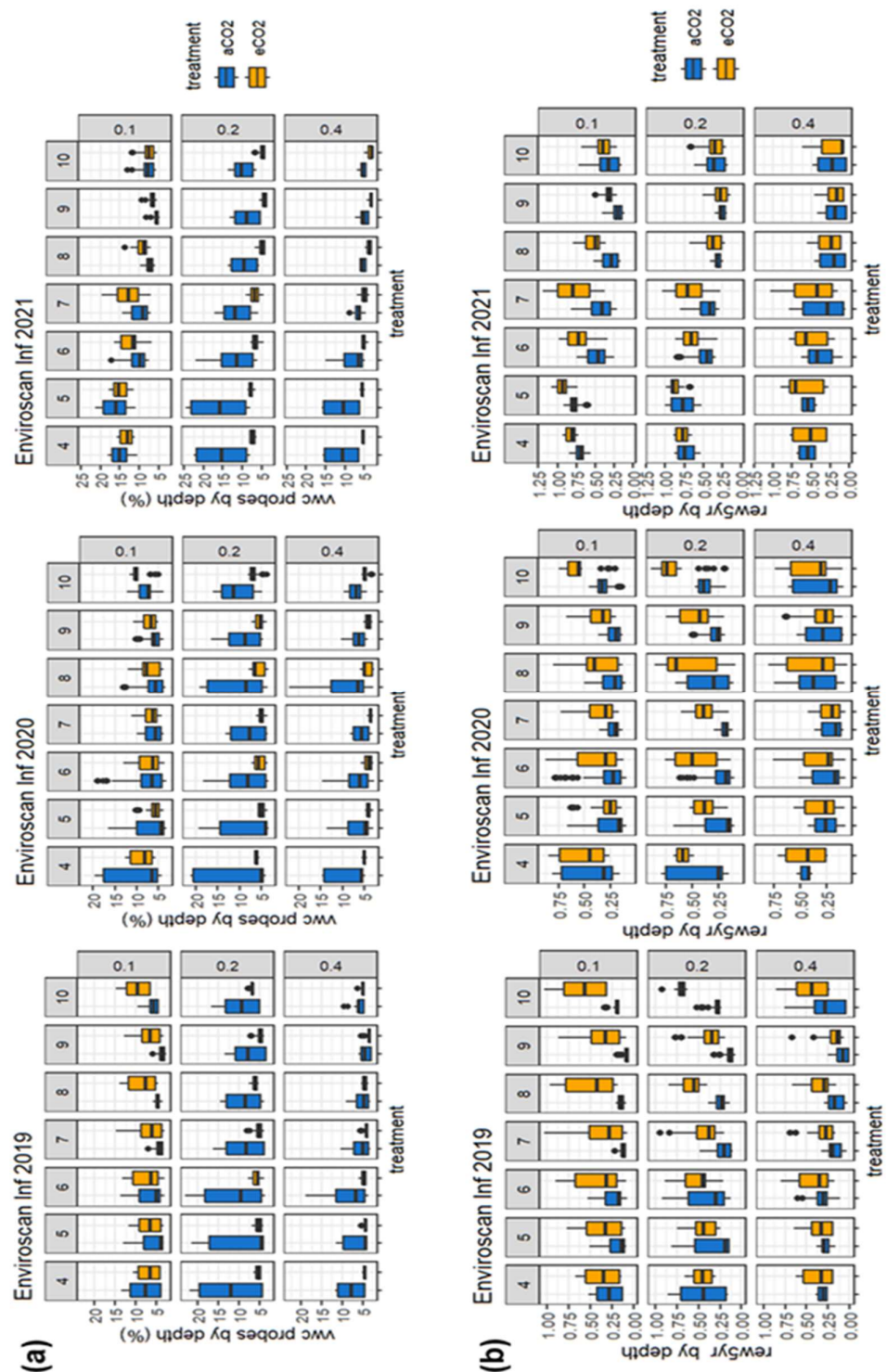


Figure 4- 7: Visual comparison of Enviroscan infrastructure treatments fine root depth data by box and whisker plots during treatment seasons 2019-2021 for probes giving reliable data (A1, A3, A4, A5). Top facet labels indicate month, side facets indicate sensor depth (to 40cm only). Top row (a) shows VWC. Bottom row (b)shows REW_{sy}. REW data include all rain period data giving REW>1 so y-axis scales differ between years (e.g. 2021).

3.2 Shallow VWC and REW treatment effect analyses

3.2.1 Shallow CS sensors REW analysis of eCO₂ effects.

Using REW_{7yr} data for 2018-2023 hydraulic years from shallow CS sensors we considered the overall trends within-season and between years (Fig. 4-8). From one-way ANOVA analysis of near equal data numbers (aCO₂:13234, eCO₂:13461) shallow REW_{7yr} for the whole hydraulic years 2018-2023 is significantly lower for eCO₂ (-2.7%, F value 8.3, p< 0.001) relative to that for the aCO₂ control. The density and histogram graphs and for the combined dataset and each of the hydraulics years are shown (Fig. 4-9).

When just the treatment seasons are considered for the same hydraulic years there is higher shallow REW_{7yr} for eCO₂ relative to that for the aCO₂ control implying a possible lower tree-water usage at those measurement positions. From one-way ANOVA analysis across leaf-on seasons two to seven (within 2018-2023 hydraulic years) infrastructure data eCO₂:aCO₂ showed a significantly higher REW_{7yr} (+2.3%, F value 4.9 , p=0.027). Table 4-3 gives both sets of results.

	Hydraulic years			Leaf-on Season Apr.-Oct.		
	F(df, value)	Pr(>F)	%	F(df, value)	Pr(>F)	%
2018-2023 Hyd. years (all:N = 26693 Season: N = 15881)	F(2, 8.3)	< 0.001***	-2.7	F(2 4.91)	0.027*	+2.3

Table 4- 3: Results from shallow CS sensors. One-way ANOVA for CO₂ effects. F(df, value), Pr(>F and % difference summary for eCO₂ REW_{7yr} compared with aCO₂ REW_{7yr} in years 2018-2023. Daily values are compared, calculated from the entire hydraulic year and for leaf-on season April–October as shown. Bold typeface indicates p-value <0.05.

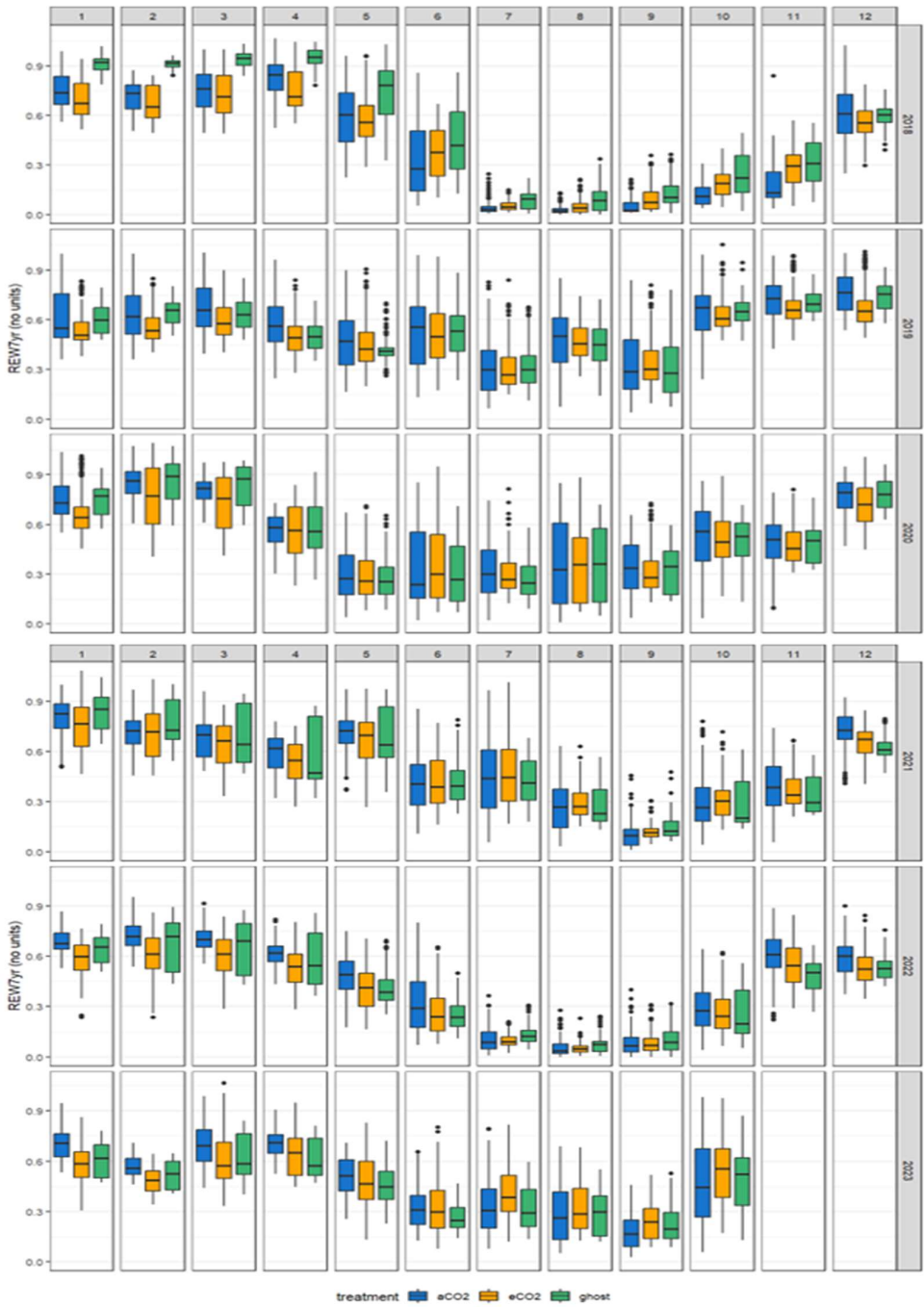


Figure 4- 8: Showing box and whisker daily REW_{7yr} plots for every month of years 2018-2023 differentiated by treatment to end of treatment season in 2023. Autumn (Nov-Dec) 2017 is omitted but forms a part of hydraulic year for 2018. Rain days are included except where they result in $REW_{7yr} > 1$.

1840

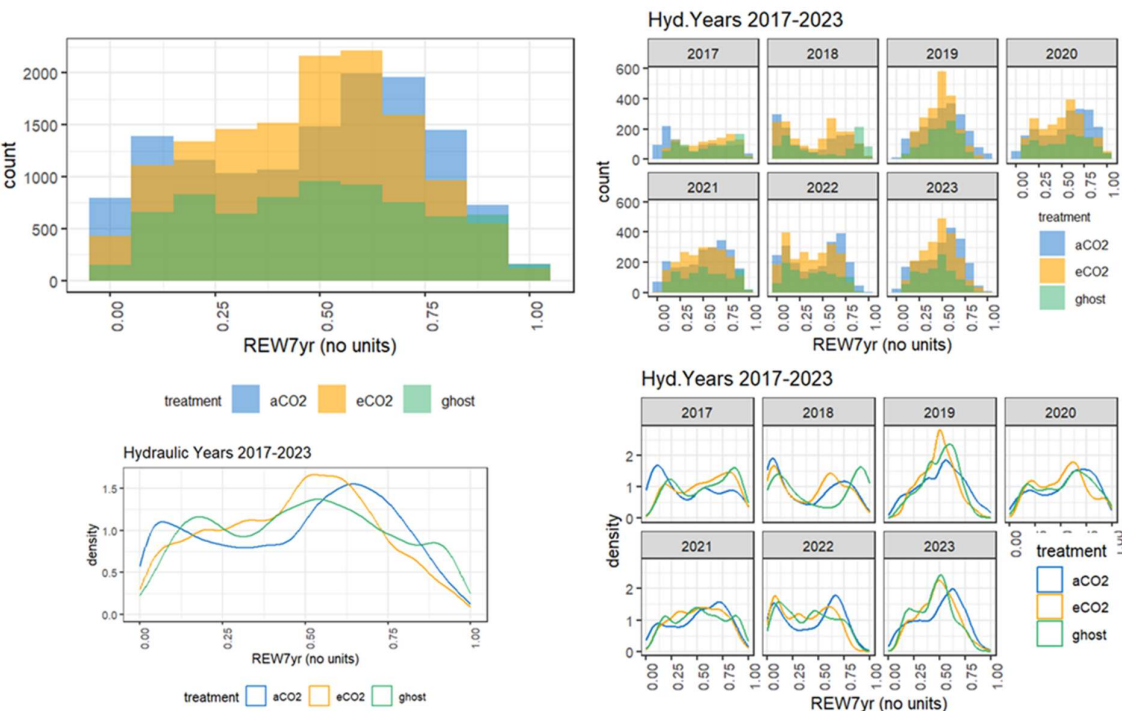


Figure 4- 9: Histograms and density plots for CS daily REW_{7yr} data for all hydraulic years 2017-2023 both (left) for combined all-year data and (right) for individual hydraulic years. Daily REW_{7yr} data >1 have been removed.

3.3 Periods of no rain and drought.

3.3.1 June to July drought periods

Using CS shallow sensor VWC data, decreases during no-rain periods in June to July were identified for seasons 2 to 7 (2018-2023). We note that, by our definition, there were shallow soil moisture droughts in each treatment season studied. Figure S8 shows the resulting VWC decrease slopes and precipitation levels before, during and after the drought periods. Table S5 shows the VWC ranges, slopes and maximum number of days of drought during these June-July periods. Figure 4-10(a) shows the correlation of VWC decrease slope ($\% \text{ d}^{-1}$) during drought against mid value of VWC (%) whereas Fig. 4-10(b) shows VWC decrease slope against average precipitation rate (mm). The patterns from these graphs tend to indicate that VWC values, and

precipitation amounts, even at a low level during drought, can influence the slope of VWC decrease.

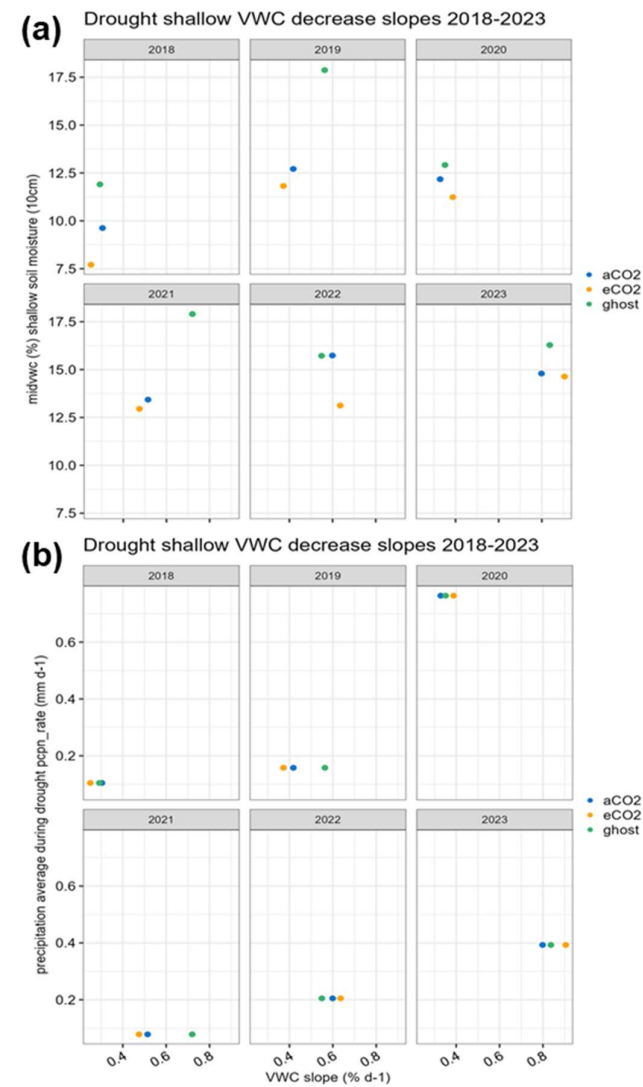


Figure 4- 10: Showing shallow VWC drought responses during the longest June-July drought periods. For (a) mid-VWC values (y-axis) during VWC decrease, versus VWC decrease slope ($\% \text{ d}^{-1}$) during drought (x-axis with suppressed zero), and bottom, (b) average precipitation rate (mm) (y-axis) against VWC decrease slope ($\% \text{ d}^{-1}$) during drought (x-axis with suppressed zero). Sub-panels are facets of seasons 2 to 7 (2018 to 2023) in both cases.

3.3.2 Legacy effects of leaf-on season droughts.

As mentioned in Methods (section 2.3.4), treatment season agricultural drought will likely affect REW available in the subsequent Leaf-on season. In Fig. 4-2 we note that, following very low values at the start of treatment, daily shallow VWC is higher at the

end of season 3 (2019) than at the start. This is reflected in the REW_{yr} figures for that season (see also Table S2). The opposite effect is evident when leaf-on season drought occurs (seasons 2 and 6, 2018 and 2022) with more balanced REW_{yr} levels during wetter summers (e.g. 2020 and 2021). in terms of the amount of water which trees might use under these differing conditions, we have revisited the results from Quick *et al.*, (2025). Figure 4-11 shows accumulative mean normalised tree water usage ($\overline{TWU_n}$) across treatment seasons 3 to 5 for consideration in the Discussion.

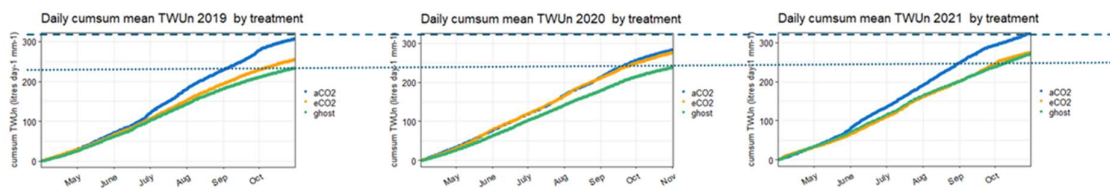


Figure 4- 11: Cumulative mean normalised tree water usage ($\overline{TWU_n}$) , (litres d⁻¹ mm⁻¹), for each of three seasons 2019-2021

3.4 Forming datasets for an LMM model.

Using data for 2019 to 2021 we first examined the correlation between shallow REW_{yr} data and oak tree TWU_n (Quick *et al.*, 2025) in the three treatments (Fig. 4-12). In 2019 we noticed a divergent tendency between the correlative relationships for eCO_2 compared with control data (aCO_2 and $Ghost$) as REW_{yr} (i.e. available plant water for roots) increases (Fig. 4-12 (a) to (f)), but this may be driven by higher numbers of rainy days, (i.e. more REW_{yr} points above 1) in a particular season for both infrastructure treatments (eCO_2 , aCO_2). For a particular year the correlations exhibit similar slopes, differentiated by treatment, when data are restricted to adjacent soil moisture sensors (Fig. S9 (b) vav Fig. S9(a)).

No-infrastructure data ($Ghost$) tend not to differ in correlative slope between years of interest (Fig. 4-12 (g), (h), (i)), neither do they exceed REW_{yr} unity value. This may mean that there are infrastructure or treatment effects of the experiment which have

not yet been accounted for (e.g. as discussed in (Quick *et al.*, 2025)). The tendency to higher REW values in eCO₂ data may reflect a better ability to replenish shallow soil moisture for trees within this treatment, or else a difference in fine root growth, but this remains to be tested. There is a clearer relationship if the data are restricted to a single tree, see Fig. S9, but further, more-focused analysis is required.

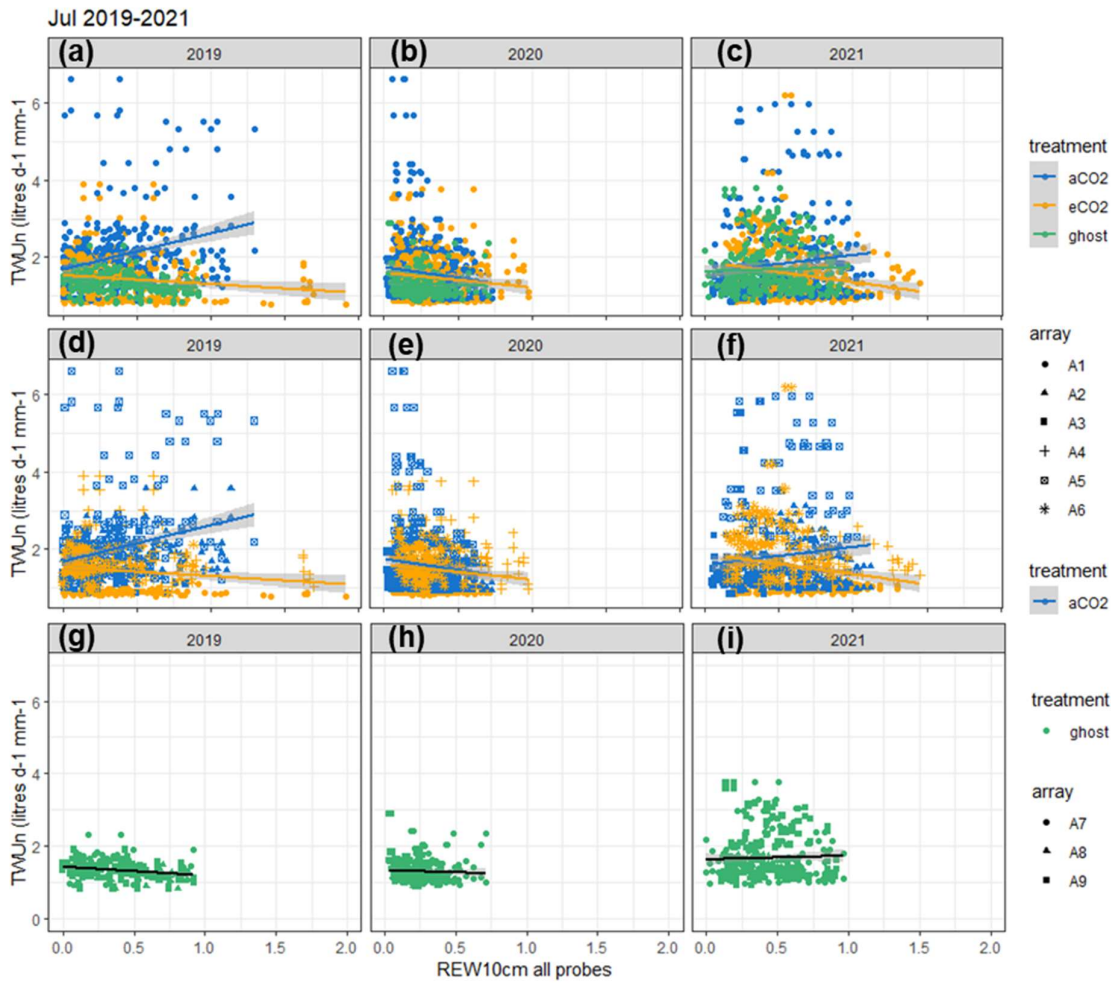


Figure 4- 12: All three years of interest, 2019, 2020, 2021. Correlative scatterplots REW10cm against TWUn. Top figures, (a), (b), (c), are all shallow sensors, middle, (d), (e), (f), are just infrastructure tree and soil data, bottom (g), (h), (i), are no-infrastructure data. (a) to (f) have suppressed y-axis to circa 0.7 l d⁻¹ mm⁻¹.

4 Discussion and conclusion.

4.1 Discussion

1905 4.1.1 Effects of eCO₂

Shallow soil-moisture VWC conversion to REW_{yr} for two sensor types (Enviroscan and CS) measuring shallow depths across our treatment arrays demonstrated that different transducer technologies producing data at the same location (here at a given oak tree position) could be combined once the process of REW has been consistently applied
1910 to good data at each source.

Similarly, using Enviroscan soil-moisture profile data, we validated REW_{yr} by comparison with data from Delta-T sensors. Consequently, we were able to use the profile REW data from the more reliable Enviroscan sensors (i.e. those which did not fail frequently) to assess of soil-water seasonal dynamics in our oak-forest experiment.

1915 Calculations of REW_{5yr} at three depths < 50 cm were valuable in indicating qualitative availability of soil-water for fine tree roots (i.e. at depths not near to a water table) under the differing treatments (Section 3.1) contributing to testing hypothesis 1, but these were insufficient to undertake quantitative analysis due to unreliability of sensors.

Bader et al., (2009), used soil-sampling and root ingrowth to examine shallow (< 18
1920 cm depth) roots of three dominant hardwood species including *Q. petraea*. during seasons 5 to 7 of WEB-FACE. They found less fine root (< 1mm diameter) biomass of c. –30% under eCO₂ in season 5 soil-samples for combined species' live roots and similar reductions in their season 7 after two years' ingrowth. BIFoR FACE work on fine root biomass is being reported separately (Handy *et al.*, no date).

1925 Using shallow CS data alone we formed REW_{7yr} and analysed treatment effects statistically across six seasons and hydraulic years (Section 3.2). For both REW_{7yr} and REW_{5yr} , we found that trees experiencing eCO_2 levels retained higher soil-moisture during each leaf-on season at shallow (< 50 cm) fine root depths compared with trees under aCO_2 , indicating differing tree-water uptake dynamics for these two treatments.

1930 Considering hypothesis 1, we found that whilst there is significantly reduced extraction of soil-water by trees (i.e. higher REW_{7yr}) at shallow (10-20 cm) depths when analysed over 5 seasons of treatment. The qualitative analysis of REW_{5yr} shows similar trends.

Under eCO_2 , the results from 40 cm depth of intensive fine-root density appear more season-specific, possibly reflecting the fact that fine root growth and death differ during

1935 drought years and that fine-root growth treatment effects may take a few years to show. Hypothesis 1 is therefore only partially confirmed. Where VWC levels are adequate (e.g. $>15\%$) for example early in the season, this needs to be tested further. Our finding tends to confirm with the finding of another FACE experiment (Tor-ngern *et al.*, 2015), where it was predicted that hydraulic effects, due to reduced transpiration by trees

1940 under eCO_2 , might only be detected by long-term rather than short-term monitoring. We consider that, in our own experiment, variation of season environmental conditions such as precipitation, heat and drought confound the inter-season comparisons, making it difficult to untangle the effects of eCO_2 on soil-water dynamics especially near soil-surface levels where evaporative processes also play a part. Separating the

1945 days further by, for example, excluding days when precipitation is greater than 2mm during the season would be best done in a glmm model which could take account of the non-normal distribution for these data.

4.1.2 Drought

Although a significant drought occurred in 2018, we could not fully explore the effects of drought on trees' water usage e.g. for eCO_2 compared to control trees (aCO_2 and *Ghost*) as we had no baseline data for the tree water usage in this season. Instead, the analysis has concentrated on two aspects. Firstly, changes in the decrease slope of VWC at differing starting VWC levels for the reliable CS sensors. REW (and hence VWC) dynamics during and just after periods with no rain, were shown to be dominated by a combination of the soil characteristics and the demands exhibited by the trees, likely affected by fine root dehydration. Secondly, the legacy effect of drought in subsequent leaf-on seasons was confirmed.

In respect to hypothesis 2, the soil-moisture dynamics during drought days showed that soil-moisture reductions (decrease slopes) are similar across the three treatments implying our hypothesis may be partially confirmed but we did not fully investigate deeper (≥ 20 cm) depths due to lack of calibration and breadth of the VWC data at these depths. In respect of rain events, we also had insufficient knowledge of the underground water flow movements which might confound the effects so did not consider these aspects here. We speculate that these effects might indicate why damage to tree xylem can occur (due to shallow soil-water depletion) not only in the year in which drought occurs, but in subsequent years, as described by many authors (e.g. (Anderegg *et al.*, 2015; Vitasse *et al.*, 2019)). In terms of the amount of water which trees might use under these differing conditions, we revisited the results from Quick *et al.*, (2025). Considering Figure 4-11 as an example, using accumulative mean normalised tree water usage ($\overline{TWU_n}$) across treatment seasons 3 to 5, (from Quick *et al.*, (2025) data), we note that, whilst *Ghost* trees had similar total water usage across

the three years, with only slight increases, there are differences for infrastructure treatment trees (eCO_2 , aCO_2) (i.e. greater tree water usage in aCO_2 trees). We conclude for infrastructure treatment experimental conditions REW and VWC dynamic analysis may in future usefully contribute to vegetation models concerning combined effects of drought and eCO_2 .

4.2 Benefits and limitations of the approach.

The use of autumn/ winter maxima θ_{max} , representing the maximum available stored soil-water present at the start of each treatment season, and θ_{min} for minimum within the leaf-on season daily VWC in lieu of accurately derived in-situ field capacity or permanent wilt point was found useful in determining the per-season effects of treatment in respect of tree-water. This is particularly important when it is not possible to accurately derive field capacity or permanent wilt point at each soil moisture monitoring position during an experiment when conditions are extremely heterogeneous or when inter-year weather conditions vary widely. This approach would be easy to implement in other seasonal studies including those controlling irrigation. The disadvantage of using annual hydraulic year parameters is that it creates stepped REW_{yr} values at the start of the treatment/ leaf-on season (April to October) change-over point (e.g. 1st Apr. each year). REW_{yr} also gives useful insight into within-season dynamics, and could be usefully deployed again especially for use in studying soil-plant fine-root interactions during summer dry periods/ droughts or during peak root growth.

To overcome the limitation of annual REW_{yr} , for this long-term seasonal manipulation experiment, we used overall maxima θ_{max} and overall minima θ_{min} from good-quality continuous data forming REW_{7yr} and REW_{5yr} from shallow and profile VWC data

respectively enabling some limited inter-seasonal and multi-year analysis of eCO_2 effects.. A rolling 7 or 5 year REW could have been used if baseline (pre-treatment) and comprehensive (all treatment type) reliable data had been available for sufficient years. Nevertheless for CS sensors, sufficient good continuous data enabled REW_{7yr}

2000 statistical analysis of treatment effects at shallow soil depth. By selecting Enviroscan sensors which worked continuously for five seasons a similar approach was applied to the fine root depths < 50 cm producing REW_{5yr} , even though probeset reliability was poor overall. Based on these results we conclude that by removing data for sensor failure, it is best to use all reliable data down to 50 cm for any models as these data

2005 reflect more closely the soil-water available to the fine tree roots. At deeper levels (0.6 and 1 m depth) comparisons between soil-water content and stem-water were impacted by the changes to water table (i.e. saturation of lower depths) and capillary fringe effects which bifurcated these data into hi-lo categories. This limitation does not impact on future work concerning fine roots.

2010 Separate soil sampling and analysis regimes (undertaken seasons 0 onwards) provided gravimetric water content, bulk density and soil texture data (e.g. see Table 4- 5 of (Kourmouli, 2022)), which could in future enable both assessment of the heterogeneity of soils at different measurement depths (at each depth across the treatment plots) and verification of data quality (e.g. to validate CS655 sensor data

2015 (Huang *et al.*, 2016)). Use of soil sampling for independent calibration of the CS and Enviroscan sensors is necessarily limited within this real-world experiment as sampling impacts on the natural soil-moisture and soil-structure condition. Sampling was not carried out near to our VWC sensors within this FACE experiment.

Whilst our data was collected across seven treatment seasons, sensor reliability limited the period used for statistical analysis. Comparisons with tree water usage were also restricted to the three year period when sufficient *TWU* data were available to test our hypotheses. Our work (see section 3.4) preparing for a future statistical model using *TWU_n* and REW data from July 2019-2021 anticipates differences between treatments, but the model has not yet been constructed. Here we use daily VWC, REW and *TWU_n* data periodicity; the latter is for daylight hours only. It is not feasible to use the tree-water data to examine nocturnal tree-water dynamics from our sap flow monitoring due to sensor type. We would recommend capturing tree water-usage data over a period of more than three years, alongside ensuring reliable soil-water profile measurement, to better examine the effects of *eCO₂*, drought and changes to soil characteristics affecting fine root growth and to enable better inter-year analysis and longer-term trends. Even the limited data provided by this study can be up-scaled as a verification tool for forest-wide studies or used to predict seasonal tree water-usage effects on available water content in similar-aged oak forests.

We find there is a paucity of detailed soil-moisture data for mature trees in temperate oak forests, so comparing VWC results from this study to those from other such forests has inherent limitations. Using REW does assist in ameliorating these limitations ((Lavergne *et al.*, 2020)) and we recommend the use of REW both for other tree hydraulic studies at BIFoR FACE and looking at other underground forest processes where absolute VWC effects are not critical, though a sufficient long-term monitoring period should be considered to assess changing field capacity and wilt points through the treatment years. This is particularly important when considering other approaches which might be used. Methods such as cross-correlation (e.g. for examining temporal

relationships among soil moisture at different depths (Gao *et al.*, 2019) rely on availability of continuous datasets of at least half a year. These methods have also not previously been rigorously tested on forested landscapes which present highly complex heterogeneous profiles, but rather in agricultural environments. Partial least squares regression (PLSR) has also been used to link soil moisture profile dynamics and environmental indices such as precipitation using in-situ data across a forested catchment, but again these techniques rely on reliable multipoint profile data (in this case measured monthly) which were not available during the whole of our core experimental period (2018-2022). Another method which might be used involves depth comparisons using available water content (AWC) at each depth per treatment season month so that a finer analysis of water-usage by fine roots at each depth could be shown as a proportion of the whole AWC.

4.3 Planned future work

4.3.1 Using soil and root analyses to inform REW treatment comparisons.

Bader *et al.*, (2009), used soil-sampling and root ingrowth to examine shallow (< 18 cm depth) roots of three dominant hardwood species including *Q. petraea*. during seasons 5 to 7 of WEB-FACE. They found less fine root (< 1mm diameter) biomass of c. -30% under eCO₂ in season 5 soil-samples for combined species' live roots. In season 7 after two years' ingrowth, new fine roots similar reductions in fine root biomass under eCO₂ were found. Ziegler, (2023) chose not to use ingrowth methods due to the complexity of the BIFoR FACE forest.

Handy *et al.*, (no date) in their analysis of BIFoR FACE deep soil-samples' show greater (c. + 41%) fine root (< 1mm diameter) biomass under eCO₂ down to 50cm depth (Table S6). However, correspondence with Handy confirmed that total

cumulative fine root amount down to 1m depth was similar in seasons 5 and 7 (2021, 2023), whilst under eCO₂ fine root proportion was biased to shallower (<40cm depths) in 2023 compared with 2021. We used these unpublished findings from our dominant oak forest to inform the interpretation of our soil-water profile data.

The use of in-situ calibrated VWC data may, in future, extend the usefulness of such VWC and REW data when considering the effects of absolute soil-moisture on underground processes (e.g. microbiota).

4.3.2 Using statistical and process models.

Following preparation of the REW datasets, we prepared datasets for use in statistical (e.g. LMMs) or process models as part of the continuing BIFoR FACE publications sequence. The correlative plots reported from these data (Fig.4-12) imply some interesting trends, but more focused analysis will be required using the models.

The REW data produced during this study could form a part of a simple process model which can be constructed in future work. An example model diagram has been prepared in the supplementary material (Fig. S10) to illustrate this approach. Previous models (e.g. (Stevenson *et al.*, 2023; Birkel *et al.*, 2024)) are mainly one-dimensional and do not always fully account for environmental drivers key to understanding plant-water. Extensions to these models to account for basic tree physical traits such as stem and canopy size are expected to improve validity.

4.3.3 Soil Temperature

Whilst Kourmouli, (2022), reported on soil surface temperature (in respect to soil respiration seasons 1 to 3) at BIFoR FACE, here we have collected profile soil-temperatures to 60cm depth. A useful future analysis might consider profile soil-

2090 temperatures relative to soil-surface and Met-tower-bottom air temperature enabling comparison between arrays and treatments. This might show differences pertinent to this experiment and in comparison with other FACE experiments (e.g. with the results from season 7 of WEB-FACE (Bader, M. K. F., & Körner, 2010)).

4.4 Conclusion

2095 In conclusion, this study, which aimed to fill knowledge gaps in the field of plant-water soil-water relations for temperate forests experiencing changes to atmospheric CO₂ has demonstrated some findings of interest in confirmation of two research questions. Use of a normalised parameter REW on good-quality data enabled statistically significant differences to be examined for the two infrastructure treatments at shallow
2100 depths. Qualitative soil-water dynamic tendencies during summer drought were also examined using shallow VWC. In future, by obtaining more reliable profile REW data, it may be possible to establish a mechanistic relationship between daily tree-water (TWU_n) and soil-water profile dynamics. Use of a combination of data from diverse transducer technologies on an equitable basis improved confidence in quality of data
2105 within this heterogeneous forest-soil environment without reliance on multiple in-situ calibrations. We have confidence that this approach can be developed further using simple models both within CO₂ temperate mature-forests manipulation experiments and in other long-term monitoring contexts.

5 Author contribution:

2110 SEQ designed and carried out this soil-water versus tree-water study and prepared the manuscript as part of the FACE programme designed by ARM. SEQ installed sap instrumentation, soil-temperature profile sensors and two types of soil-water transducer (CS and Enviroscan) following on from the start-up project (MacKenzie *et*

al., 2021). SQ programmed the data loggers for multiple data collection during 2017-
2115 2018, curated, visualised and analysed the sap and soil data and physical tree data.
GC and FT curated the reference and on-site weather data, as well as all core soil
data. RG created the Array diagrams. ARM and SK supervised the project.

6 Acknowledgements

All authors acknowledge support from the Birmingham Institute of Forest Research.
2120 BIFoR FACE facility is a research infrastructure project supported by the JABBS
Foundation and the University of Birmingham. ARM gratefully acknowledges support
from NERC (grant nos. NE/S015833/1 and NE/S002189/1). SEQ acknowledges
BIFoR FACE Operations team's contribution to logger and instrumentation
implementation, data visualisation and data curation. Laboratories and workshops
2125 were provided at BIFoR FACE. Shawbury historical precipitation data provided by the
National Meteorological Library and Archive – Met Office, UK.

References Chapter 4

- Anderegg, W. R. L. et al. (2015) 'Pervasive drought legacies in forest ecosystems and their implications for carbon cycle models', *Science*. American Association for the Advancement of Science, 349(6247), pp. 528–532. doi: 10.1126/science.aab1833.
- 2130
- Bachofen, C. et al. (2023) 'Stand structure of Central European forests matters more than climate for transpiration sensitivity to VPD', *Journal of Applied Ecology*. John Wiley & Sons, Ltd, 60(5), pp. 886–897. doi: <https://doi.org/10.1111/1365-2664.14383>.
- Bachofen, C. et al. (2024) 'Tree water uptake patterns across the globe', *New Phytologist*. John Wiley & Sons, Ltd, 242(5), pp. 1891–1910. doi: <https://doi.org/10.1111/nph.19762>.
- 2135
- Bader, M. K. F., & Körner, C. (2010) 'No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO₂ enrichment', *Global Change Biology*. John Wiley & Sons, Ltd, 16(10), pp. 2830–2843. doi: <https://doi.org/10.1111/j.1365-2486.2010.02159.x>.
- 2140
- Bader, M., Hiltbrunner, E. and Körner, C. (2009) 'Fine root responses of mature deciduous forest trees to free air carbon dioxide enrichment (FACE)', *Functional Ecology*. John Wiley & Sons, Ltd, 23(5), pp. 913–921. doi: <https://doi.org/10.1111/j.1365-2435.2009.01574.x>.
- 2145
- Baert, A. et al. (2015) 'Variable hydraulic resistances and their impact on plant drought response modelling', *Tree Physiology*, 35(4). doi: 10.1093/treephys/tpu078.
- Barataud, F. et al. (1995) 'Soil water dynamics in an oak stand. II. A model of the soil-root network compared with experimental data', *Plant and Soil*, 172, pp. 29–43. doi: 10.1007/BF00020857.

- 2150 Birkel, C. et al. (2024) 'Importance of measured transpiration fluxes for modelled ecohydrological partitioning in a tropical agroforestry system', *Agricultural and Forest Meteorology*, 346, p. 109870. doi: <https://doi.org/10.1016/j.agrformet.2023.109870>.
- Black, T. A. (1979) 'Evapotranspiration from Douglas fir stands exposed to soil water deficits', *Water Resources Research*. John Wiley & Sons, Ltd, 15(1), pp. 164–170. doi: <https://doi.org/10.1029/WR015i001p00164>.
- 2155
- Blaen, P. J. et al. (2017) 'High-frequency monitoring of catchment nutrient exports reveals highly variable storm event responses and dynamic source zone activation', *Journal of Geophysical Research: Biogeosciences*. John Wiley & Sons, Ltd, 122(9), pp. 2265–2281. doi: <https://doi.org/10.1002/2017JG003904>.
- 2160 Bréda, N. et al. (1995) 'Soil water dynamics in an oak stand: I. Soil moisture, water potentials and water uptake by roots', *Plant and Soil*. Springer, 172(1), pp. 17–27. Available at: <http://www.jstor.org/stable/42947459>.
- Bréda, N. et al. (2006) 'Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences', *Ann. For. Sci.*, 63(6), pp. 625–644. Available at: <https://doi.org/10.1051/forest:2006042>.
- 2165
- Buckley, T. N. (2019) 'How do stomata respond to water status?', *New Phytologist*. John Wiley & Sons, Ltd, 224(1), pp. 21–36. doi: <https://doi.org/10.1111/nph.15899>.
- Fares, A. and Alva, A. K. (2000) 'Soil Water Components Based on Capacitance Probes in a Sandy Soil', *Soil Science Society of America Journal*. John Wiley & Sons, Ltd, 64(1), pp. 311–318. doi: <https://doi.org/10.2136/sssaj2000.641311x>.
- 2170

De Frenne, P. et al. (2021) 'Forest microclimates and climate change: Importance, drivers and future research agenda', *Global Change Biology*. John Wiley & Sons, Ltd, 27(11), pp. 2279–2297. doi: <https://doi.org/10.1111/gcb.15569>.

- 2175 Gao, X. et al. (2019) 'Testing of observation operators designed to estimate profile soil moisture from surface measurements', *Hydrological Processes*. John Wiley & Sons, Ltd, 33(4), pp. 575–584. doi: <https://doi.org/10.1002/hyp.13344>.

Handy, G. et al. (no date) 'Changed Fine Root Biomass and Morphology in a Mature Temperate Forest Under Elevated CO₂', (in draft).

- 2180 Hanson, P. J. and Walker, A. P. (2020) 'Advancing global change biology through experimental manipulations: Where have we been and where might we go?', *Global Change Biology*. John Wiley & Sons, Ltd (10.1111), 26(1), pp. 287–299. doi: [10.1111/gcb.14894](https://doi.org/10.1111/gcb.14894).

- Hart, K. M. et al. (2020) 'Characteristics of free air carbon dioxide enrichment of a northern temperate mature forest', *Global Change Biology*, 26(2), pp. 1023–1037. doi: [10.1111/gcb.14786](https://doi.org/10.1111/gcb.14786).

Huang, X. et al. (2016) 'Soil moisture dynamics within soil profiles and associated environmental controls', *CATENA*, 136, pp. 189–196. doi: <https://doi.org/10.1016/j.catena.2015.01.014>.

- 2190 IPCC (2023) 'Technical Summary', in *Intergovernmental Panel on Climate Change (IPCC) (ed.) Climate Change 2021 – The Physical Science Basis: Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press, pp. 35–144. doi: DOI: [10.1017/9781009157896.002](https://doi.org/10.1017/9781009157896.002).

- 2195 Iqbal, S. et al. (2021) 'Interannual variation in sap flow response in three xeric shrub species to periodic drought', *Agricultural and Forest Meteorology*, 297, p. 108276. doi: <https://doi.org/10.1016/j.agrformet.2020.108276>.
- Köcher, P., Horna, V. and Leuschner, C. (2013) 'Stem water storage in five coexisting temperate broad-leaved tree species: significance, temporal dynamics and
2200 dependence on tree functional traits', *Tree Physiology*, 33(8), pp. 817–832. doi: 10.1093/treephys/tpt055.
- Kourmouli, A. (2022) Soil respiration and nutrient dynamics at a mature temperate woodland - the BIFoR FACE experiment. University of Birmingham. Available at: <https://etheses.bham.ac.uk/id/eprint/11835/>.
- 2205 Landsberg, J., Waring, R. and Ryan, M. (2017) 'Water relations in tree physiology: where to from here?', *Tree Physiology*, 37(1), pp. 18–32. doi: 10.1093/treephys/tpw102.
- Lavergne, A. et al. (2020) 'Impacts of soil water stress on the acclimated stomatal limitation of photosynthesis: Insights from stable carbon isotope data', *Global Change
2210 Biology*. John Wiley & Sons, Ltd, 26(12)(7158–7172). doi: <https://doi.org/10.1111/gcb.15364>.
- Leuzinger, S. and Körner, C. (2007) 'Water savings in mature deciduous forest trees under elevated CO₂', *Global Change Biology*. John Wiley & Sons, Ltd, 13(12), pp. 2498–2508. doi: 10.1111/j.1365-2486.2007.01467.x.
- 2215 MacKenzie, A. R. et al. (2021) 'BIFoR FACE: Water–soil–vegetation–atmosphere data from a temperate deciduous forest catchment, including under elevated CO₂',

Hydrological Processes. John Wiley & Sons, Ltd, 35(3), p. e14096. doi: 10.1002/hyp.14096.

2220 MacKenzie, R. et al. (2021) 'BIFoR FACE: Water-soil-vegetation-atmosphere research in a temperate deciduous forest catchment, including under elevated CO₂', Hydrological Processes, pp. 1–9.

McGill, R., Tukey, J. W. and Larsen, W. A. (1978) 'Variations of Box Plots', The American Statistician. [American Statistical Association, Taylor & Francis, Ltd.], 32(1), pp. 12–16. doi: 10.2307/2683468.

2225 Mishra, V. et al. (2020) 'Performance evaluation of soil moisture profile estimation through entropy-based and exponential filter models', Hydrological Sciences Journal. Taylor & Francis, 65(6), pp. 1036–1048. doi: 10.1080/02626667.2020.1730846.

2230 Nehemy, M. F. et al. (2021) 'Tree water deficit and dynamic source water partitioning', Hydrological Processes. John Wiley & Sons, Ltd, 35(1), p. e14004. doi: <https://doi.org/10.1002/hyp.14004>.

Norby, R. J. et al. (2016) 'Model-data synthesis for the next generation of forest free-air CO₂ enrichment (FACE) experiments', New Phytologist, 209(1), pp. 17–28. doi: 10.1111/nph.13593.

2235 Oishi, A. C. et al. (2013) 'Spatial and temporal variability of soil CO₂ efflux in three proximate temperate forest ecosystems', Agricultural and Forest Meteorology, 171–172, pp. 256–269. doi: <https://doi.org/10.1016/j.agrformet.2012.12.007>.

Oishi, A. C. et al. (2014) 'Sustained effects of atmospheric [CO₂] and nitrogen availability on forest soil CO₂ efflux', *Global Change Biology*. John Wiley & Sons, Ltd, 20(4), pp. 1146–1160. doi: <https://doi.org/10.1111/gcb.12414>.

- 2240 Orlowski, N. et al. (2023) 'Challenges in studying water fluxes within the soil-plant-atmosphere continuum: A tracer-based perspective on pathways to progress', *Science of The Total Environment*, 881, p. 163510. doi: <https://doi.org/10.1016/j.scitotenv.2023.163510>.

- Pedersen, B. S. (1999) 'THE MORTALITY OF MIDWESTERN OVERSTORY OAKS
2245 AS A BIOINDICATOR OF ENVIRONMENTAL STRESS', *Ecological Applications*. John Wiley & Sons, Ltd, 9(3), pp. 1017–1027. doi: [https://doi.org/10.1890/1051-0761\(1999\)009\[1017:TMOMOO\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1999)009[1017:TMOMOO]2.0.CO;2).

- Perkins, D. et al. (2018) 'Impact of Climate Trends and Drought Events on the Growth of Oaks (*Quercus robur* L. and *Quercus petraea* (Matt.) Liebl.) within and beyond Their
2250 Natural Range', *Forests*, 9(3), p. 108. doi: 10.3390/f9030108.

Posit Team (2024) 'RStudio: Integrated Development Environment for R'. Boston, MA: R. Posit Software, PBC. Available at: <http://www.posit.co/>.

Quick, S. E. (2025) *Tree-soil-water relations under elevated CO₂ – following the flow*. University of Birmingham. doi: tba.

- 2255 Quick, S. E. et al. (2025) 'Water usage of old-growth oak at elevated CO₂ in the FACE (Free-Air CO₂ Enrichment) of climate change', *Biogeosciences*. Copernicus Publications Göttingen, Germany, 22(6), pp. 1557–1581. doi: 10.5194/bg-22-1557-2025.

R Core Team (2024) 'R version 4.4.1'. Vienna, Austria: R Foundation for Statistical
2260 Computing. Available at: <https://www.r-project.org/>.

Reich, P. B. et al. (2018) 'Effects of climate warming on photosynthesis in boreal tree species depend on soil moisture', *Nature*. Springer US, 562(7726), pp. 263–267. doi: 10.1038/s41586-018-0582-4.

Renninger, H. J. et al. (2014) 'Physiological strategies of co-occurring oaks in a water-
2265 and nutrient-limited ecosystem', *Tree Physiology*, 34(2), pp. 159–173. doi: 10.1093/treephys/tpt122.

Robinson, J. L., Slater, L. D. and Schäfer, K. V. R. (2012) 'Evidence for spatial variability in hydraulic redistribution within an oak–pine forest from resistivity imaging', *Journal of Hydrology*, 430–431, pp. 69–79. doi:
2270 <https://doi.org/10.1016/j.jhydrol.2012.02.002>.

Salomón, R. L. et al. (2022) 'The 2018 European heatwave led to stem dehydration but not to consistent growth reductions in forests', *Nature Communications*, 13(1), p. 28. doi: 10.1038/s41467-021-27579-9.

Sánchez-Pérez, J. M. et al. (2008) 'Water uptake by trees in a riparian hardwood forest
2275 (Rhine floodplain, France)', *Hydrological Processes*, 22(3), pp. 366–375. doi: 10.1002/hyp.6604.

Saxton, K. E. and Rawls, W. J. (2006) 'Soil Water Characteristic Estimates by Texture and Organic Matter for Hydrologic Solutions', *Soil Science Society of America Journal*. John Wiley & Sons, Ltd, 70(5), pp. 1569–1578. doi:
2280 <https://doi.org/10.2136/sssaj2005.0117>.

Schäfer, Karina V R et al. (2002) 'Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO₂ concentration', *Global Change Biology*, 8(9), pp. 895–911.

2285 Schmidt, M. (2007) Canopy transpiration of beech forests in Northern Bavaria –
Structure and function in pure and mixed stands with oak at colline and montane sites.
Bayreuth ,.

Seneviratne, S. I. et al. (2010) 'Investigating soil moisture–climate interactions in a changing climate: A review', *Earth-Science Reviews*, 99(3), pp. 125–161. doi: 10.1016/j.earscirev.2010.02.004.

2290 Stagge, J. H. et al. (2017) 'Observed drought indices show increasing divergence
across Europe', *Scientific Reports*, 7(1), p. 14045. doi: 10.1038/s41598-017-14283-2.

Steger, D. N. et al. (2024) 'Site matters - Canopy conductance regulation in mature
temperate trees diverges at two sites with contrasting soil water availability',
Agricultural and Forest Meteorology, 345, p. 109850. doi:
2295 <https://doi.org/10.1016/j.agrformet.2023.109850>.

Stevenson, J. L. et al. (2023) 'Quantifying heterogeneity in ecohydrological partitioning
in urban green spaces through the integration of empirical and modelling approaches',
Environmental Monitoring and Assessment, 195(4), p. 468. doi: 10.1007/s10661-023-
11055-6.

2300 Teepe, R., Dilling, H. and Beese, F. (2003) 'Estimating water retention curves of forest
soils from soil texture and bulk density', *Journal of Plant Nutrition and Soil Science*.
John Wiley & Sons, Ltd, 166(1), pp. 111–119. doi:
<https://doi.org/10.1002/jpln.200390001>.

- Tor-ngern, P. et al. (2015) 'Increases in atmospheric CO₂ have little influence on transpiration of a temperate forest canopy', *New Phytologist*. John Wiley & Sons, Ltd, 2305 205(2), pp. 518–525. doi: <https://doi.org/10.1111/nph.13148>.
- Verstraeten, W. W., Veroustraete, F. and Feyen, J. (2008) 'Assessment of Evapotranspiration and Soil Moisture Content Across Different Scales of Observation', *Sensors*, 8(1), pp. 70–117. doi: 10.3390/s8010070.
- 2310 Vicca, S. et al. (2012) 'Urgent need for a common metric to make precipitation manipulation experiments comparable', *New Phytologist*. John Wiley & Sons, Ltd, 195(3), pp. 518–522. doi: <https://doi.org/10.1111/j.1469-8137.2012.04224.x>.
- Vitasse, Y. et al. (2019) 'Contrasting resistance and resilience to extreme drought and late spring frost in five major European tree species', *Global Change Biology*. John 2315 Wiley & Sons, Ltd, 25(11), pp. 3781–3792. doi: <https://doi.org/10.1111/gcb.14803>.
- Warren, J. M., Norby, R. J. and Wullschleger, S. D. (2011) 'Elevated CO₂ enhances leaf senescence during extreme drought in a temperate forest', *Tree Physiology*, 31(2), pp. 117–130. doi: 10.1093/treephys/tpr002.
- Whalley, W. R., Ober, E. S. and Jenkins, M. (2013) 'Measurement of the matrix 2320 potential of soil water in the rhizosphere', *Journal of Experimental Botany*, 64(13), pp. 3951–3963. doi: 10.1093/jxb/ert044.
- Wickham, H. (2016) 'ggplot2: Elegant Graphics for Data Analysis.' New York: Springer-Verlag .

- Wullschleger, S. D., Tschaplinski, T. J. and Norby, R. J. (2002) 'Plant water relations
2325 at elevated CO₂ - Implications for water-limited environments', *Plant, Cell and*
Environment, 25(2), pp. 319–331. doi: 10.1046/j.1365-3040.2002.00796.x.
- Zhang, L., Dawes, W. R. and Walker, G. R. (2001) 'Response of mean annual
evapotranspiration to vegetation changes at catchment scale', *Water Resources*
Research. John Wiley & Sons, Ltd, 37(3), pp. 701–708. doi: 10.1029/2000WR900325.
- 2330 Zhang, M. et al. (2017) 'A global review on hydrological responses to forest change
across multiple spatial scales: Importance of scale, climate, forest type and
hydrological regime', *Journal of Hydrology*. Elsevier, 546, pp. 44–59. doi:
10.1016/J.JHYDROL.2016.12.040.
- Ziegler, C. et al. (2023) 'Quantification and uncertainty of root growth stimulation by
2335 elevated CO₂ in a mature temperate deciduous forest', *Science of The Total*
Environment, 854, p. 158661. doi: <https://doi.org/10.1016/j.scitotenv.2022.158661>.
- Ziegler, C. (2023) Quantitative modelling of root growth and carbon allocation: bridging
theory and experiment. University of Birmingham.

Chapter 5 : Subdominant tree sap flow

- 2340 This chapter contains a skeleton draft data-based article formatted with a view to future publication. Data related to this chapter also inform the leaf analyses in Chapter 6.

Comparison of dominant and subdominant tree sap flux patterns at BIFoR FACE forest during CO₂ treatment seasons two to seven.

2345 Susan Quick^{1 2}, Stefan Krause^{1 2 3 4}, A. Robert MacKenzie^{1 2}

¹School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, B15 2TT, UK

²Birmingham Institute of Forest Research, University of Birmingham, Birmingham, B15 2TT, UK

2350 ³Laboratoire d'écologie des hydrosystèmes naturels et anthropisés (LEHNA), University Claude Bernard, Lyon1, Lyon, France

⁴Institute for Global Innovation, University of Birmingham, Birmingham, B15 2TT, UK

Correspondence to: (Susan Quick (SEQ616@alumni.bham.ac.uk), - proofs etc.

Keywords: sap flux, oak, sycamore, hawthorn, eCO₂, FACE experiment

2355 **Abstract.** o/s

1 Introduction

Different plant species respond differently to changing climate. Most tree species, including *Acer* and *Fagus*, adjust their stomatal response to heat and water stress (Oren *et al.*, 1999; Tang *et al.*, 2006; Bader *et al.*, 2022) as well as to atmospheric CO₂ increases by changing their photosynthetic mechanisms during carbon assimilation, affecting gas exchange at leaf level i.e. stomatal conductance and leaf transpiration (Barton *et al.*, 2012; Batke *et al.*, 2020; Gardner *et al.*, 2022, 2023). These isohydric tendencies are not universal with oak tending to anisohydric mechanisms.

2365 Large leaf area can bring benefits for photosynthesis but can increase susceptibility to leaf wilt and stomata closure for protection as the compromise of these genera's adaptation for heat and full sun. In contrast small leaf area provides greater frost protection. Leaf and canopy traits of trees conform to an economy of resource balance

(Givnish, 1986; Wright *et al.*, 2004). Isohydric tendencies of some genera imply a tendency to close stomata for self-protection during extremes of heat and/ or low soil moisture conditions, which reduces transpiration and may result in leaf temperature increase. Another factor in self-protection during heat might be leaf curl which reduces the surface area of larger leaves, though this is not so relevant for species adapted to northern climates, where smaller leaves (for deciduous trees such as hawthorn) or needles, waxes or scales (of evergreen trees) give the double benefit of resistance to leaf wilt and protection from frost.

Root depth patterns can also influence accessibility of essential water and nutrient supply (Givnish *et al.*, 1986) influencing responses to stress. Shallow root domination may increase immediate access to soil moisture following rainfall given suitable light and vapour pressure deficit (VPD) conditions, whilst deeper roots may protect against effects of surface soil moisture deficit. On a global basis the variation of above and below ground tree traits, alongside mean rooting depth, has been seen to vary with climatic zone (Tumber-Dávila *et al.*, 2022) as well as species (Thomas, 2000), but these studies do not characterize plant volumes above and belowground explicitly which are likely dependent on available plant water (Ch.4) or nitrogen levels (Thomas, 2000). Despite using water from the same soil, there is also evidence to show that different tree species in mixed forest exploit different depths of soil according to soil dryness (Smith *et al.*, 2013; Fan *et al.*, 2017; Bachofen *et al.*, 2024) and tolerate differing amount of soil water saturation and flooding (Sánchez-Pérez *et al.*, 2008).

With the climate emergency now progressing apace, atmospheric CO₂ levels continuing to rise, extreme weather patterns emerging and flood, drought and heatwaves reaching unprecedented extremes, there is an urgent need to share all

results from controlled climate and CO₂ experiments to aid efforts to mitigate the effects of this emergency. Here we supplement our findings concerning oak (*Quercus robur* L.) water usage under elevated CO₂ (eCO₂) (Ch.3) by focusing on sap flow data from monitoring of two subdominant species, sycamore (*Acer pseudoplatanus* L.) and hawthorn (*Crataegus monogyna*) within our Birmingham Institute of Forest Research (BIFoR) Free-air CO₂ enrichment (FACE) experiment in central England, UK, (Hart *et al.*, 2020). These two species were chosen as they present larger numbers of significant stem size (>10 cm DBH) for subdominant trees throughout the experimental arrays. At BIFoR FACE, sycamore (large leaves, shallow roots) presence as a subdominant tree may perform sub-optimally, whereas hawthorn (small leaves) may be better adapted to shade. Both species have isohydric tendencies. *Quercus* genus deciduous trees are not isohydric so do not use stomata as a protective measure until extreme (e.g. leaf wilt) conditions are met.

Spring and autumn phenology may have an influence on the ability of leaves to survive weather extremes as younger leaves are more vulnerable to frost. Early wilt or senescence may reduce within-season access to nutrients. In deciduous forest, some species such as oak respond primarily to prior years' autumn / winter chill in respect of their spring phenology (Roberts *et al.*, 2015; Tansey, Hadfield and Phillimore, 2017) and exhibit partial plasticity, tracking the optimum conditions each spring. Others (such as sycamore and bluebell) are predominantly driven by a combination of daylength and temperature balance (Fu *et al.*, 2019) and considered maladaptive during spring forcing. Preceding autumn warmth can also delay the onset of budburst (Roberts *et al.*, 2015). The opportunistic use of early spring light levels by subdominant hawthorn and sycamore species prior to dominant oak budburst mean they can utilise water and

nutrient resources early in the season. Whilst it is known that, under the dominant canopy, such trees may suffer from low light and energy levels during the summer season, there is less evidence concerning their stem sap flow and transpiration under these mid-canopy spring and autumn conditions.

2420 Collection of phenological empirical (individual tree) data can be labour intensive and often carried out by citizen scientists (e.g. Nature's Calendar). At our site we have phenocam monitoring enabling whole forest data to be collected (Delpierre *et al.*, 2024). Whilst this does have the advantage of identification of the band of responses (e.g. for first leaf, or autumn tint) across the experimental forested landscape this does
2425 not enable species-specific data to be collected and may miss events occurring beneath the canopy.

Previous research (e.g Herbst *et al.*, 2008; Tumber-Dávila *et al.*, 2022) has shown that temperate broadleaf forests containing differing dominant and understorey species have quite different responses to light levels across the leaf-on season. Responses to
2430 drought and heat have also been studied, especially under recent weather extremes (e.g. Bennett *et al.*, 2015; Martínez-Sancho *et al.*, 2022; Gauthey *et al.*, 2024).

In respect of eCO_2 each species has different traits which lead to specific preferred environmental preferences and differing response to eCO_2 , so comparative species' responses (e.g. from results within a single forest experiment) might be difficult to
2435 generalise for ecological, vegetation or climate modelling purposes, especially where there is a mixture of young and mature trees present (Norby *et al.*, 1999). The predictions may also be confounded by the variation of environmental factors such as MAT and MAP during the experimental period.

2440 Researchers (e.g. Valladares *et al.*, 2016; Kemppinen *et al.*, 2024) have found that understorey species experienced different environmental stress factors. under dominant species' canopies relative to the dominant species, Factors include air temperature, VPD, throughfall and light levels, affecting both growth and survival in high-canopy forest environments, but measurement limitations may mean detection of only a limited range of variation. Comparison of accumulative water usage of two
2445 forests in the south of the UK containing different dominant and subdominant species mixtures was undertaken by Herbst *et al.*, (2007). They found little net difference in total stand water usage despite considerable variation total by individual species of tree, suggesting a balancing effect between leaf area index (LAI), stomatal characteristics and species water usage. Other stressors affecting survival (e.g. insect defoliation,
2450 pollinator depletion) might differ for subdominant species (Roberts *et al.*, 2015; Valladares *et al.*, 2016) relative to dominant species under stress.

There are few studies concerning mature subdominant trees' responses to eCO₂ within manipulation experiments (e.g. Leuzinger and Körner, 2007). Carbon assimilation changes within FACE experiments provide more valuable pointers to the effects of
2455 climate change, with plant water usage taking a secondary place. By highlighting plant water effects of eCO₂, during the differing range of environmental and soil factors experienced by larger subdominant compared to dominant tree species, it is hoped that a more complete picture of both stressors and benefits to trees might be portrayed.

1.1 Aims and objectives of this research

2460 The main aim of this xylem sap flow extension work was to provide sap flux data for larger subdominant trees within arrays at BIFoR FACE experiment, enabling inter-

species and intra-species comparison water usage response to eCO_2 (e.g. comparison of subdominant tree species to dominant oak, comparison of sycamore to hawthorn) during spring (pre-budburst, flowering and early leaf) and during the eCO_2 treatment
2465 season. A secondary purpose might be (see Renninger *et al.*, 2014) derivation of leaf transpiration or hydraulic conductance from stem sap flux data.

By selecting and monitoring subdominant species within the FACE experimental arrays (eCO_2 , ambient infrastructure control (aCO_2) and no-infrastructure control (*Ghost*)), we generated new canopy-transpiration-related data, derived from the sap flux. We also
2470 aimed to compare species' whole tree and leaf-level transpiration results under the differing experimental treatments (see Chapter 6 which illustrates the potential for stem-based sap flux/ canopy transpiration and leaf-level transpiration to be compared).

We considered the following research questions. We expect subdominant tree transpiration to complement that of dominant trees in time (peak time of day, season
2475 of year, and over successive treatment seasons) and positioning, due to differences in shading, phenology (but see Wolkovich *et al.*, 2025), leaf size, stomatal behaviour and likely depth of soil-water exploitation:

Q1: How do subdominant species trees responses differ inter and intra specifically and to the response of dominant ring porous oak?

2480 Hypothesis 1: For diffuse-porous (sycamore) and hybrid-porous (hawthorn), tree size (both stem size and above ground total biomass) influences the intraspecific tree water needs on a daily and seasonal basis. There are set relationships between each species' patterns of total tree water usage (and/ or sap flux) and tree size (e.g. by height and DBH) enabling a normalisation factor to be applied before interspecies

2485 comparison. Subdominant sap flux is also less affected by the presence of
infrastructure than dominant oak due to tree height and size differences.

2 Methods

2.1 BIFoR FACE habitats and species

Further details of the BIFoR FACE experiment are described in Chapters 2, 3 and 4
2490 and FACE publications (e.g. Hart et al., 2020). Here we use subdominant species trees
from each treatment type (i.e. (*eCO₂*, *aCO₂* and *Ghost*) enabling measurement of sap
flux in sufficient numbers for each selected subdominant species (hawthorn and
sycamore).

A pre-build botanical survey (BIFoR., 2014) of the FACE forest site, which is
2495 designated as 'W10 *Quercus robur* – *Pteridium aquilinum* - *Rubus
fruticosus*' woodland, was undertaken in 2014. This resulted in selection of appropriate
habitat areas (predominantly type 1 and 3), shown in Fig. 5-1, in response to these
pre-build reports commissioned by University of Birmingham, with the aim of providing
comparable plant habitats in the experiment arrays. These habitat types, in the
2500 enclosed BIFoR FACE facility, are all semi-natural broadleaf forest, with the arrays
selected according to oak plantation areas (planted mid-19th century).

Before selecting larger subdominant trees for sap monitoring an ad hoc survey of
species spread across the infrastructure arrays was undertaken to determine which
species to focus on. Figure 5-2 shows the results of that survey with % frequency in
2505 each species separated into *eCO₂* Treatment (T) and all treatments (T+C, i.e. *eCO₂*,
aCO₂ and *Ghost*). As a result of this survey we initially selected sycamore, hawthorn
and hazel (*Corillus avellana*) as potential candidates for sap monitoring. We later found

during a more detailed survey that some elm (*Ulmus* sp.) tree species had been misidentified as hazel. Subsequently, due to manpower, time and equipment resource limitations (as well as access problems during the COVID_19 pandemic), we decided that it would not be practical to complete the monitoring of hazel stem sap flux within this project. During early seasons walkways were established and the outline boundaries of all the arrays (*eCO₂*, *aCO₂* and *Ghost*) were better defined. A second survey of such trees contained within these new boundaries was used to select oaks for other concurrent experiments (e.g. see Norby et al., 2024). Species' trees > 10cm DBH within the new boundaries have dendrometer bands fitted. A summary of large tree numbers is shown in Table 5-1.

2.2 Measurements overview for subdominant trees

In Chapter 3 we described the methods used for xylem sap flow monitoring of oak trees within the three manipulation type arrays. Chapter 5 describes the subdominant trees monitored and the two types of sap flow probeset lengths used.

Here in our comparison of subdominant trees to previous reported results from oak we note that hawthorn spring changes (budburst/ first -leaf March) will occur earlier in the year than sycamore (budburst/ first -leaf March/ April) and oak (budburst/ first -leaf April/ May as shown in Ch.3).

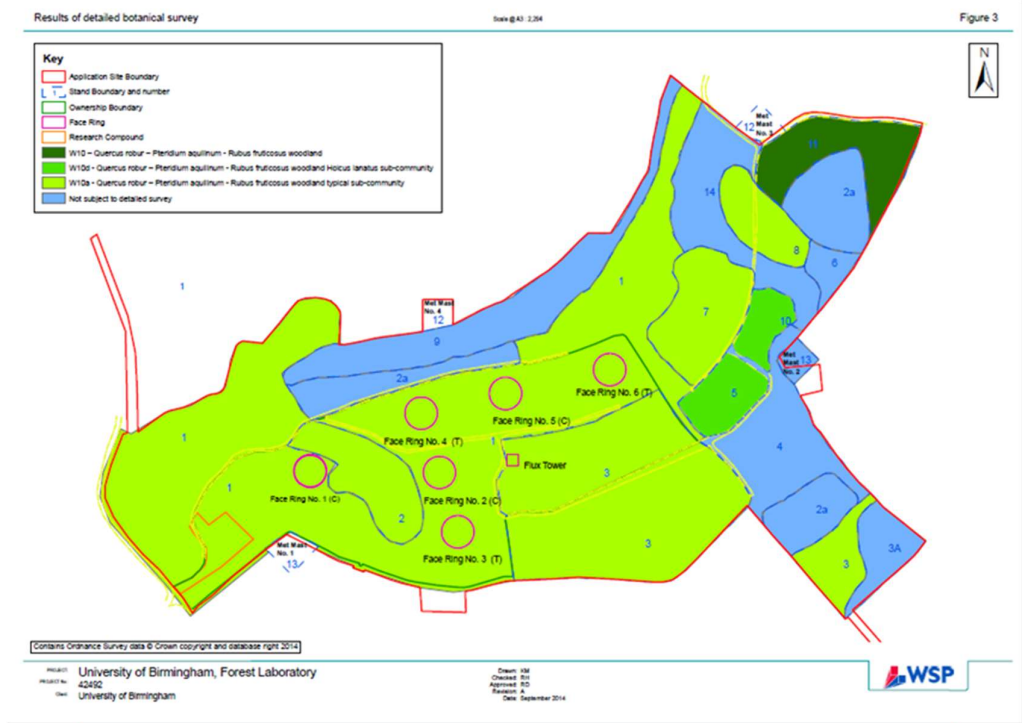


Table 2: Homogenous Stand Descriptions

Stand	Description	Further Survey Completed
1	Mature oak woodland with a dense hazel <i>Corylus avellana</i> dominated understorey, though containing extensive sycamore <i>Acer pseudoplatanus</i> or wych elm <i>Ulmus glabra</i> in localised areas. Shading by this shrub layer reduces ground layer cover.	Yes
2	Younger plantation oak with an open to largely absent understorey and a bramble <i>Rubus fruticosus</i> spp. field layer. Stands numbered 2a were not subject to further detailed survey as they are not due to be directly affected by the Proposed Development.	Yes
3	Mature oak woodland similar to Stand 1, though the hazel and sycamore has been coppiced, with stool re-growth extending to approximately 2m in most areas. A bramble dominated ground layer is present, with male fern <i>Dryopteris filix-mas</i> and bluebell <i>Hyacinthoides non-scripta</i> also locally extensive. Sycamore coppice is more mature in one area (3a) extending to approximately 6m and the ground layer cover consequently reduced beneath through shading.	Yes
4	Scot's pine <i>Pinus sylvestris</i> plantation with a recently coppiced sycamore understorey and bracken <i>Pteridium aquilinum</i> , bluebell and male fern ground layer.	No
5	An open young oak plantation with some pine and birch <i>Betula pendula</i> and a grass dominated ground layer.	Yes
6	Area of sycamore and sweet chestnut <i>Castanea sativa</i> plantation, including some very mature specimens. Bramble, bluebell and male fern understorey.	No
7	A young ash <i>Fraxinus excelsior</i> plantation with a bluebell and fern dominated ground layer.	Yes
8	A small area of plantation with a larch <i>Larix spp.</i> , sycamore and sweet chestnut canopy.	Yes
9	Semi-natural broadleaf woodland following the stream corridor in which alder <i>Alnus glutinosa</i> , hazel, willow <i>Salix sp.</i> and sycamore are more prevalent within mature oak woodland. The ground layer comprises nettle <i>Urtica dioica</i> , dog's mercury <i>Mercurialis perennis</i> and wood anemone <i>Cicuta urbanum</i> , with hogweed <i>Heracleum sphondylium</i> present along an embankment.	No
10	An open oak, ash and sitka spruce <i>Picea sitchensis</i> woodland, with a bluebell understorey.	
11	Dense young oak plantation with a bluebell, bramble, turf	

Figure 5- 1: Showing pre-build habitat survey results. Note array positions have been changed for build. The figure is based on Table 2 and Figure 2 from WSP Phase 1 Botanical survey A9RCB9B 2014 (BIFoR., 2014).

2530

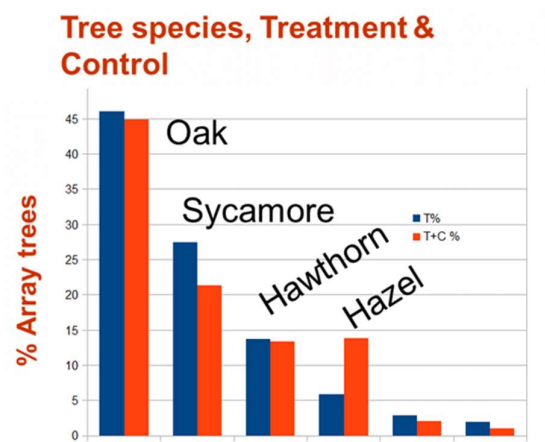


Figure 5- 2: Initial infrastructure array species survey results for larger trees > 10 cm DBH shown as proportion (%) of total large trees. T indicates all such trees in eCO₂ arrays. T + C indicates all such trees in infrastructure arrays. Note some mistaken species identity occurred for hazel trees during first survey.

2535 Subdominant species (with the exception of hazel) are not equally spread across the BIFoR FACE forest as they would be in a plantation, but rather self-regenerating on an ad hoc opportunistic basis. We report raw data from seven treatment seasons, May 2018 to end of October 2024 but have only fully processed sap flux data for selected (large probeset) data prior to this thesis being presented. Apart from the two failed
2540 installations in sycamore (one in Array 9 and one in Array 1) all installations worked continuously from commissioning date (starting for a few trees in 2018-2020) until end of 2024 season (when decommissioning was being planned). Table 5-2 summarises the raw and processed data available at end of 2024 treatment season.

We focus on diurnal (i.e. daylight) responses within our experimental treatment season
2545 April–October inclusive. Individual long probeset sap flux density datasets are processed for those four trees derived from HPC transducers. For all xylem sap flux monitored subdominant trees, tree identification, treatment type, array number, along with their stem circumference and average Rb, at probeset insertion point are shown in Table 5-3. Height was also measured (see Ch.6).

Species	Total number of eCO ₂ , aCO ₂ (infrastructure array) trees (A1-6) – Norby et al. (2024)	Total number of Ghost (no-infrastructure array) trees (A7-A9) 2024	All array trees	Average eCO ₂ , aCO ₂ DBH	Total eCO ₂ , aCO ₂ Biomass – Norby et al. (2024)
Oak	47 (55%)	19 (33%)		65.2	259778 (98%)
Sycamore	15 (18%)	30 (52%)		23.7	3579 (1.4%)
Hawthorn	20 (24%)	3 (5%)		16.2	2204 (0.8%)
Hazel	3 (4%)	5 (9%)		15.55	262(0.1%)
	85 (59% of total)	57(40% of total)	142		265,823

Table 5- 1: Species fitted with dendrometer bands (i.e. greater than 10cm DBH) sited within the new array boundaries. Numbers of trees (% of all species in brackets, average DBH, total biomass are shown as at summer 2023. Infrastructure figures are taken from Norby *et al.*, 2024.

Species	Number of stems, (type of probesets, long or short)	Raw data date range	Processed (Y or N)
Sycamore	3 (long)	May 2018 to present	Y
Hawthorn	1(long)	Mar 2020 to present	Y
Sycamore	4 (short)	Oct 2020 to present	N
Hawthorn	6 (short)	Oct 2020 to present	N

Table 5- 2: Raw and processed data as of January 2025. Note, one hawthorn tree is multi-stemmed with two stems monitored.

Tree ID	Array No.	Treatment	Species	Installation date	Probe type	Initial Circumference (m)	Initial Average R _b at probes et (m)	2022 Circumference (m)	2022 Average R _b at probes et (m)
8435A	1	eCO ₂	hawthorn	21/10/2020	short	0.372	0.059	0.364	0.058
8435B	1	eCO ₂	hawthorn	22/10/2020	short	0.368	0.059	0.369	0.059
9378	1	eCO ₂	sycamore	12/05/2021	short	0.325	0.052	0.358	0.057
8350	3	aCO ₂	hawthorn	14/04/2021	short	0.345	0.055	0.342	0.054
8352	3	aCO ₂	hawthorn	22/04/2021	short	0.56	0.089	0.57	0.091
9982	4	eCO ₂	hawthorn	18/03/2020	long	0.54	0.086	0.538	0.086
6638	4	eCO ₂	sycamore	03/11/2020	short	0.65	0.103	0.678	0.108
6385	5	aCO ₂	hawthorn	24/09/2020	short	0.528	0.084	0.548	0.087
6386	5	aCO ₂	hawthorn	24/09/2020	short	0.5	0.080	0.504	0.080
6381	5	aCO ₂	sycamore	27/10/2020	short	0.43	0.068	0.46	0.073
5848	6	eCO ₂	sycamore	31/05/2018	long	1.17	0.186	1.19	0.189
5847	6	eCO ₂	sycamore	12/05/2021	short	0.685	0.109	0.68	0.108
6026	7	none/ Ghost	sycamore	18/03/2020	long	0.442	0.070	0.463	0.074
7482	9	none/ Ghost	sycamore	09/07/2019	long	0.57	0.091	0.578	0.092

Table 5- 3: Subdominant trees selected for sap flow monitoring. Long probes have four thermocouples radially into the tree, short have two radially into the tree. Two probesets are fitted to each stem at East-facing and West-facing positions.

2560 2.3 Seasonal definitions

The definitions of seasons are contained within section 2.3 of Chapter 3. Phenological data collected for the two subdominant species is available on request. Table 5-4 shows sycamore leaf-season length, defined as first leaf to first leaf tint, in seasons 2017 to 2022.

Year	1 st leaf	1 st leaf-tint	Leaf-season length (i.e. 1 st leaf to 1 st leaf-tint	Longest (L) and shortest (S) seasons
2017	27 th March	4 th October	6mth 8days	
2018	5 th April	3 rd October	5mths 28days	S
2019	15 th March	24 th September	6mths 10days	
2020	11 th March	17 th September	6mths 7days	
2021	29 th March	28 th September	6mths 0days	
2022	18 th March	5 th October	6mths 18days	L

2565 Table 5- 4: Sycamore 1st leaf, 1st leaf-tint and calculated leaf-on season lengths for seasons 2017 to 2022 based on physical observations of first of such events across the experimental woodland.

2.4 FACE and meteorological measurements

These details are described in section 2.4 of Chs. 3, 4 and 6. Of particular interest for phenology are Ch.4 parameters (e.g. soil moisture/ temperature) and Ch.5 parameters
 2570 (e.g. air temperature, RH near to ground level, also key for transpiration).

2.5 Tree selection

The selection of subdominant trees is described in Section 2.3 of Ch. 6 (Tables 6-1 and 6-2). Table 6-2 is repeated here in part as Table 5-3. There were limitations on the available trees from which we could select due both to lack of uniform species spread
 2575 across the forest and the minimum stem size required for use of the smaller sap flow probesets.

Whilst all oaks were of similar height (circa 25 m), there was a spread of heights for the selected subdominant trees. One hawthorn tree selected for monitoring (in array 1) had multiple stems (natural coppice).

2580 **2.6 Xylem sap flux**

The xylem sap flux measurement method (in summary) and associated calculations (Table 5-4) used are repeated here from Ch.3 for convenience. At stage 4 woody matrix factor $c1$ varies by species (derived from wood cores) as described in Ch.2 Methods. At stage 5, heartwood boundary H is assumed at the centre of the stem for
2585 the subdominant diffuse or hybrid porous species. It is also noted that hawthorn presents a much more irregularly shaped tree stem than either sycamore or oak. Bark thickness is estimated to be 1 mm thick for sycamore and 2 mm thick for all hawthorn stems.

Each target subdominant tree had two probesets, East- and West-facing, using long
2590 (7 cm four-sensor) probes (for four larger trees) or shorter probesets with only two radial measurement points (4cm two-sensor positions at 5mm and 15mm) for smaller sub-dominant species installation (data from 2021 treatment season). The shorter probesets had cabling length to suits the distance between the tree and the array logger so did not need extension enclosures for the heater pulse equipment. Each
2595 probeset was inserted at a stem height between 1.1 and 1.3 m and contained a central heat pulse probe and two measurement probes upstream and downstream of the heater. Each probeset was protected from natural heating by reflective insulation covers during the treatment season.

During monitoring, a heater pulse of duration 1.5–2.5 secs was applied half-hourly through a heater box (one per tree) to the heater probes. The pulse duration was dependant on the number of heaters pulsed simultaneously. A two second pulse was standard for all tree configurations. Each thermocouple pair in the upstream and downstream positions took up to 330sec (5.5 minutes) to reach a differential heat balance point and this time determined the minimum detectable heat velocity, for a time just within this timeout period. The thermocouple datalogger sampling rate of 0.5 secs determined the maximum detectable speed (minimum time-to-balance), which, given normal interference levels, was adequate for deriving maximum heat velocity. Up to 16 differential thermocouple configurations were sampled per array in one 6 minute timeslot every 30 minutes, giving time-to-balance (t_z , secs) data. Up to three start times were available to accommodate oaks and subdominant trees. Pulsing and multiplexing of the sets of subdominant data was delayed for at least ten minutes after an oak set to ensure the heater battery had recovered.

Two sycamore tree sap installations (two long and two shorter probesets) failed, but no cause was found in either case despite a concerted fault-finding effort. The longer probesets from one sycamore tree were successfully redeployed in a different tree in early 2020.

2.7 Raw file processing

Logger data processing followed similar processes to those described in other chapters.

2.8 Xylem sap flux calculations

Following raw data quality checks, each stage of calculation to produce wound-corrected sap velocity and sap flux density at each transducer position (four or two per probeset) was performed in stages in the same sequence as described in Ch.3 (see Table A2 of Ch.3 publication) and Table 5-4 of this chapter). The methodology and equations were similar at each stage, but adjustments were made for the diffuse-porous nature and other xylem specific characteristics of the subdominant species. Sap flux data outliers may have potential for further analysis alongside studies of embolism so have currently been retained in the datasets.

At stage 3 (Table 5-4), the (Green and Clothier, 1988) Green and Clothier (1988) polynomial factors were used for wound compensation. For stage 4, the conversion factor c_1 was derived by measurement of wet and dry woodcores and microcores (Eq.(A4) and Eq.(A5) of Ch.3) to calculate xylem sap velocity from heat velocity in each species (see Appendix 2-C to Ch. 2).

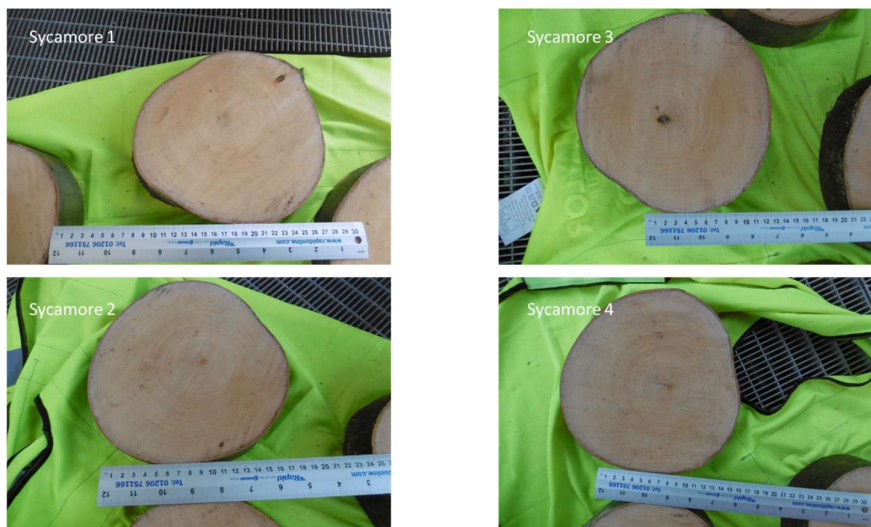


Figure 5- 3: Sycamore woodslices showing diffuse nature and asymmetry.

Short incremental wood cores (circa 10 cm long, 4 mm diameter) were taken from at least two trees per species (see Ch.2 Methods and Ch.3) outside of the experimental

Stage	Parameter	Relationship equation	References
Stage 1	t_z Time to heat balance point	At each position $z = 1$ to 4	Tranzflo Manual
Stage 2	V_s Raw heat velocity (uncompensated)	At each position $z = 1$ to 4 $V_s = \frac{(X_d + X_u)}{2t_z}$ (A1) For all probe lengths $X_d = 20$, $X_u = 5$ in mm $V_s = 12.5 / t_z$	Swanson, 1962 Tranzflo Manual
Stage 3	V_c Wound compensated heat velocity	At each position $z = 1$ to 4 $V_c = a + bV + cV^2 + dV^3$ (A2) Where V is V_s in $m\ s^{-1}$. Empirical parameters a , b , c and d are chosen for probe diameter 2 mm.	Green and Clothier, 1988
Stage 4	J_z Probeset (4 point) Sap flux density for each radius transducer position	$J_z = \{(0.505 F_M + F_L) V_{c,z}\}; z = 1:4$ (A3) Where J_z is the sap flux density at each position $z = 1$ to 4 Defining conversion factor $c1$ as $c1 = (0.505 F_M + F_L)$ (A4) gives $J = c1 V_{c,z}$ (A5)	Edwards and Warwick, 1984; Marshall, 1958
Stage 5	Q_p Probeset Volumetric Sap flux across sapwood	For each probeset $Q_p = \sum_{z=1}^{z=4} A_z J_z; p=E \text{ (east) or } W \text{ (west)}$ (A6) Area-weighted sum of sap flux density, where associated sapwood areas, A_z for $z = 1$ to 4 for long probes, is calculated from R , r_z , $r_{z+1}, \dots, r_{z+3}$ (radii) and H .	Hatton et al., 1990; Tranzflo Manual
Stage 6	Q_T Whole tree Sap flux	For each probeset at each sample time: $(Q_{p1} + Q_{p2})/2$ simplified model $Q_T = \frac{(Q_E + Q_W)}{2}$ (A7) Where E and W indicate east -facing and west-facing probes.	
Stage 7	TWU Tree diurnal water usage	$TWU = N \sum_{i=id_a}^{i=idusk} Q_{Ti}$ (A8) Where i is the 30-minute sample time of Q_{Ti} , N is conversion factor from per second (Q_T) to per diurnal day dawn to dusk	

Table 5- 5: Calculations stages 1 to 7 showing flow of data processing to obtain TWU from time to heat balance t_z from all differential HPC probeset thermocouple radial positions (from Quick *et al.*, 2025).

2640 arrays. Microcores were also taken from a few probeset-fitted trees near to installation positions. These cores were used to determine wood hydraulic properties (Marshall, 1958; Edwards and Warwick, 1984) for sap velocity and flux calculations (see stage 4, Table 5-4 and definitions in Ch.3 of this thesis). Some measurements

were also taken from felled stems of sycamore to verify estimates of heartwood

2645 radius (H (m) and bark thickness in the live trees were appropriate. (Photos in Fig.5-3).

2.9 Xylem sap flux density to sap flux calculations.

Stage 5 the calculations assume that there is minimal heartwood (i.e. H , m, is at the centre of the stem) in both subdominant species (sycamore and hawthorn).

2650 Calculations are performed for each probeset position separately as with oak monitoring, combining sap flux densities using the Hatton et al., (1990.) method.

Future calculations of short probeset data may use a different algorithm which is under development. We note (Fig. 5-4) that there is little change in the range of median and mean probeset sap flux and, as with oak, the outliers dominate the data.

2655 2.10 Data processing, visualization and analysis

Details are outlined in Ch.3.

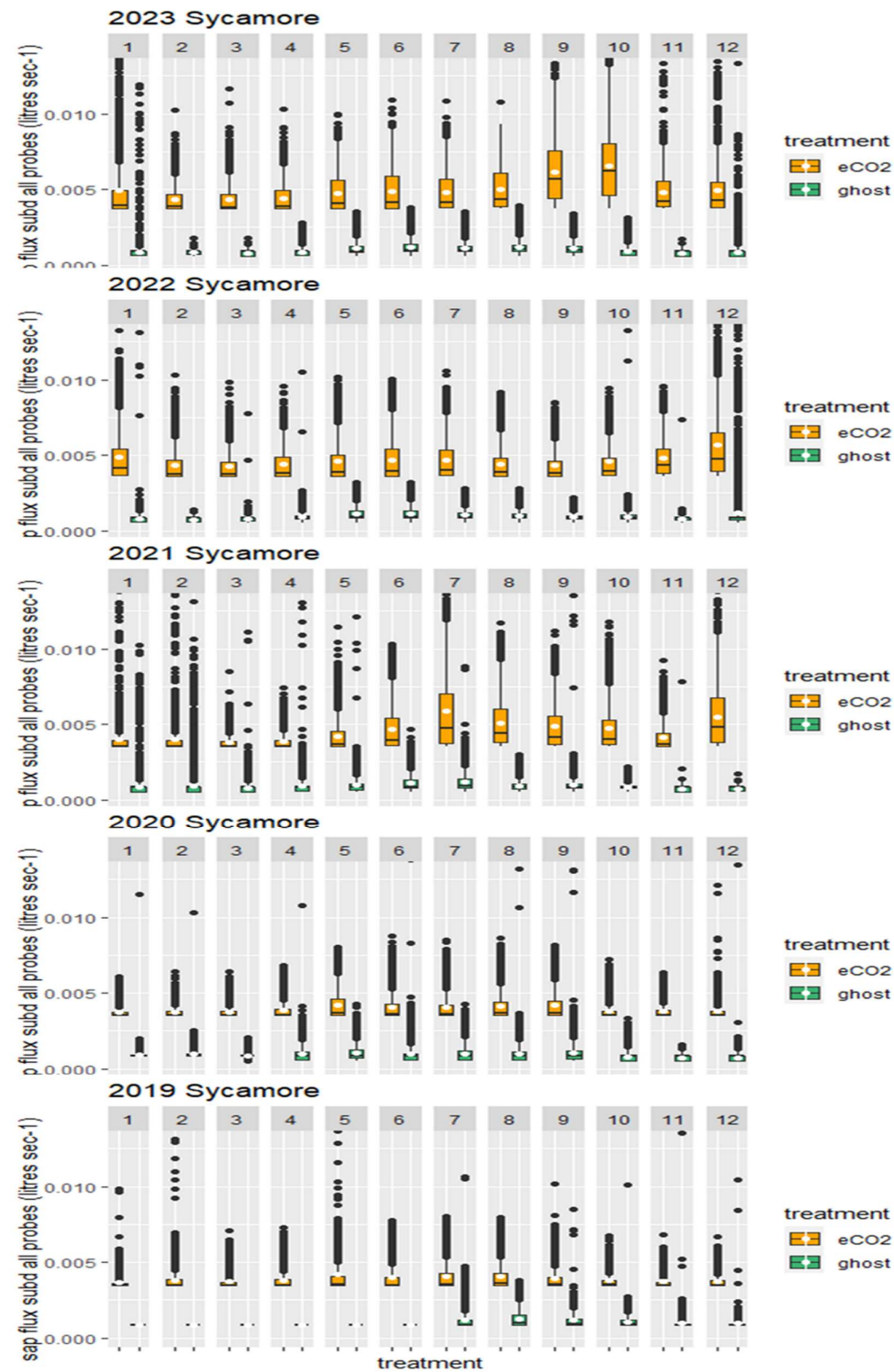


Figure 5- 4: Stage 6 output diurnal sap flux (l s^{-1}) in monthly periods across whole years for two sycamore trees with long probesets. Years 2019-2023 (including treatment seasons 3 to 7) are shown starting at the bottom with 2019 (season 3).

2660

3 Results

The results are not yet fully quantified as there are insufficient long probeset processed data. Here (Fig. 5-5) we illustrate the differing sap flux results from three sycamore and one hawthorn tree with long probesets prior to normalisation across July in years 2019 to 2021. We observe the differing maxima values of sap flux which will require normalisation by species and tree size.

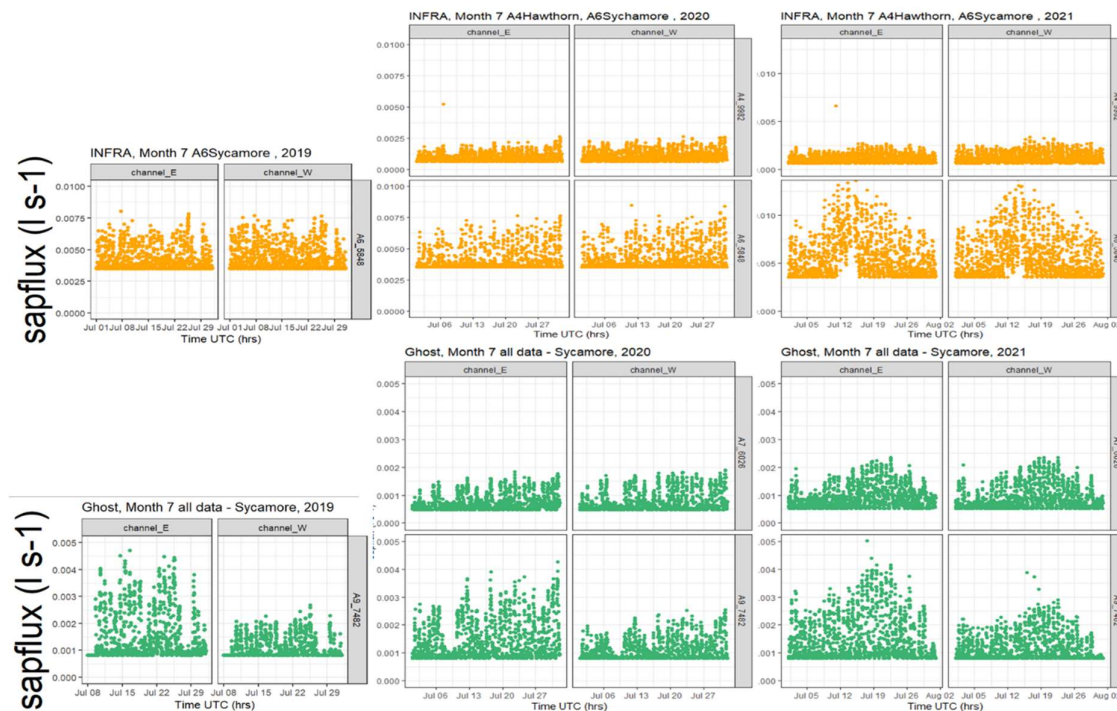


Figure 5- 5: Example sap flux results from individual (E-facing and W-facing) long probesets for three sycamore and one hawthorn tree during July in years 2019 to 2021. Top graphics (orange) are for eCO₂ tree probesets and bottom (green) are for aCO₂ tree probesets.

A second graphic (Fig. 5-6) illustrates all July daylight sap flux data for the same four trees for years 2019, 2020 and 2021 with all data superimposed across daytime hours.

It is expected that short probeset sycamore data will give results similar to those of the longer probesets as shown in Figs. 5-4 to 5-6 with differences between results from hawthorn and those shown for sycamore for a given stem size.

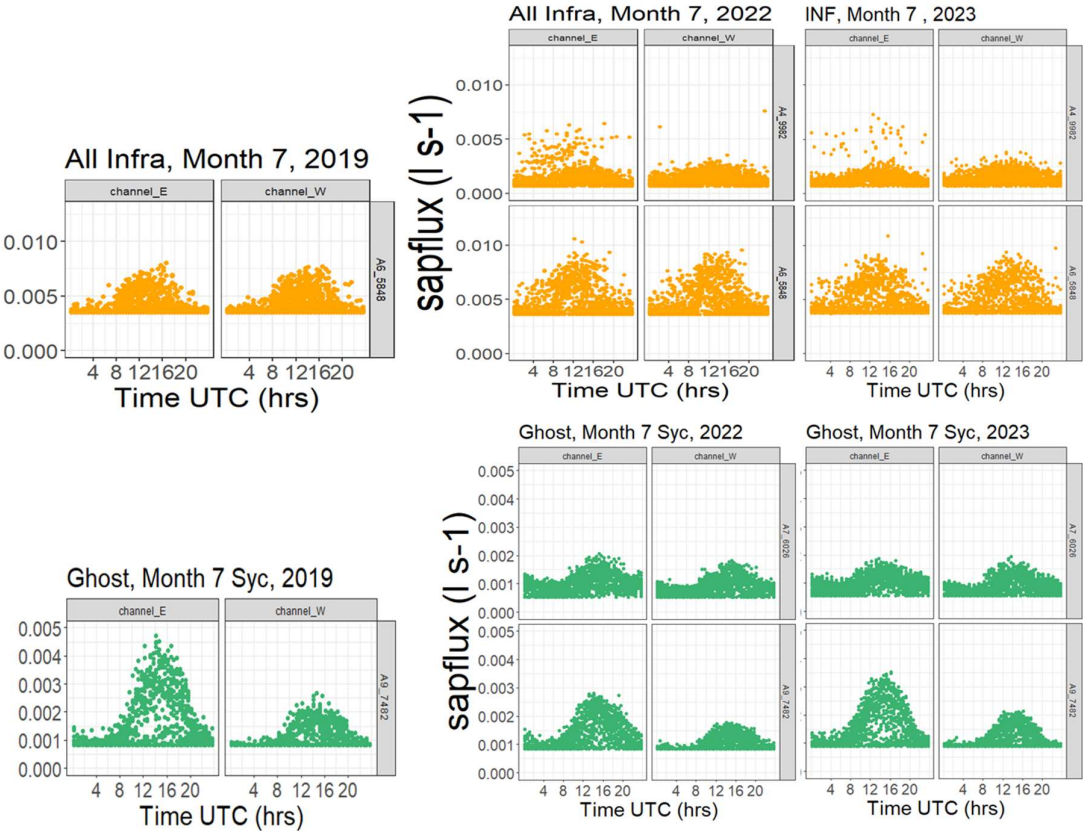


Figure 5- 6: Example sap flux results from individual (E-facing and W-facing) long probesets for three sycamore and one hawthorn tree (A4_9982) during July in years 2019, 2022 and 2023. Top graphics (orange) are for eCO₂ tree probesets and bottom (green) are for aCO₂ tree probesets.

Typical areas of analysis which might ensue with a larger dataset include:

- 2680
- Pre- budburst sap flux in dominant and subdominant species.
 - Sap flux within the leaf-on season only and between years.
 - Diurnal sap flux & water usage variation between tree species.
 - Intra- and inter- species normalisation approach.
 - Yearly and seasonal variation.

2685

4 Discussion

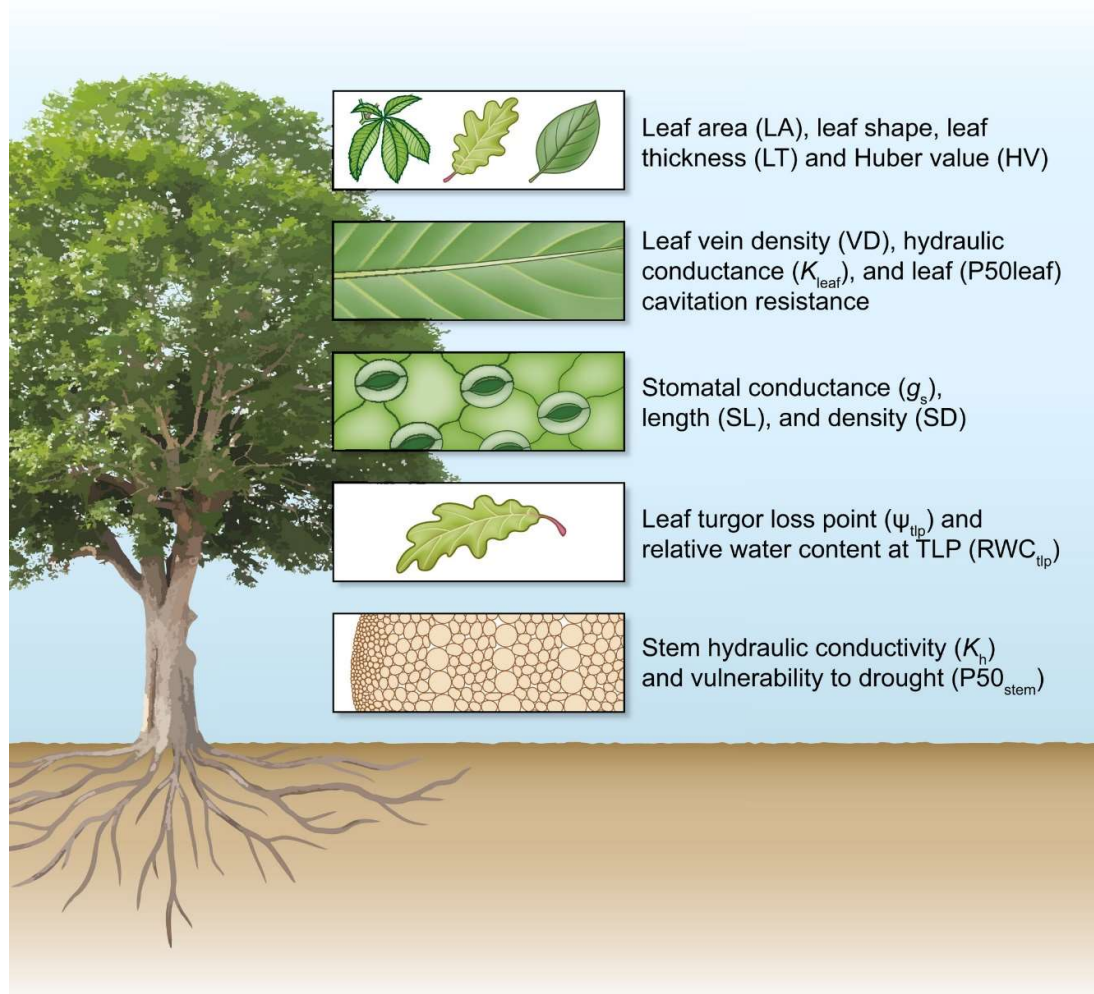


Figure 5- 7: (based on (Ávila-Lovera, Winter and Goldsmith, 2023), Fig. 2) Showing conceptual representation of the key plant–water relation traits typically analysed. Trait abbreviations: stomatal length (SL), stomatal density (SD), stomatal conductance (g_s), leaf hydraulic conductance (K_{leaf}), vein density (VD), leaf cavitation resistance ($P50_{leaf}$), leaf area (LA), leaf thickness (LT), leaf turgor loss point (Ψ_{tlp}), relative water content at turgor loss point (RWC_{tlp}), stem hydraulic conductivity (K_h), stem cavitation resistance ($P50_{stem}$), and Huber value (HV).

Interspecies comparisons of sap flux, tree water usage or canopy transpiration depend on selection of a suitable normalisation approach, for example using stem hydraulic conductivity (K_h) calculated from sap flux or leaf transpiration calculated from stomatal conductance (g_s) and upscaled to whole canopy transpiration. In the latter case additional data such as leaf area and wood density are required. This will differ from the intra-species normalisation I have used for oak (see Chapter 3) as absolute values

of water usage are probably only directly comparable (under different treatments) within species for same age trees. It seems likely that a single normalisation factor for stem sap flux will not resolve causation of differences between species' response to the experiment (Ávila-Lovera, Winter and Goldsmith, 2023 and Fig.5-6).

Data availability

Data will be made available in an open access database.

Acknowledgements

All authors acknowledge support from the Birmingham Institute of Forest Research. BIFoR FACE facility is a research infrastructure project supported by the JABBS Foundation and the University of Birmingham. ARM gratefully acknowledges support from NERC (grant nos. NE/S015833/1 and NE/S002189/1). SEQ acknowledges BIFoR FACE Operations team's contribution to facilitating access to arrays enabling me to install transducers. Also for maintenance of the data loggers and data collection systems across the site. Special thanks to Robert Grzesik for provision of regular updates to the dendrometer band and tree size information for oaks and larger subdominant trees. We gratefully acknowledge the Woodland Trust and the Centre for Ecology and Hydrology for enabling use of site phenology data collected 2016-2022 for submission to Nature's Calendar by SEQ as a citizen scientist.

References Chapter 5.

Websites

Nature's Calendar: <https://naturescalendar.woodlandtrust.org.uk/>.

2720 **Bibliography**

Ávila-Lovera, E., Winter, K. and Goldsmith, G. R. (2023) 'Evidence for phylogenetic signal and correlated evolution in plant–water relation traits', *New Phytologist*. John Wiley & Sons, Ltd, 237(2), pp. 392–407. doi: 10.1111/nph.18565.

2725 Bachofen, C. et al. (2024) 'Tree water uptake patterns across the globe', *New Phytologist*. John Wiley & Sons, Ltd, 242(5), pp. 1891–1910. doi: 10.1111/nph.19762.

Bader, M. K.-F. et al. (2022) 'Less pronounced drought responses in ring-porous than in diffuse-porous temperate tree species', *Agricultural and Forest Meteorology*, 327, p. 109184. doi: 10.1016/j.agrformet.2022.109184.

2730 Barton, C. V. M. et al. (2012) 'Effects of elevated atmospheric [CO₂] on instantaneous transpiration efficiency at leaf and canopy scales in *Eucalyptus saligna*', *Global Change Biology*. John Wiley & Sons, Ltd, 18(2), pp. 585–595. doi: 10.1111/j.1365-2486.2011.02526.x.

2735 Batke, S. P. et al. (2020) 'Plant responses to decadal scale increments in atmospheric CO₂ concentration: comparing two stomatal conductance sampling methods', *Planta*, 251(2), p. 52. doi: 10.1007/s00425-020-03343-z.

Bennett, A. C. et al. (2015) 'Larger trees suffer most during drought in forests worldwide', *Nature Plants*, 1(10), p. 15139. doi: 10.1038/nplants.2015.139.

BIFoR. (2014) WSP Phase 1 Botanical survey A9RCB9B 2014.

- 2740 Delpierre, N. et al. (2024) 'Phenology Across Scales: An Intercontinental Analysis of Leaf-Out Dates in Temperate Deciduous Tree Communities', *Global Ecology and Biogeography*. John Wiley & Sons, Ltd, 33(12), p. e13910. doi: 10.1111/geb.13910.
- 2745 Edwards, W. R. N. and Warwick, N. W. M. (1984) 'Transpiration from a kiwifruit vine as estimated by the heat pulse technique and the penman-monteith equation', *New Zealand Journal of Agricultural Research*, 27:4, pp. 537-543,. doi: 10.1080/00288233.1984.10418016.
- Fan, Y. et al. (2017) 'Hydrologic regulation of plant rooting depth', *Proceedings of the National Academy of Sciences of the United States of America*, 114(40), pp. 10572–10577. doi: 10.1073/pnas.1712381114.
- 2750 Fu, Y. H. et al. (2019) 'Daylength helps temperate deciduous trees to leaf-out at the optimal time', *Global Change Biology*. Wiley Online Library. doi: 10.1111/gcb.14633.
- Gardner, A. et al. (2022) 'Is photosynthetic enhancement sustained through three years of elevated CO₂ exposure in 175-year-old *Quercus robur*?', *Tree Physiology*, 42(1), pp. 130–144. doi: 10.1093/treephys/tpab090.
- 2755 Gardner, A. et al. (2023) 'Optimal stomatal theory predicts CO₂ responses of stomatal conductance in both gymnosperm and angiosperm trees', *New Phytologist*. John Wiley & Sons, Ltd, 237(4), pp. 1229–1241. doi: 10.1111/nph.18618.
- 2760 Gauthey, A. et al. (2024) 'High heat tolerance, evaporative cooling, and stomatal decoupling regulate canopy temperature and their safety margins in three European oak species', *Global Change Biology*. John Wiley & Sons, Ltd, 30(8), p. e17439. doi: 10.1111/gcb.17439.

Givnish, T. J. (ed. . (1986) *On the Economy of Plant Form and Function*. Cambridge/New York: Cambridge Univ. Press.

Green, S. R. and Clothier, B. E. (1988) 'Water use of kiwifruit vines and apple trees by the heat-pulse technique', *Journal of Experimental Botany*, 39(1), pp. 115–123. doi: 10.1093/jxb/39.1.115.

Hart, K. M. et al. (2020) 'Characteristics of free air carbon dioxide enrichment of a northern temperate mature forest', *Global Change Biology*, 26(2), pp. 1023–1037. doi: 10.1111/gcb.14786.

Hatton, T. J., Catchpole, E. A. and Vertessy, R. A. (1990) 'Integration of sapflow velocity to estimate plant water use', *Tree Physiology*, 6(2), pp. 201–209. doi: 10.1093/treephys/6.2.201.

Herbst, M. et al. (2007) 'Edge effects and forest water use: A field study in a mixed deciduous woodland', *Forest Ecology and Management*, 250(3), pp. 176–186. doi: 10.1016/j.foreco.2007.05.013.

Herbst, M. et al. (2008) 'Comparative measurements of transpiration and canopy conductance in two mixed deciduous woodlands differing in structure and species composition', *Tree Physiology*, 28(6), pp. 959–970. doi: 10.1093/treephys/28.6.959.

Kemppinen, J. et al. (2024) 'Microclimate, an important part of ecology and biogeography', *Global Ecology and Biogeography*. John Wiley & Sons, Ltd, n/a(n/a), p. e13834. doi: 10.1111/geb.13834.

Leuzinger, S. and Körner, C. (2007) 'Water savings in mature deciduous forest trees under elevated CO₂', *Global Change Biology*. John Wiley & Sons, Ltd, 13(12), pp. 2498–2508. doi: 10.1111/j.1365-2486.2007.01467.x.

2785 Marshall, D. C. (1958) 'Measurement of Sap Flow in Conifers by Heat Transport.', *Plant Physiology*, 33(6), pp. 385 LP – 396. doi: 10.1104/pp.33.6.385.

Martínez-Sancho, E. et al. (2022) 'Drought impacts on tree carbon sequestration and water use – evidence from intra-annual tree-ring characteristics', *New Phytologist*. John Wiley & Sons, Ltd, n/a(n/a). doi: 10.1111/nph.18224.

2790 Norby, R. J. et al. (1999) 'Tree responses to rising CO₂ in field experiments: implications for the future forest', *Plant, Cell & Environment*, (22), pp. 683–714.

Norby, R. J. et al. (2024) 'Enhanced woody biomass production in a mature temperate forest under elevated CO₂', *Nature Climate Change*. doi: 10.1038/s41558-024-02090-3.

2795 Oren, R. et al. (1999) 'Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit', *Plant, Cell & Environment*. John Wiley & Sons, Ltd, 22(12), pp. 1515–1526. doi: 10.1046/j.1365-3040.1999.00513.x.

2800 Quick, S. E. et al. (2025) 'Water usage of old-growth oak at elevated CO₂ in the FACE (Free-Air CO₂ Enrichment) of climate change', *Biogeosciences*. Copernicus Publications Göttingen, Germany, 22(6), pp. 1557–1581. doi: 10.5194/bg-22-1557-2025.

Renninger, H. J. et al. (2014) 'Physiological strategies of co-occurring oaks in a water- and nutrient-limited ecosystem', *Tree Physiology*, 34(2), pp. 159–173. doi: 10.1093/treephys/tpt122.

2805 Roberts, A. M. I. et al. (2015) 'Predicting a change in the order of spring phenology in temperate forests', *Global Change Biology*. doi: 10.1111/gcb.12896.

Sánchez-Pérez, J. M. et al. (2008) 'Water uptake by trees in a riparian hardwood forest (Rhine floodplain, France)', *Hydrological Processes*, 22(3), pp. 366–375. doi: 10.1002/hyp.6604.

2810 Smith, A. R. et al. (2013) 'Tree species diversity interacts with elevated CO₂ to induce a greater root system response', *Global Change Biology*, 19(1), pp. 217–228. doi: 10.1111/gcb.12039.

Tang, J. et al. (2006) 'Sap flux–upscaled canopy transpiration, stomatal conductance, and water use efficiency in an old growth forest in the Great Lakes region of the United States', *Journal of Geophysical Research: Biogeosciences*. John Wiley & Sons, Ltd, 2815 111(G2). doi: 10.1029/2005JG000083.

Tansey, C. J., Hadfield, J. D. and Phillimore, A. B. (2017) 'Estimating the ability of plants to plastically track temperature-mediated shifts in the spring phenological optimum', *Global Change Biology*. Wiley Online Library. doi: 10.1111/gcb.13624.

2820 Thomas, F. (2000) 'Vertical rooting patterns of mature *Quercus* trees growing on different soil types in northern Germany', *Plant Ecology*, 147, pp. 95–103. doi: 10.1023/A:1009841921261.

Tumber-Dávila, S. J. et al. (2022) 'Plant sizes and shapes above and belowground and their interactions with climate', *New Phytologist*. John Wiley & Sons, Ltd, 235(3), pp. 1032–1056. doi: 10.1111/nph.18031.

2825 Valladares, F. et al. (2016) 'Shedding light on shade: ecological perspectives of understorey plant life', *Plant Ecology & Diversity*. Taylor & Francis, 9(3), pp. 237–251. doi: 10.1080/17550874.2016.1210262.

Wolkovich, E. M. et al. (2025) 'Why longer seasons with climate change may not increase tree growth', *Nature Climate Change*, 15(12), pp. 1283–1292. doi: 2830 10.1038/s41558-025-02476-x.

Wright, I. J. et al. (2004) 'The worldwide leaf economics spectrum', *Nature*, 428(6985), pp. 821–827. doi: 10.1038/nature02403.

Chapter 6 : Leaf and stem: transpiration flow dynamics

- 2835 This chapter contains a draft article formatted with a view to future publication. Figures and Tables prefaced with 'S' appear in Appendix 5 (Supplement to this chapter).

Leaf and stem: transpiration dynamics of dominant oak and sub-dominant trees at BIFoR FACE experiment.

2840 Susan Quick^{1 2}, Stefan Krause^{1 2 3 4}, A. Robert MacKenzie^{1 2}

¹School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, B15 2TT, UK

²Birmingham Institute of Forest Research, University of Birmingham, Birmingham, B15 2TT, UK

2845 ³Laboratoire d'écologie des hydrosystèmes naturels et anthropisés (LEHNA), University Claude Bernard, Lyon1, Lyon, France

⁴Institute for Global Innovation, University of Birmingham, Birmingham, B15 2TT, UK

Correspondence to: (Susan Quick (SEQ616@student.bham.ac.uk), - proofs etc. only), A. Robert MacKenzie (A.R.Mackenzie@bham.ac.uk) official correspondence author

2850 **Keywords:** FACE, temperate, transpiration, oak, Quercus, sycamore, Acer, hawthorn, Crataegus, old growth, stomatal conductance, leaf temperature.

Abstract.

1 Introduction

Free-Air CO₂ Enrichment (FACE) forest experiments (Norby and Luo, 2004) play a key
2855 role in enabling predictions of vegetation responses to increasing atmospheric CO₂
levels (IPCC, 2023). Most tree species, including *Acer* and *Fagus*, adjust their stomatal
response to heat and water stress (Oren *et al.*, 2001; Tang *et al.*, 2006; Bader *et al.*,
2022) as well as to atmospheric CO₂ increases by changing their photosynthetic
mechanisms during carbon assimilation, affecting gas exchange at leaf level i.e.
2860 stomatal conductance and leaf transpiration (Barton *et al.*, 2012; Batke *et al.*, 2020;
Gardner *et al.*, 2022, 2023) demonstrating isohydric tendencies. Ring-porous trees
such as *Quercus robur* L. (oak) in contrast are more anisohydric (i.e. no apparent
protective closure of stomata except under extreme leaf heat stress (Gauthey *et al.*,
2024; Gardner *et al.*, 2025)). Trees may also adjust stomatal density and stomatal pore
2865 size (Xu *et al.*, 2016; Batke *et al.*, 2020) as a part of these co-coordinated responses
(Haworth, Elliott-Kingston and McElwain, 2013). Stomatal conductance changes are
particularly noticeable during dry periods (Ainsworth & Rogers, 2007). Photosynthesis
and transpiration are driven by light energy levels, humidity and air temperature under
conditions of adequate soil moisture, but during soil drought (Warren, Norby and
2870 Wullschleger, 2011) tree responses change. To interpret leaf-level and canopy-level
responses to elevated CO₂ (eCO₂, ppmv) it is necessary to untangle these various
drivers. Collecting new field experimental data fills gaps, assists in vegetation model
verification and enables more accurate projection of future of ecosystem water use in
a changing global ecosystem environment. Alignment of climate change land surface
2875 models with observations can also (Paschalis *et al.*, 2024), improve this accuracy.
Equally process models (e.g. (Warren *et al.*, 2011; Wang, Guan and Simmons, 2016;

Birkel *et al.*, 2024)) can be improved by using canopy transpiration and leaf-level empirical data.

Various scales of water use efficiency (WUE) reflecting tree responses are defined:

2880 intrinsic (iWUE, leaf-level), inherent (tree and ecosystem scale) and instantaneous (forest scale). Transpiration changes were reported overall for mature deciduous tree species under eCO₂ in the Swiss FACE experiment (Bader *et al.*, 2013), with an average water usage reduction of 10–15%. Within these results mature deciduous members of the *Quercus* genus (e.g. *Quercus robur*, *Quercus petraea*) there were zero

2885 transpiration changes under eCO₂. Gimeno *et al.*, (2016) at EucFACE focused on stomatal conductances from leaf chamber measurements testing the unified stomatal optimization (USO) theory. Gardner *et al.*, (2023) extended the work on iWUE by undertaking a meta-analysis from different vegetation types' field experiments. Torgern *et al.*, (2015) found that leaf level responses in young plantations may not

2890 transfer to canopy-wide effects due to increasing leaf area index (LAI) compensating for reduced canopy conductance, hence leaving net canopy transpiration unaltered. Wullschleger *et al.*, (2002) emphasised that soil–root and leaf–atmosphere responses must be integrated and scaled to predict eCO₂ effects, and Warren *et al.*, (2011a) predicted that forest site water balance will change under eCO₂ reducing relative

2895 humidity (*RH*, %) and enhancing vapour pressure deficit (*VPD*, kPa) when canopy transpiration reduces. In a synthesis of an open-topped chamber experiment findings, Barton *et al.*, (2012) explored both leaf and canopy scale transpiration effects using instantaneous transpiration efficiency (ITE) – the ratio of photosynthesis to transpiration – and noted the varied effects of VPD on this characteristic under elevated

2900 and ambient CO₂.

The subdominant species large-stemmed trees (e.g. *Acer*, *Crataegus* and *Corylus* species), present in small numbers at the Birmingham Institute of Forest Research (BIFoR) FACE experiment, have diffuse or hybrid porous xylems in contrast to the ring porous oak. These species are expected to respond to eCO_2 with stomatal conductance and leaf-transpiration reductions in mid-season (July – August) full leaf sunshine and heat but, being subdominant species, they experience greater shading than the dominant oak (Stokes, Morecroft and Morison, 2010), and a sub-canopy microclimate, affecting the responses. For these tree families (Aceraceae, Rosaceae, Betulaceae) there are fewer stomatal conductance or transpiration studies than for oak. Multispecies (e.g. (Morecroft and Roberts, 1999; Akhmatov, M. K., Salaš, 2005; Stokes, Morecroft and Morison, 2010; Renninger *et al.*, 2014; Yi *et al.*, 2017; Granda *et al.*, 2020; Fabiani *et al.*, 2022)), and *Acer* (Morecroft and Roberts, 1999; Wang *et al.*, 2020) mature tree studies have considered transpiration calculated from leaf or stem parameters across seasonal changes and differing daylength, but not in conjunction with eCO_2 .

Focusing on the role of water, some research (e.g. (Bernacchi and VanLoocke, 2015; Liang *et al.*, 2023)) highlights the problems of scale when modelling. One scale in time or space is often selected to determine transpiration from large trees within forest environments (e.g. (Aranda *et al.*, 2012)). The limitations and relationships of different temporal and spatial parameters have been widely described (Beer *et al.*, 2009; Donohue *et al.*, 2017; Gardner *et al.*, 2023 ; Guerrieri *et al.*, 2016; Hassler, Weiler and Blume, 2018; Renninger *et al.*, 2015) and averaging of parameters over differing timescales can lead to misleading comparisons (Murray, 1967; Wullschlegel *et al.*, 2002). Comparison between model and empirical results (Harley and Baldocchi, 1995;

- 2925 Leuning *et al.*, 1995; Oren and Pataki, 2001; Bader *et al.*, 2013; Monteith and
Unsworth, 2013; Renninger *et al.*, 2014; Ward *et al.*, 2018) can, if suitably defined,
inform future field study design and model improvements. Verification of data across
different spatial scales (e.g. stem, canopy, leaf), from differing soil types (e.g. mesic
versus xeric), or different species, remains problematic.
- 2930 Models which can mimic empirical results, or which link two or more scales are rare
(but see (Herbst *et al.*, 2008; Stokes, Morecroft and Morison, 2010; Ward *et al.*, 2018)).
It is recognised that there are mismatches of time and space scales between model
processes and empirical parametric data for carbon and water fluxes in forests (De
Kauwe *et al.*, 2013; Medlyn *et al.*, 2015; Norby *et al.*, 2016; Zhang *et al.*, 2017). Multiple
2935 parameter capture to populate models is costly and often available for limited periods
only. Longer-term studies enable examination of inter-seasonal and inter-year
characteristics but may collect parameters on an intermittent or limited basis. Despite
the use of manipulation experiment findings to improve models, interpretation of
responses within the natural environment can be challenging.
- 2940 Renninger *et al.*, (2014) have clarified pathways and triggers for differing sensitivity of
stomatal conductance to VPD drivers for *Quercus* species. They recommend careful
experimental design (e.g. to frequency of parameter measurement) to ensure inter-
scale comparisons can be made. Most air-to-leaf VPD values are calculated from
canopy (average) air temperatures (e.g. see (Shirke and Pathre, 2004)), which may be
2945 appropriate for tree-scale transpiration. Leaf abaxial temperatures give a more exact
leaf-level air-to-leaf VPD. Alternative whole-tree measures such as stem sap flux (e.g.
(Oren and Pataki, 2001; Herbst *et al.*, 2008; Schäfer, 2011; Quick, 2025)) can be used

as a proxy for canopy transpiration during low VPD when there are leaf-chamber measurement difficulties, but the ability to capture low plant-water flux (Forster, 2019) may be limited by logger sampling rate or sap flow transducer type.

Linking whole tree stem sap flux (as a proxy for canopy transpiration, Fig. 6-1) to leaf transpiration measurements under eCO₂ has benefits. For mature tree field experiments no simple continuous measurement of whole-tree carbon assimilation exists, meaning that interpretation of related WUE is subject to errors of dynamic variation at each scale and, measurement approach (e.g. (Keenan, Sabaté and Gracia, 2008; Bernacchi and VanLoocke, 2015; Guerrieri *et al.*, 2016; Lavergne *et al.*, 2019)). Differing water storage characteristics (Goldstein *et al.*, 1998; Meinzer, James and Goldstein, 2004; Keenan, Sabaté and Gracia, 2008; Schäfer, 2011) can be assessed by considering both sapwood area and leaf hydraulic traits during intra- or inter-species' individual tree- to leaf-transpiration data. Interspecific differences based solely on field measurements of single leaves may be questionable by failing to consider variation in the stem water storage capacity of different-size trees (Goldstein *et al.*, 1998), relying solely on species-specific leaf characteristics. The variation in microclimate from bottom-canopy to top-canopy may also affect stem-to-leaf water flow dynamics.

In exploring some of these issues here, we wish to determine whether findings concerning tropical species (Meinzer, James and Goldstein, 2004) and global changes to terrestrial ecosystems (described e.g. (Bernacchi and VanLoocke, 2015)) are replicated here within the BIFoR FACE CO₂ manipulation experiment.

1.1 Objectives and expected outcomes.

This study had two primary aims: (i) to determine whether the response of individual tree species to eCO₂ differs and (ii) to investigate plant-water relations across longer periods of monitoring at both leaf and canopy scales, enabling inter-species and canopy-to-leaf scale comparisons in three species with differing xylem anatomy: ring-porous oak, diffuse-porous sycamore, and semi-diffuse hawthorn.

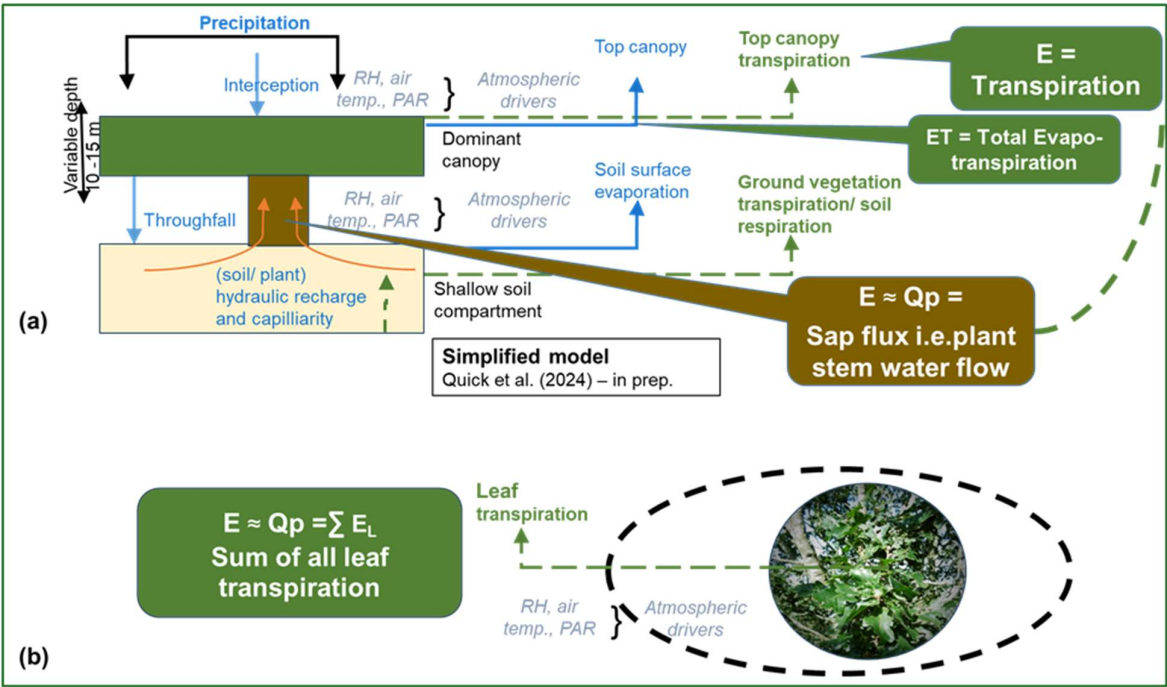


Figure 6- 1: Schematic showing a) an extract from a model of water flows to/ from the dominant canopy and b) detail of leaf transpiration contribution to the plant -water flow.

The following specific expected outcomes are addressed by the analyses in this paper:

- 1: We expect a detectable eCO₂ treatment effect on transpiration to be present at leaf level, varying by species monitored.
- 2: In oak peak values of daily leaf transpiration might increase with daylength to a summer maximum at midsummer's day and decrease again towards senescence.

Peak leaf transpiration at a given canopy height may be correlated positively with tree size, daylength, light levels and air temperature, and negatively with *RH* for all species.

3: Some measurable time delay might occur between the peak transpiration measured at leaf compared to stem sap flow. Delayed stem response relative to leaf could indicate use of stored stem water.

2 Materials and Methods

Leaf – atmospheric water vapour exchange (leaf-water flow) was monitored for a subset of oak (*Quercus robur* L.), sycamore (*Acer pseudoplatanus*) and hawthorn (*Crataegus monogyna*) trees present at the BIFoR FACE experiment during seasons 3 to 5 (2019-2021). Stomatal conductance and leaf temperature data were collected from both in-situ and cut-twig leaves under the three experimental conditions and used to calculate and compare within-day transpiration. Oak data were available for all three years, whilst subdominant species (sycamore and hawthorn) were monitored during 2020-2021 only. Oak leaf-level transpiration patterns were compared with concurrent whole-tree sap flux data (Quick *et al.*, 2025) to assess temporal variation and synchrony between leaf and whole-canopy transpiration.

2.1 BIFoR FACE experimental site and species

The BIFoR FACE experiment is located in a temperate deciduous forest in Staffordshire, England, UK (52.801° N, 2.301° W, altitude +108 ± 2.7 m a.s.l.) as described in Hart *et al.*, (2020). Forest slope and soil characteristics are detailed elsewhere (e.g. (Kourmouli *et al.*, 2025)). The climate is maritime, moist and temperate with a mean annual temperature of 10.3 °C and mean annual precipitation of 760 mm

over the 2019–2021 study period ((Quick *et al.*, 2025), Table S14). The forest is a circa 1840 plantation of oak with hazel (*Corylus avellana*) coppice. Naturally propagated subdominant sycamore and hawthorn trees varying in age up to circa 100 years, are sufficiently abundant to be included in this transpiration study alongside dominant oak trees.

The experimental platform consists of nine approximately circular 30 m diameter arrays under three FACE treatment conditions: Three no-infrastructure ambient-air (natural/*ghost*) arrays, three ambient-air infrastructure FACE arrays (*aCO*₂) and three elevated CO₂ (*eCO*₂) arrays (ambient + 150 ppmv CO₂). Infrastructure treatments (i.e. to *aCO*₂ and *eCO*₂ arrays) are applied during daylight hours throughout the leaf-on season (April to October).

2.2 Trees monitored

Target trees for leaf transpiration monitoring were selected from those previously instrumented for stem sap flow measurements (18 oak, eight sycamore - of which two failed installations - and seven hawthorn stems from six trees) (Quick, 2025, Ch.5 - sycamore & hawthorn, (Quick *et al.*, 2025) - oak). Tree ID's are listed in Table S1 (Supplement).

For subdominant species, the study was restricted to sycamore and hawthorn trees with a DBH >10 cm and height > 8 m. Only limited numbers of trees met these criteria.

Structural traits including tree stem radius (*R*_b, m), projected canopy area (*A*_c, m²) and height (TH, m) were measured for all monitored subdominant trees ranging as follows: *R*_b 0.05 to 0.19 m; *A*_c 12 to 121 m²; TH 8 to 17 m (Table S2). Equivalent structural traits for oak sap flow trees (all similar height) were provided from Quick *et al.*, (2025).

2.3 Leaf measurements

3030 The leaf data (for dominant old-growth oak and subdominant species) were collected
for daytime hours around midday, to compare between-individual, within-species
(intraspecies) and between-species (interspecies) variability under the three FACE
treatment conditions. Oak tree top-canopy leaves and subdominant bottom-canopy
leaves were measured enabling both within-season and inter-annual responses to be
3035 assessed.

Stomatal conductance and abaxial leaf temperature was measured using a Leaf
Porometer (Decagon Devices Inc., now METER, USA and Germany). Measurements
were taken either in-situ, where canopy or ladder access was possible, or, on top-
canopy cut-twig samples measured at ground level with stems kept hydrated by
3040 placing in deionised water. The porometer was calibrated daily against a standard (see
Decagon Manual) before measurements commenced. No measurements were taken
when leaves were wet or covered with dew, or when ambient air temperature was < 5
 $^{\circ}\text{C}$.

In-situ top-canopy leaves within infrastructure arrays were measured using a custom
3045 built canopy access system (CAS (Total Access Ltd, UK)) as described by Gardner et
al., (2022). Six oak trees and one sycamore (array 6) tree top-canopies were measured
during 2020-2021 but only four oaks in 2019. For subdominant trees, bottom-canopy
stomatal conductance was measured in-situ on east- and west- facing sprigs, using
ladders, deer platforms, or from the ground, with triplicate measurements taken on
3050 each leaf. Sprigs were labelled with coloured wool enabling repeat monthly
measurements. Epicormic leaves were avoided. At least three healthy leaves per tree

were selected, with triplicate measurements taken per leaf choosing different leaf areas to minimise leaf drying effects. Target sprigs were labelled with tape to ensure consistent measurement positions across monthly campaigns. Only one side of the top-canopy was accessible (mainly south-facing), but leaves of different orientation were selected within the accessible area.

Cut-twig sampling was available for up to 15 oak trees across the nine arrays as a part of a fixed schedule of monthly green-leaf sampling during the leaf-on season. For two infrastructure arrays, cut-twig sampling was for oak trees not fitted with sap flow probes (Table S1). In all arrays cut-twigs were randomly selected by arborists at set top-canopy positions (south-facing side), excluding areas accessed by CAS.

In addition to abaxial leaf temperature data from the in-built temperature sensors of the Decagon Leaf porometer, from 2020 onwards independent infrared (IR) laser thermometer measurements were introduced to provide greater accuracy outside of the instrument porometry chamber. These temperature readings also minimised artefacts caused by boundary layer disruption. To account for discrepancies between the two measurements methods, paired comparisons were conducted on 2020 data (Fig. S1) and porometer temperature data were adjusted to infill missing IR readings in 2019 and 2021.

For oak, leaf-level to same-day tree-stem sap flux comparison was used to examine whole-tree smoothing effect of canopy transpiration vav. leaf transpiration dynamics and look for water storage delays. Temporal differences were assessed by normalising day-specific data and mapping transpiration by time of day.

2.4 Sap flux data

3075 Quick *et al.*, (2025) reported stem sap flux data determining tree-based water usage characteristics of oak. Here, 30-minute resolution sap flux data from that dataset were re-used to further explore within-day variation enabling comparison of canopy-level with leaf-level transpiration. Subdominant ((Quick, 2025), Ch. 5) sap flux data from long-probe installations were processed to generate diurnal subdominant sap flux
3080 datasets (Table S2). To look at water storage in oak, sap flux data were extracted for individual days on which leaf stomatal conductance was measured, then normalised by median to facilitate comparison of transpiration patterns. The middle-day period (08:00hrs to 16:00hrs UTC) was selected to align with the core period of leaf measurement. We followed previous stored water usage estimation methods (Meinzer
3085 *et al.*, 2004; Goldstein *et al.*, 1998) for identifying time lags between crown and basal sap flux signals. Here leaf transpiration was used as a proxy for branch-level sap flux.

2.5 Other parameters – FACE data, soil, precipitation and environmental variables (2019 onwards).

From 2019 season onwards, forest-edge atmospheric variables (Table S3), including
3090 *RH*, (%) and air temperature ($^{\circ}\text{C}$), top-canopy total and net solar radiation (W m^{-2}) and photosynthetically active radiation (PAR, $\mu\text{mol of photons m}^{-2} \text{s}^{-1}$) were measured at four meteorological (Met) towers (MacKenzie *et al.*, 2021). Figure 6-2 gives an overview. It was assumed similar wind and precipitation conditions were present across the small research forest within a given canopy layer.

3095 **Additional parameters, including CO₂ concentration** (ppm) were monitored through the FACE instrumentation within *aCO₂* and *eCO₂* arrays (Hart *et al.*, 2020; Quick *et al.*, 2025) and *ghost* (Harper, 2024).

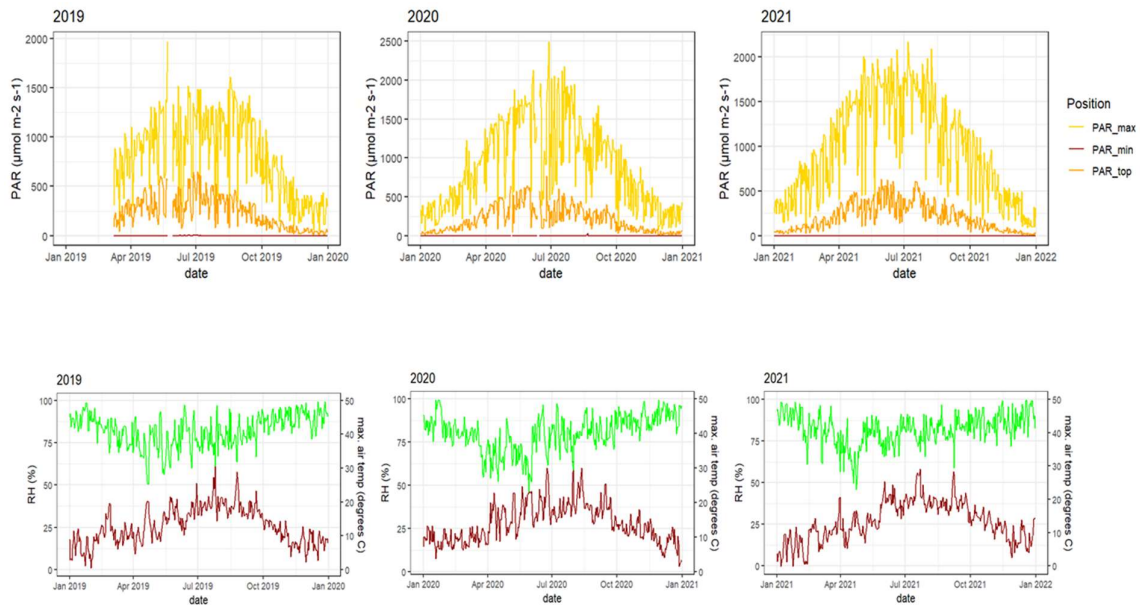


Figure 6- 2: Environmental daytime canopy parameters across years 2019-2021. Top row: Maximum (yellow) mean (orange) and minimum (brown) daily PAR. 2nd row: maximum T_{top} , dark red, minimum RH_{top} , green.

2.6 Calculation Methods.

Leaf transpiration calculation

Leaf transpiration (E , $\text{mmol m}^{-2} \text{s}^{-1}$) was derived from stomatal conductance to H_2O (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$), leaf-to-air vapour pressure deficit (VPD_L , kPa), and barometric pressure (p , kPa) following Equation (1) (Ehleringer, J. R. Hall, A. E. Farquhar, 1993):

$$E = \frac{g_s \times VPD_L}{p} \quad (1)$$

Barometric pressure measured at top-canopy height (Table S3) was adjusted for use in bottom-canopy transpiration calculations (Equation 1) using standard methods (i.e. 1 hPa or 1 millibar) reduction per for each 10 m height reduction). Transpiration units were converted to $\text{mg H}_2\text{O m}^{-2} \text{s}^{-1}$, using the molar mass of H_2O (18.014g mol^{-1}).

Calculations were applied to all leaf data. This simple model has limitations (it omits boundary layer decoupling) but is deemed adequate (Stoy *et al.*, 2006). The method was chosen as a first estimate as we expected that broadleaf forest trees were highly decoupled (e.g. (Stoy *et al.*, 2006; De Kauwe *et al.*, 2017)). Our data also lacked
 3115 sufficiently localised wind data at canopy measurement height (which might affect decoupling) to use the more accurate Penman-Montieth model approach.

Saturated vapour pressure (SVP , kPa) and air VPD (VPD_{air} , kPa) were calculated using mean air temperature (T_{air} , °C) or leaf temperature (T_L , °C), and RH following Tetens' (1930) equation, which provides sufficient accuracy (better than +/- 0.1%,
 3120 (Buck, 1981); (Murray, 1967)) across temperatures from -5°C to 50 °C.

$$SVP = 0.61078 \times e^{\left\{\frac{(17.27 \times T_x)}{(T_x + 237.3)}\right\}} \quad (2)$$

where T_x is the temperature in °C. (Note: we use $T_x = T_{air}$ to get air saturated vapour pressure ($ASVP$, kPa) and $T_x = T_L$ to get leaf saturated vapour pressure ($LSVP$, kPa).

Mean RH and T_{air} (top- or bottom-canopy, depending on porometry measurement
 3125 height) from the met towers (Table S3) were used to calculate $ASVP$ and $LSVP$ (Equation 2). VPD_L was then determined as:

$$VPD_L = LSVP - (ASVP \times RH/100) \quad (3)$$

where VPD_L is in kPa. These calculations were then applied consistently for all porometer and leaf temperature data to ensure comparability of results.

Following calculation of leaf transpiration, correlation visualisations were conducted showing relationships between leaf transpiration and stomatal conductance (e.g. for oak, Fig. S2), and leaf transpiration and leaf temperature (e.g. for oak leaves Fig. S3; subdominant Fig. S4). Leaf transpiration relationships with PAR levels were analysed for covariance.

2.7 Data processing, visualization and analysis

All analysis was in 'R' (R Core Team, 2024) using RStudio (Posit Team, 2024) with full lists of packages provided in scripts. Manually collected data were pre-processed as .csv files for import. Box and whisker plots to visualise sap flux and leaf transpiration details, between treatments, were generated in *ggplot* where standard Tukey (McGill, Tukey and Larsen, 1978) percentiles (median + interquartile range) whiskers ($1.5 \times$ IQR from each hinge, where IQR is the inter-quartile range) and points for outliers are used. For exploratory analysis of diurnal patterns and non-linear relationships, we used LOESS (locally estimated scatterplot smoothing) for exploratory analysis of data patterns, an approach that, does not rely on specific assumptions about the distributions from which observations are drawn. Further statistical tests (e.g. Levene homogeneity of variance and Shapiro-Wilk normality checks) were applied where appropriate. We used Wilcoxon rank sum tests for statistical comparisons of transpiration and stomatal conductance between treatment and species types. Linear models of PAR versus transpiration were utilised to indicate likely statistical relationships.

3 Results

3.1 Leaf transpiration

Box and whisker plots of oak leaf transpiration in-situ data (Figs. 6-3(a), (b)), cut-twig
data (Fig. 6-3(c)) and in-situ subdominant species' leaf transpiration data (Figs. 6-3(d))
are shown.

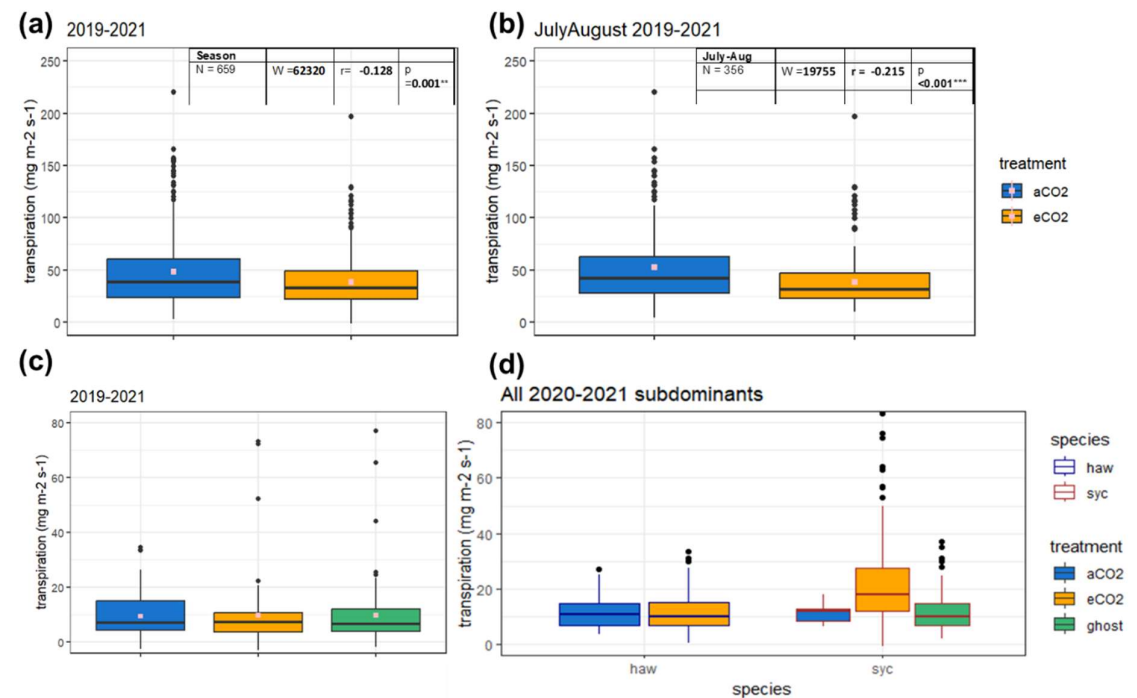


Figure 6- 3: Leaf transpiration (mg m⁻² s⁻¹). Oak panels: (a) infrastructure (aCO₂ and eCO₂) dominant oak data derived from in-situ measurements, all three seasons 2019-2021, (b) oak infrastructure in-situ measurements July and August 2019-2021, and (c) derived from all cut-twig measurements, three seasons 2019-2021 in all treatments. Subdominant hawthorn and sycamore bottom-canopy in-situ leaf transpiration data box and whisker plots from 2020 and 2021 are shown similarly in (d) separated by treatment and species. Note maximum transpiration (y-axis) is 250 mg H₂O m⁻² s⁻¹ for (a) and (b), but 80 mg H₂O m⁻² s⁻¹ for (c) and (d). For (a) and (b) Wilcoxon rank sum test results are embedded. Box and whisker plots show median and interquartile range (IQR, 25%ile to 75%ile) with whiskers calculated as 1.5 x IQR from the hinge and points for outliers. Mean values are calculated from the entire range of data and shown as spots (pink).

Each whole-season, and mid-season infrastructure (eCO₂, aCO₂) array's oak leaf transpiration data were tested by Levene tests, separately for each year (2019, 2020, 2021) (Table S4). For 2021 season and mid-season 2020 the test was not significant indicating homogeneity of variance assumption was met. Seasonal 2019 and 2020 and

3170 mid-season 2019 and 2021 Levene results were significant indicating homogeneity of
variance assumption was not met between the infrastructure treatments (eCO_2 and
 aCO_2). These results align with similar tests for sap flux (Table S5).

Shapiro-Wilk normality tests were conducted on the same infrastructure oak leaf data
for both transpiration and stomatal conductance, for each season and mid-season
3175 period indicating that each whole-season and mid-season specific transpiration data
were not normally distributed ($p < 0.001$) (Table S6). Stomatal conductance seasonal
data were also not normally distributed (marginally significant ($p = 0.066$) in 2019 and
highly significant in 2020, 2022). Mid-season stomatal conductances demonstrated
normality in 2019, 2020 and non-normality in 2021 (marginal significance, Table S6).

	2019			2020			2021		
Season	W =	r =	P =	W =	r =	P =	W =	r =	P =
N =		172			354			301	
transpiration	4196	-0.12	0.12 ns	17013	-0.08	0.15 ns	12638	-0.10	0.081.
stomatal conductance	4469	-0.18	0.018*	18955	-0.18	<0.001***	14886	-0.27	<0.001***
July-Aug	W =	r =	P =	W =	r =	P =	W =	r =	P =
N =		42			167			153	
transpiration	354	-0.56	<0.001***	4360	-0.23	0.003**	3440	-0.15	0.06.
stomatal conductance	251	-0.13	0.39 ns	5026	-0.40	<0.001***	4373	-0.43	<0.001***
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1									

3180 Table 6- 1: Wilcoxon rank sum test for effects of eCO_2 on oak-leaf transpiration during 2019 to 2021.
Whole season and July-Aug only infrastructure (eCO_2 and aCO_2) leaf data are tested for differences.
Transpiration and stomatal conductance were separately tested. In-situ and cut-twig data are included.

Wilcoxon rank sum test results for oak leaf transpiration infrastructure treatment
comparison (eCO_2 versus aCO_2) are shown in Table 6-1. There was significant
3185 reduction of leaf transpiration in response to eCO_2 treatment in all mid-season periods

(median % differences: 2019: ~ -40%, 2020: ~ -23%, 2021: ~ -27%) with low to medium effect sizes (2019: $r = -0.56$, 2020: $r = -0.4$, 2021: $r = -0.15$) though in 2021 significance was only marginal ($p = 0.06$). For the whole-season data, only 2021 showed significant (median % difference ~ -9%) reduction in eCO_2 leaf transpiration with a low to medium effect size ($W = 12638$, $r = -0.1$, $p = 0.081$).

In contrast, Wilcoxon test comparisons of infrastructure treatment stomatal conductance data were significant for each whole-season's data (median % differences: 2019: ~ -16%, 2020: ~ -17%, 2021: ~ -26%) with low to medium effect size (Table 6-1). During mid-season periods stomatal conductance data reductions (median % differences: 2019: ~ -18%, 2020: ~ -19%, 2021: ~ -25%) were only significant in 2020 and 2021 with low to medium effect size (Table 6-1).

Wilcoxon rank sum results from in-situ infrastructure array leaf flux transpiration data									
	2019			2020			2021		
Season	W =	r =	p =	W =	r =	p =	W =	r =	p =
N =		172			249			238	
transpiration	4196	-0.12	0.12	9525	-0.20	0.0014**	7856	-0.10	0.14
July-Aug	W =	r =	p =	W =	r =	p =	W =	r =	p =
N =		42			167			147	
transpiration	354	-0.56	<0.001***	4360	-0.23	0.0031**	3193	-0.15	0.057.
ANOVA results from sap flux data (from Quick et al., (2025) Supplementary Information)									
	2019	2019	2019	2020	2020	2020	2021	2021	2021
	%	F ratio	p value	%	F ratio	p value	%	F ratio	p value
Season	-19%	91.90	<0.001***	-3%	3.09	$p > 0.05$, actual value 0.079	-13.9%	32.27	<0.001***
July only	-26%	35.61	<0.001***	-4.5%	0.80	$p > 0.05$, actual value 0.37	-7.3%	1.71	$p > 0.05$, actual value 0.19

Significance codes: 0 '****' 0.001 '***' 0.01 '**' 0.05 '.' 0.1 ' ' 1

Table 6- 2: Wilcoxon rank sum test for effects of eCO₂ in 2019 to 2021 from in-situ leaf transpiration data only (top eight rows) compared with ANOVA results from sap flux data (mean eCO₂ TWU_n, compared with mean aCO₂ TWU_n) in same seasons (bottom four rows). For Wilcoxon tests whole-season and July-Aug only infrastructure (eCO₂ and aCO₂) leaf data are tested for differences.

There was significant reduction of leaf transpiration in response to eCO₂ treatment for in-situ data (Table 6-2) in season 2020 with lesser reductions in other whole-season's data (median % differences: 2019: ~ -11%, 2020: ~ -21%, 2021: ~ -9%) with low to medium effect sizes (2019: $r = -0.12$, 2020: $r = -0.20$, 2021: $r = -0.10$). Mid-season periods exhibited similar results to the whole datasets (due to small numbers of mid-season cut-twig data), but all were significant (median % differences: 2019: ~ -40%, 2020: ~ -23%, 2021: ~ -30%) with low to medium effect sizes (2019: $r = -0.56$, 2020: $r = -0.23$, 2021: $r = -0.15$). There were insufficient cut-twig data for statistical analysis.

	slope	SE	R ²	Adj R ²	intercept	t	df	Pr(> t)
	E / PAR (mg μmol^{-1})				(mg H ₂ O m ⁻² s ⁻¹)			
2019 season	0.0539	0.0100	0.15	0.14	3.773	5.39	170	<0.001 ***
2020 season	0.0331	0.0063	0.102	0.099	23.40	5.30	247	<0.001 ***
2021 season	0.0391	0.0055	0.175	0.171	2.731	7.07	236	<0.001 ***
2019-2021 seasons	0.0297	0.0035	0.100	0.098	19.95	8.52	657	<0.001 ***
2019 July & August	-0.005	0.05	0.00025	-0.025	68.90	-0.10	40	0.921 ns
2020 July & August	0.0124	0.0065	0.022	0.016	29.51	1.903	165	0.0587 .
2021 July & August	0.0447	0.0082	0.17	0.164	3.162	5.451	145	<0.001 ***
2019-2021 July & August	0.0336	0.0046	0.133	0.131	17.55	7.38	354	<0.001 ***

Table 6- 3: In-situ Infrastructure only (i.e. combined aCO₂ and eCO₂) oak data linear model for leaf transpiration versus top-canopy PAR. Top four rows show results for each season and combined results for all years. Bottom four rows show results for each mid-season (July-August), and combined results for all years.

Correlative graphics of leaf transpiration data against PAR are shown for all oak-leaf data by year (Fig. S5), then just the in-situ data from the infrastructure arrays (Fig. S6) faceted by year and grouped for all years. A linear regression model for each year and all years (Table 6-3) shows differing slopes by treatment in 2019 for the whole- season infrastructure data, but very similar slopes in 2020 and 2021. Using in-situ data from all years (2019-2021) we tested the hypothesis that the effect of top-canopy PAR on leaf transpiration depends on infrastructure treatment and found no influence between PAR and treatment ($F=0.04$; $df = 1$), indicating that the effects of PAR and treatment are additive.

The model for transpiration under aCO_2 is:

$$E_{aCO_2} = 24.32 + 0.03 * PAR \quad (4)$$

The change in transpiration (in $mg\ m^{-2}\ s^{-2}$) intercept arising from elevation of CO_2 is (-7.93) and the change in slope is (-0.0014) $mg\ \mu mol^{-1}$, so:

$$E_{eCO_2} = 16.39 + 0.03 * PAR \quad (5)$$

Although the intercept difference between treatments appears large (>30%), the t value is quite small and the p value is >0.1 so we cannot reject the null hypothesis (i.e. that the intercepts are the same). Furthermore there are likely to be other drivers to the transpiration differences as the ANOVA gives a residual standard error of 0.29 on 655 degrees of freedom.

Mid-season data PAR versus transpiration linear regression model for July and August data in all years are also reported (Table 6-3). Analysis of treatment effects in mid-

season only found significant differences in years 2020 and 2021. For July and August
 3235 in all years 2019 to 2021 we again tested the hypothesis that the effect of top-canopy
 PAR on leaf transpiration depends on infrastructure treatment and again found no
 influence between PAR and treatment ($F = 2.67$; $df = 1$), indicating that the effects of
 PAR and treatment are additive.

Sycamore							Hawthorn		
	2020		2020	2021		2021	2020- 2021		2020- 2021
Season	W =	r =	P =	W =	r =	P =	W =	r =	p =
N =		99			164			247	
transpiration	1216	= -0.19	0.056.	4264	-0.24	0.0023**	26	= -0.040	0.53
July-Aug	W =		P =	W =		P =	W =		P =
N =		42			13			54	
transpiration	174	-0.013	0.93	26	-0.17	0.5338	418	-0.13	= 0.3573
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1									

3240 **Table 6- 4: Wilcoxon rank sum test for effects of eCO_2 on subdominant-leaf in-situ bottom-canopy transpiration during 2020 to 2021. For sycamore (left side of table) whole-season and July-Aug eCO_2 versus all control (i.e. aCO_2 and *ghost*) leaf data are tested for differences. For hawthorn all data from years 2020-2021 are combined by treatment (right of table) as no control data were captured during 2020.**

Repeating the homogeneity of variance and normality tests for the 2020 and 2021
 season subdominant bottom-canopy leaf data, independently for each species
 3245 (hawthorn and sycamore), showed similar non-linearities to those exhibited by oak top-
 canopy data. The Wilcoxon rank sum test results for subdominant leaf transpiration
 differ to those for oak due to the inability to compare equal quantities of data for
 infrastructure treatment from all arrays. Instead, for sycamore, the comparison was
 between eCO_2 and both controls (aCO_2 , *ghost*) combined. For hawthorn there were no
 3250 control leaf data during 2020 (and delays in installation of the sap sensors), so leaf
 results for 2020 and 2021 were combined testing eCO_2 versus aCO_2 . All Wilcoxon
 results for subdominant trees are shown are shown in Table 6-4.

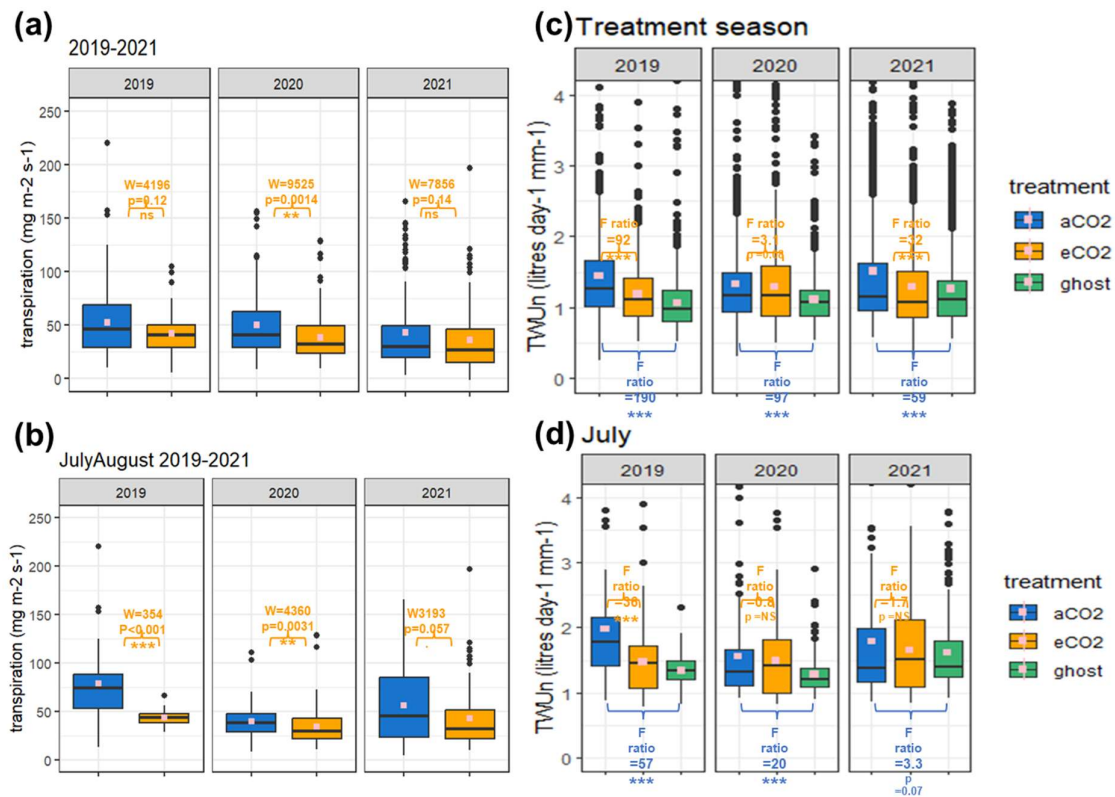


Figure 6- 4: Box and whisker plots showing median and interquartile range (IQR, 25%ile to 75%ile) with whiskers calculated as 1.5 x IQR from the hinge and points for outliers. Mean values (panels (a), (b) and (c), are calculated from the entire range of data and shown as spots (pink). Comparison of middle-day infrastructure (aCO₂ and eCO₂) in-situ oak leaf transpiration results through (a) season and (b) July and August with previous whole-tree normalised daylight tree water usage results ((c) season and (d) July) derived from 30-minute sap flux data (based on Quick et al. 2025, in supplementary information Fig. S 8).

For bottom-canopy sycamore leaves there was significant increase of leaf transpiration in response to eCO₂ treatment compared to pooled control leaves' whole-season data (median % differences: 2020: ~ +39%, 2021: ~ +21%) with low to medium effect sizes (2020: $r = -0.19$, 2021: $r = -0.24$) though in 2020 significance was only marginal ($p = 0.056$). Mid-season data showed no significant differences. We noted that in 2021 ambient infrastructure (aCO₂) leaf transpiration results were ~ +6% higher than those in *ghost* leaves though we did not test the significance of this. For hawthorn there appeared to be no effects of eCO₂ treatment on leaf transpiration (Table 6-4).

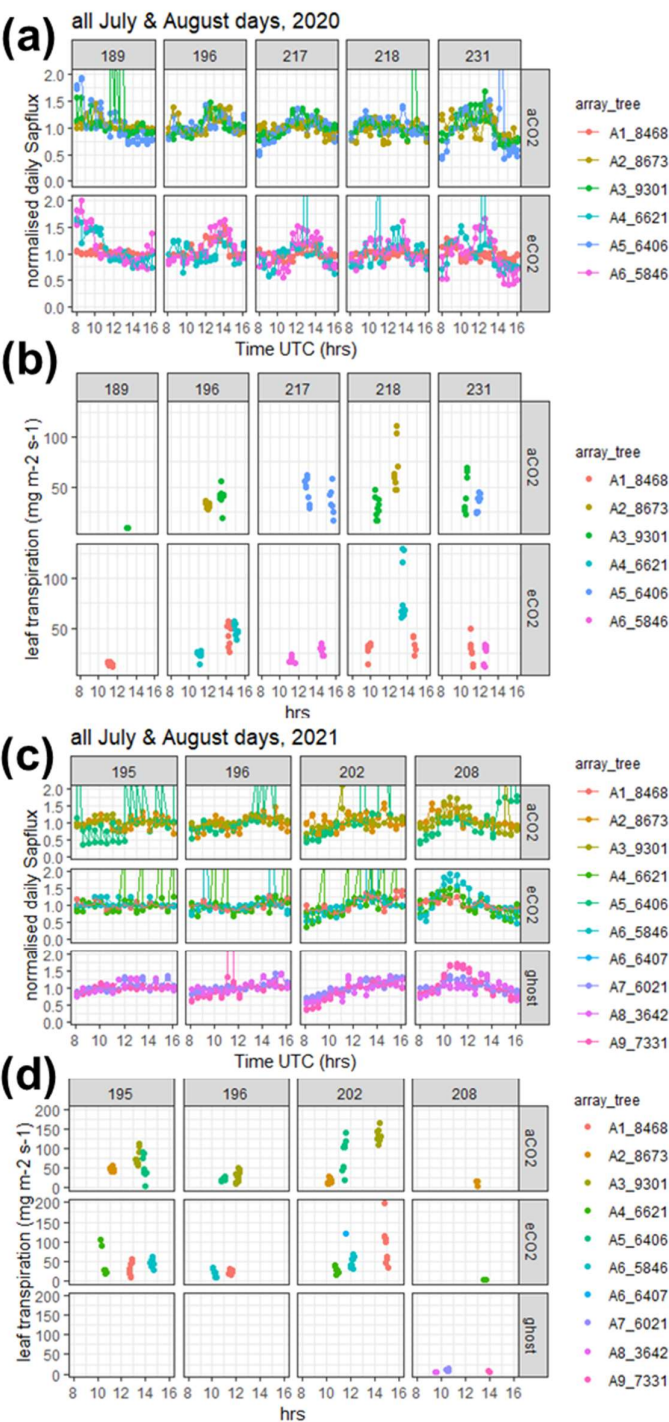
3.2 Comparison of leaf transpiration results with whole-tree oak results

Table 6-2 and Box plots (Fig. 6-4) compare middle-day in-situ oak leaf transpiration results with previous whole-tree results from 30-minute sap flux data (based on (Quick *et al.*, 2025), supplementary information Fig. S 8).

3.3 Dynamics of stem to leaf.

Using sap flux data for oak from Quick *et al.*, (2025) from the middle of the day (08:00hrs to 16:00hrs UCT) we normalised these data (Step 3, Table S2). There are difficulties with comparing small samples of leaf transpiration data to continuously monitored stem data even when the data has been normalised. Figure 6-4 illustrates the box and whisker plots for both data sources. To overcome these difficulties, the outputs from sap flux normalisation were compared with pooled data from all oak porometry for the mid-season (July and August) by treatment and day of year (DOY) to aid comparison of the daytime shape of response.

Following normalisation we show comparison of oak whole tree transpiration to (leaf transpiration for days in mid-season (July & August) 2020 and 2021 (Fig. 6-5). Results are shown initially with all outliers. (N.B. extra work needed to define straight-line relationship in Figure 6-6).



3285 Figure 6- 5: Comparison of oak (a), (c) middle-day whole-tree transpiration (i.e. sapflux) to (b), (d) middle-day leaf-transpiration for days in mid-season (July & August) during 2020 (a) & (b) and 2021 (c) & (d).

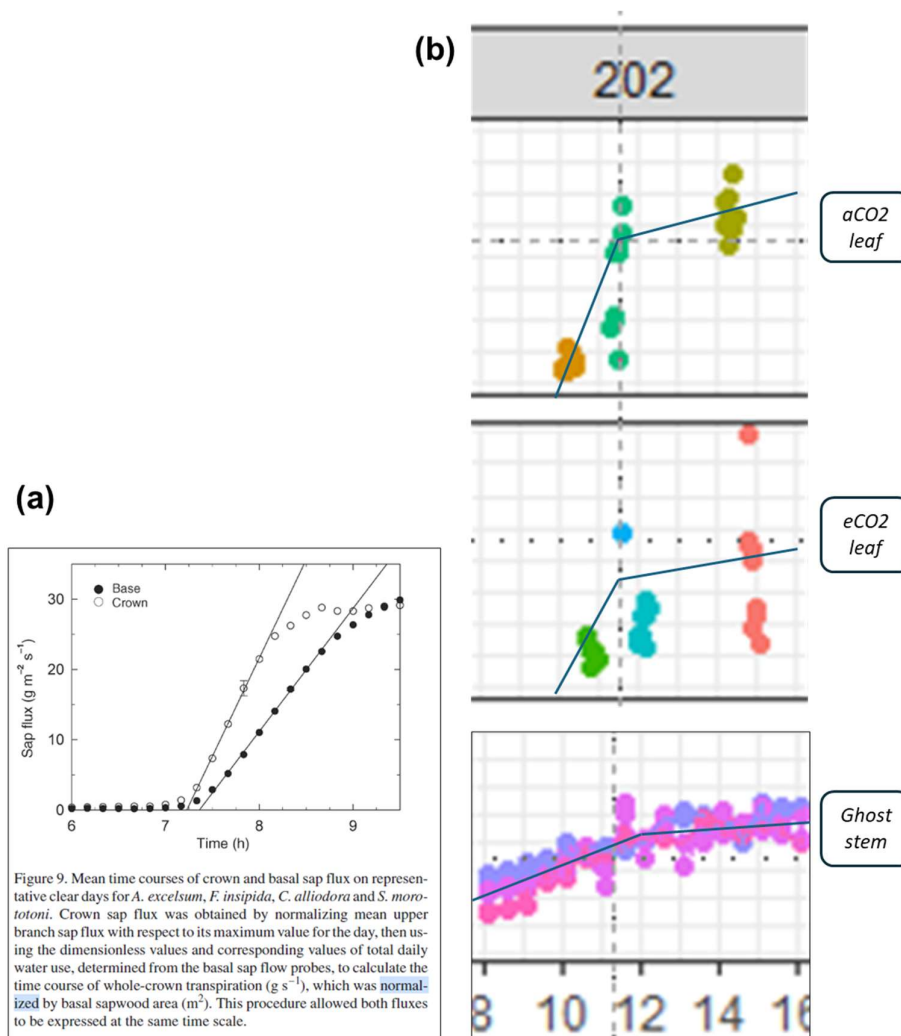


Figure 6- 6: Comparison between (a) sap flux results from Meinzer et al., (2004) and (b) leaf (top two panels) and sap fluxes of transpiration from BIFoR FACE showing potential characteristics of morning slope and levelling. Data for (b) is taken from 2021 results shown Figure 6-5.

4 Discussion

4.1 eCO₂ treatment effect on oak leaf transpiration

In-situ infrastructure leaf eCO₂ treatment results for top oak canopy transpiration partially align with those for whole-tree (Quick *et al.*, 2025) but differ in many ways. This may be due: to differences in data time of day (leaf data only covers 09:00hrs to 16:00hrs whereas whole-tree analysis covers down to dusk); or smaller sample of trees

measured for leaf transpiration. During mid-season there are closer alignments of leaf transpiration results with carbon assimilation results at the same location (Gardner *et al.*, 2022), though we recognise that the mechanisms for water loss and carbon gain differ.

We found significant median reductions in stomatal conductance to water of -19% and -25% under eCO_2 during mid-seasons 2020 and 2021. The mid-season results differ from oak stomatal conductance findings from Web-FACE in a dry summer and mid-season whole-tree oak water savings at the same site (Keel *et al.*, 2007; Leuzinger and Körner, 2007) where no significant reductions were found for *Quercus* species. We might assume that the higher ambient levels of CO_2 now present (circa 425 ppmv CO_2 2019-2021 compared to circa 370 ppmv CO_2 at Web-FACE), combined with different environmental conditions, might have affected stomatal response (and consequently leaf transpiration) in oaks, but we have no evidence to explain the apparent differences. Similarly whilst Ainsworth and Rogers, (2007), summarising previous FACE experiments, reported reductions in stomatal conductance of 16–23% (trees having a mean of 20%), that study included all types of vegetation and they did not study the consequent reductions in transpiration.

The differences between whole-season and mid-season might indicate that, even in middle day, PAR levels have covariant effect on the transpiration response, but when we tested this hypothesis using a linear model we found that the effects of PAR and eCO_2 are additive (Table 6-3 and Fig. S6). Renninger *et al.*, (2014) in that study, used daily PAR values when comparing leaf transpiration in two *Quercus* species and found a similar increase in transpiration for increasing PAR, but those leaf-related results

were derived from stem sap flux data. Morecroft and Roberts, (1999) considered differing response of stomatal conductance for oak and sycamore under differing light conditions but found the PAR effects on assimilation were inconclusive. We are not aware of any other detailed analysis of the effects of PAR on leaf transpiration for

3325 Quercus species in a mature forest, though researchers may assume that reduction in stomatal conductance results in reduced leaf transpiration (Medlyn *et al.*, 2001; Keel *et al.*, 2007; Bader *et al.*, 2013). Certainly the increasing extreme variation in seasonal temperatures and amounts of sunshine under global climate change might be expected to explain inter-season differences in leaf-water fluxes, but disentangling the

3330 environmental drivers and experimental equipment effects within a field experiment requires a more focused and complex measurement and verification regime than this project was able to provide.

Comparing middle-day oak-leaf Wilcoxon rank sum test results from in-situ transpiration measurements with whole-tree daylight sap flux ANOVA results (see

3335 Table 6-2 and (Quick *et al.*, 2025)) we find that both mid-season and whole-season eCO_2 leaf transpiration results only exhibits similar trends in 2019 mid-summer. For 2020, 2021 mid-summer and 2019 season we conclude that results from top-canopy leaves do not reflect the whole canopy response, confirming the findings of other research (e.g. (Donohue *et al.*, 2017; Gardner *et al.*, 2025) which shows such disparity

3340 between leaf and whole-tree responses to eCO_2 . We suggest that, for dominant oak, eCO_2 may only result in significant stomatal responses which affect transpiration when exposed to high PAR levels and/ or increased ambient temperatures or VPD. Other concurrent research considered whether leaf traits (stomatal density, stomatal length, stomatal size) might differ in oak trees under eCO_2 . (Gardner *et al.*, 2025) found no

significant differences whilst (Hagan Brown *et al.*, 2025) found increases in stomatal size and reduction in stomatal density in top canopy eCO_2 leaves compared to aCO_2 leaves, with dissimilar non-significant trends for bottom canopy. These findings might in future better inform our leaf transpiration results.

4.2 Subdominant species leaf response

Under eCO_2 , bottom-canopy leaf transpiration results for subdominant species, sycamore and hawthorn, differed to oak top-canopy results and were similar magnitude to results from cut-twigg oak (Fig. 6-3 (c) and (d)). Results from leaf transpiration in the subdominant species (Fig. 6-3(d), Table 6-4) show differing responses for sycamore and hawthorn across the treatments. Whilst hawthorn does not appear to respond to the eCO_2 , there are indications that sycamore trees may exhibit greater leaf transpiration at the bottom-canopy under eCO_2 , despite experiencing similar environmental conditions to hawthorn. Morecroft and Roberts', (1999) study of oak and sycamore leaves under ambient conditions found similar stomatal conductance ranges under full sun ($PAR = 200-1600 \mu mol m^{-2} s^{-1}$) for both oak (our results IQR $g_s = \sim 170$ to $\sim 470 mmol m^{-2} s^{-1}$) and sycamore (our results IQR g_s , ~ 60 to $\sim 210 mmol m^{-2} s^{-1}$), with proportionate reductions under increasing shade. The deepest shade leaves for oak in that study showed higher g_s , than leaves in the intermediate canopy, a feature which might align to our increased g_s and transpiration for sycamore at bottom-canopy under eCO_2 . We interpret this as a probable response to the very different microclimate at the bottom of the canopy. This response can usefully be the studied further in laboratory and field research as there are no equivalent eCO_2 studies of sycamore to date.

Since both subdominant species experience similar microclimatic effects, we speculate that specific leaf area and/ or stomatal density may differ in sycamore vav hawthorn, which could influence eCO_2 results. We recommend inclusion of such measurements in future studies. Other limitations might be that, under the experimental treatment conditions, some aCO_2 arrays experience greater or lesser ambient CO_2 levels within the two – eight metre height range of bottom-canopy measurements compared to the average value (Hart *et al.*, 2020; Harper, 2024). There are also unusual unexplained leaf responses during senescence.

We compared eCO_2 effects on sycamore top and bottom-canopy leaves on a paired basis with responses to top-canopy oak leaves (in-situ and cut-twigs) in the same array (Array 6 (eCO_2) and Array 7 (*ghost*)) but did not report these as the measurement days were different for bottom-canopy and there were insufficient same-day data to form realistic comparisons between the dominant and subdominant species. The effects of differing VPD (Valladares *et al.*, 2016) and PAR (Valladares and Niinemets, 2008) in the understorey may be the strongest influence on these differences. More detailed field work would be required to interpret these results.

To account for decoupling, a better transpiration model such as Penman-Monteith (or Priestley-Taylor which allows for lack of wind data) might improve interspecies comparisons but these models are focused on upscaling from leaf/ tree to landscape not leaf/ tree comparisons. Another model decoupling factor, especially during drought, might be the presence of deep roots in oak which increase decoupling (Flo *et al.*, 2022).

We might expect that top-canopy oak leaves would generally exhibit resilience to decreasing soil-water availability as the summer progressed with greater resilience

from eCO_2 leaves, as shown in Aranda et al. (2012) study of oak and beech,. This can best be studied further in a separate article (see Ch.4). Here we can only compare oak leaf transpiration with one top-canopy sycamore tree (Array 6, eCO_2), results of which are not yet conclusive.

3395 **4.3 Peak values of daily leaf transpiration**

Most of these data were collected from June to September, but the DOY was not consistent (oak, Fig. S7(a)) across the three years of interest due to restrictions on fieldwork and manpower. Oak leaf transpiration data over the three years (Fig. S7) indicates a peak occurring between days 202 (20th July) and 232 (20th Aug) in any one
3400 year, though a high transpiration day also occurred in September 2020. For sycamore and hawthorn leaf a more complex picture emerges. In May at first-leaf these trees take advantage of the more open canopy (prior to oak budburst) achieving an initial peak. This is followed in late August/ early September with a second peak aligning with higher temperatures and longer days. So this leaf-data cannot fully predict when peak
3405 leaf transpiration might occur in future years without reference to sap flux results.

4.4 Water storage

Some measurable time delay occurs between the peak values relating to water fluxes (i.e. sap flux and transpiration) measured at stem and leaf. Stem sap flux peak is delayed relative to leaf transpiration but future per-tree diurnal measurements, starting
3410 earlier on sunny days, would more clearly define these characteristics.

From visualisations of stem sap flux (measured at two positions on 18 trees), normalised by each probeset middle-day median value, and leaf transpiration (derived

from stomatal conductances measured in 9 trees), we can see consistent intraspecies (oak) patterns. These patterns are unique to the day on which the data are collected due to perturbations of VPD and PAR. Figure 6-6 compares an outline of one day's findings from this paper compared with the results from Meinzer et al., (2004).

The rate at which stomatal conductance (and hence leaf transpiration) values change is faster than the frequency of porometer measurement rate (maximum frequency of about 20 times per hour under low humidity conditions). In contrast, sap flux data is only collected every 30 minutes, and there is a smoothing effect across the period of measurement (1 to 300 secs) despite that period being short (e.g. <120 seconds) for high flow rates. By considering a number of normalised results of sap flux from the same species (Fig.6-6(b)) we could, given more leaf data, characterise the delay between stem (measured and sap flux) and leaf (measured from leaf stomatal conductance) fluxes of water/ water vapour. A better way of normalising the data might be used to inspect sap flux. For example sub-daily sap flux time-series could be aggregated to middle-day averages (e.g. 08:00–16:00 h solar time), using the R package *sapfluxnetr* (Flo et al., 2021), for normalisation purposes.

The middle-day leaf data collected are randomly variant around the median, therefore, from this experiment, it was possible to estimate a measurable time delay occurring (of less than 1 hour) between the changes in initial to afternoon slopes relating to transpiration as measured at stem level (a proxy for whole canopy transpiration) and at leaf level. This estimate is not yet sufficiently ruggedised and requires more leaf data as previously discussed.

3435 We speculate that high VPD early in the day limits loss of water from the leaf, whilst
high leaf water potential starts to drive the xylem sap flow which has greater inertia.
The steeper leaf-transpiration slope indicates a faster dynamic response at top-canopy
leaves. It appears, in contrast to Meinzer et al., (2004), that there is considerable delay
(circa two hours) between early morning stem and leaf initial responses, but only less
3440 that one hour delay for reaching the middle day median. A limitation of our calculations
may be, that *RH* changes alongside air temperature changes near to and surrounding
the leaf are more influential in determining VPD than previously thought.

5 Concluding summary and further work

This study has assessed the effects of *eCO*₂ on oak and subdominant species'
3445 transpiration focusing at the leaf scale. The comparison of transpiration dynamics at
leaf and whole tree scale has illuminated some limitations of different measurement
methods and varying maximum sampling rates. More targeted future field studies
aligned with the appropriate timescales and equipment could test this more fully
including undertake more intensive in-situ leaf measurement campaigns during July to
3450 August under consistent concurrent conditions.

The findings of a significant reduction in oak top-canopy transpiration under *eCO*₂
across (pooling data from all years) during July and August may not be replicated in
future. Nevertheless these results might indicate how to improve validation of
vegetation and landscape models of oak under future-forest environments.

3455 In contrast, hawthorn bottom-canopy transpiration remains unchanged, while
sycamore may exhibit increased leaf transpiration during mid-summer, possibly due to
shading effects, differing stomatal traits or treatment anomalies in the understorey.

Future use of sap flux data from short probesets could help clarify inter- and intra-species' variations in response to CO₂.

- 3460 These findings provide insight into how different tree species may respond to rising CO₂ levels and changing environmental conditions. While reduced transpiration could lead to reduced water loss and greater drought resilience in some species, it may also impair cooling and increase the risk of heat stress in others, affecting overall forest health and productivity.

3465 **References Chapter 6**

Ainsworth, E. A., & Rogers, A. (2007) 'The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions.', *Plant, Cell & Environment*, 30(3), pp. 258–270. doi: 10.1111/j.1365-3040.2007.01641.x.

Ainsworth, E. A. and Rogers, A. (2007) 'The response of photosynthesis and stomatal
3470 conductance to rising [CO₂]: Mechanisms and environmental interactions', *Plant, Cell and Environment*, pp. 258–270. doi: 10.1111/j.1365-3040.2007.01641.x.

Akhmatov, M. K., Salaš, P. (2005) 'The daytime woody plants water output by transpiration.', *Acta univ. agric. et silvic. Mendel. Brun.*, LIII(No. 2,), pp. 163–176.

Aranda, I. et al. (2012) 'Species-specific water use by forest tree species: From the
3475 tree to the stand', *Agricultural Water Management*. Elsevier, 114, pp. 67–77. doi: 10.1016/J.AGWAT.2012.06.024.

Bader, M. K.-F. et al. (2013) 'Central European hardwood trees in a high-CO₂ future: synthesis of an 8-year forest canopy CO₂ enrichment project', *Journal of Ecology*. John Wiley & Sons, Ltd, 101(6), pp. 1509–1519. doi: 10.1111/1365-2745.12149.

3480 Bader, M. K.-F. et al. (2022) 'Less pronounced drought responses in ring-porous than in diffuse-porous temperate tree species', *Agricultural and Forest Meteorology*, 327, p. 109184. doi: 10.1016/j.agrformet.2022.109184.

Barton, C. V. M. et al. (2012) 'Effects of elevated atmospheric [CO₂] on instantaneous transpiration efficiency at leaf and canopy scales in *Eucalyptus saligna*', *Global
3485 Change Biology*. John Wiley & Sons, Ltd, 18(2), pp. 585–595. doi: 10.1111/j.1365-2486.2011.02526.x.

Batke, S. P. et al. (2020) 'Plant responses to decadal scale increments in atmospheric CO₂ concentration: comparing two stomatal conductance sampling methods', *Planta*, 251(2), p. 52. doi: 10.1007/s00425-020-03343-z.

3490 Beer, C. et al. (2009) 'Temporal and among-site variability of inherent water use efficiency at the ecosystem level', *Global Biogeochemical Cycles*. John Wiley & Sons, Ltd, 23(2). doi: 10.1029/2008GB003233.

Bernacchi, C. J. and VanLoocke, A. (2015) 'Terrestrial Ecosystems in a Changing Environment: A Dominant Role for Water', *Annual Review of Plant Biology*. Annual
3495 Reviews, 66(1), pp. 599–622. doi: 10.1146/annurev-arplant-043014-114834.

Birkel, C. et al. (2024) 'Importance of measured transpiration fluxes for modelled ecohydrological partitioning in a tropical agroforestry system', *Agricultural and Forest Meteorology*, 346, p. 109870. doi: 10.1016/j.agrformet.2023.109870.

Buck, A. L. (1981) "New Equations for Computing Vapor Pressure and Enhancement
3500 Factor.", *Journal of Applied Meteorology* (1962-1982), 20(12), pp. 1527–32. Available at: www.jstor.org/stable/26180379.

Donohue, R. J. et al. (2017) 'A simple hypothesis of how leaf and canopy-level transpiration and assimilation respond to elevated CO₂ reveals distinct response patterns between disturbed and undisturbed vegetation', *Journal of Geophysical
3505 Research: Biogeosciences*, 122(1), pp. 168–184. doi: 10.1002/2016JG003505.

Ehleringer, J. R. Hall, A. E. Farquhar, G. D. (1993) "Introduction: Water use in relation to productivity", in *Stable Isotopes and Plant Carbon-water Relations*, pp. 3–8.

Fabiani, G. et al. (2022) 'Transpiration patterns and water use strategies of beech and oak trees along a hillslope', *Ecohydrology*. John Wiley & Sons, Ltd, 15(2), p. e2382.

3510 doi: 10.1002/eco.2382.

Flo, V. et al. (2021) 'Climate and functional traits jointly mediate tree water-use strategies', *New Phytologist*. John Wiley & Sons, Ltd, 231(2), pp. 617–630. doi: 10.1111/nph.17404.

Flo, V. et al. (2022) 'Vapour pressure deficit is the main driver of tree canopy conductance across biomes', *Agricultural and Forest Meteorology*, 322, p. 109029. doi: 10.1016/j.agrformet.2022.109029.

Forster, A. M. (2019) 'The Dual Method Approach (DMA) Resolves Measurement Range Limitations of Heat Pulse Velocity Sap Flow Sensors', *Forests* . doi: 10.3390/f10010046.

3520 Gardner, A. et al. (2022) 'Is photosynthetic enhancement sustained through three years of elevated CO₂ exposure in 175-year-old *Quercus robur*?', *Tree Physiology*, 42(1), pp. 130–144. doi: 10.1093/treephys/tpab090.

Gardner, A. et al. (2023) 'Optimal stomatal theory predicts CO₂ responses of stomatal conductance in both gymnosperm and angiosperm trees', *New Phytologist*. John Wiley
3525 & Sons, Ltd, 237(4), pp. 1229–1241. doi: 10.1111/nph.18618.

Gardner, A. et al. (2025) 'Elevated CO₂ buffers negative impacts of heat stress on growth of 180 years-old *Quercus robur*', *Plant Physiology*, (in review).

Gauthey, A. et al. (2024) 'High heat tolerance, evaporative cooling, and stomatal decoupling regulate canopy temperature and their safety margins in three European

- 3530 oak species', *Global Change Biology*. John Wiley & Sons, Ltd, 30(8), p. e17439. doi: 10.1111/gcb.17439.
- Gimeno, T. E. et al. (2016) 'Conserved stomatal behaviour under elevated CO₂ and varying water availability in a mature woodland', *Functional Ecology*, 30(5), pp. 700–709. doi: 10.1111/1365-2435.12532.
- 3535 Goldstein, G. et al. (1998) 'Stem water storage and diurnal patterns of water use in tropical forest canopy trees', *Plant, Cell & Environment*. John Wiley & Sons, Ltd, 21(4), pp. 397–406. doi: 10.1046/j.1365-3040.1998.00273.x.
- Granda, E. et al. (2020) 'Day length regulates seasonal patterns of stomatal conductance in *Quercus* species', *Plant, Cell & Environment*. John Wiley & Sons, Ltd, 3540 43(1), pp. 28–39. doi: 10.1111/pce.13665.
- Guerrieri, R. et al. (2016) 'Evapotranspiration and water use efficiency in relation to climate and canopy nitrogen in U.S. forests', *Journal of Geophysical Research: Biogeosciences*, 121(10), pp. 2610–2629. doi: 10.1002/2016JG003415.
- Hagan Brown, W. et al. (2025) 'Elevated CO₂ Increases the Canopy Temperature of 3545 Mature *Quercus robur* (Pedunculate Oak)', *Global Change Biology*. John Wiley & Sons, Ltd, 31(11), p. e70565. doi: 10.1111/gcb.70565.
- Harley, P. C. and Baldocchi, D. D. (1995) 'Scaling carbon dioxide and water vapour exchange from leaf to canopy in a deciduous forest. I. Leaf model parametrization', *Plant, Cell & Environment*. John Wiley & Sons, Ltd, 18(10), pp. 1146–1156. doi: 3550 10.1111/j.1365-3040.1995.tb00625.x.

Harper, N. J. (2024) Are the baseline control spaces at the BIFoR Free Air Carbon Enrichment experiment being polluted? University of Birmingham.

Hart, K. M. et al. (2020) 'Characteristics of free air carbon dioxide enrichment of a northern temperate mature forest', *Global Change Biology*, 26(2), pp. 1023–1037. doi: 10.1111/gcb.14786.

Hassler, S. K., Weiler, M. and Blume, T. (2018) 'Tree-, stand- and site-specific controls on landscape-scale patterns of transpiration', *Hydrology and Earth System Sciences*, 22(1), pp. 13–30. doi: 10.5194/hess-22-13-2018.

Haworth, M., Elliott-Kingston, C. and McElwain, J. (2013) 'Haworth, M., Elliott-Kingston, C., McElwain, J.C. (2013) Co-ordination of physiological and morphological responses of stomata to elevated [CO₂] in vascular plants. *Oecologia* 171(1):71-82', *Oecologia*, 171, pp. 71–82.

Herbst, M. et al. (2008) 'Comparative measurements of transpiration and canopy conductance in two mixed deciduous woodlands differing in structure and species composition', *Tree Physiology*, 28(6), pp. 959–970. doi: 10.1093/treephys/28.6.959.

IPCC (2023) 'Technical Summary', in Intergovernmental Panel on Climate Change (IPCC) (ed.) *Climate Change 2021 – The Physical Science Basis: Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press, pp. 35–144. doi: DOI: 10.1017/9781009157896.002.

De Kauwe, M. G. et al. (2013) 'Forest water use and water use efficiency at elevated CO₂: a model-data intercomparison at two contrasting temperate forest FACE sites',

Global Change Biology. John Wiley & Sons, Ltd, 19(6), pp. 1759–1779. doi: 10.1111/gcb.12164.

- 3575 De Kauwe, M. G. et al. (2017) 'Ideas and perspectives: how coupled is the vegetation to the boundary layer?', *Biogeosciences*. Copernicus Publications, 14(19), pp. 4435–4453. doi: 10.5194/bg-14-4435-2017.

- Keel, S. G. et al. (2007) 'Stomatal conductance in mature deciduous forest trees exposed to elevated CO₂', *Trees - Structure and Function*, 21(2), pp. 151–159. doi: 3580 10.1007/s00468-006-0106-y.

Keenan, T., Sabaté, S. and Gracia, C. (2008) 'Forest Eco-physiological Models and Carbon Sequestration BT - Managing Forest Ecosystems: The Challenge of Climate Change', in Bravo, F. et al. (eds). Dordrecht: Springer Netherlands, pp. 83–102. doi: 10.1007/978-1-4020-8343-3_5.

- 3585 Kourmouli, A. et al. (2025) 'Rapid Increase in Soil Respiration and Reduction in Soil Nitrate Availability Following CO₂ Enrichment in a Mature Oak Forest', *ACS Omega*. American Chemical Society, 10(1), pp. 1624–1634. doi: 10.1021/acsomega.4c09495.

- Lavergne, A. et al. (2019) 'Observed and modelled historical trends in the water use efficiency of plants and ecosystems', *Global Change Biology*. John Wiley & Sons, Ltd 3590 (10.1111), 0(ja). doi: 10.1111/gcb.14634.

Leuning, R. et al. (1995) 'Leaf nitrogen, photosynthesis, conductance and transpiration: scaling from leaves to canopies', *Plant, Cell & Environment*. John Wiley & Sons, Ltd, 18(10), pp. 1183–1200. doi: 10.1111/j.1365-3040.1995.tb00628.x.

- Leuzinger, S. and Körner, C. (2007) 'Water savings in mature deciduous forest trees
3595 under elevated CO₂', *Global Change Biology*. John Wiley & Sons, Ltd, 13(12), pp.
2498–2508. doi: 10.1111/j.1365-2486.2007.01467.x.
- Liang, J. et al. (2023) 'Linking water use efficiency with water use strategy from leaves
to communities', *New Phytologist*, 240. doi: 10.1111/nph.19308.
- MacKenzie, A. R. et al. (2021) 'BIFoR FACE: Water–soil–vegetation–atmosphere data
3600 from a temperate deciduous forest catchment, including under elevated CO₂',
Hydrological Processes. John Wiley & Sons, Ltd, 35(3), p. e14096. doi:
10.1002/hyp.14096.
- McGill, R., Tukey, J. W. and Larsen, W. A. (1978) 'Variations of Box Plots', *The
American Statistician*. [American Statistical Association, Taylor & Francis, Ltd.], 32(1),
3605 pp. 12–16. doi: 10.2307/2683468.
- Medlyn, B. E. et al. (2001) 'Stomatal conductance of forest species after long-term
exposure to elevated CO₂ concentration: a synthesis', *New Phytologist*, 149, pp. 247–
264.
- Medlyn, B. E. et al. (2015) 'Using ecosystem experiments to improve vegetation
3610 models', *Nature Clim. Change*. Nature Publishing Group, a division of Macmillan
Publishers Limited. All Rights Reserved., 5(6), pp. 528–534. doi:
10.1038/nclimate2621.
- Meinzer, F. C., James, S. A. and Goldstein, G. (2004) 'Dynamics of transpiration, sap
flow and use of stored water in tropical forest canopy trees', in *Tree Physiology*. doi:
3615 10.1093/treephys/24.8.901.

Monteith, J. L. and Unsworth, M. H. (2013) 'Principles of Environmental Physics: Plants, Animals, and the Atmosphere', in. Available at: <https://api.semanticscholar.org/CorpusID:126432457>.

Morecroft, M. D. and Roberts, J. M. (1999) 'Photosynthesis and stomatal conductance
3620 of mature canopy Oak (*Quercus robur*) and Sycamore (*Acer pseudoplatanus*) trees
throughout the growing season', *Functional Ecology*. John Wiley & Sons, Ltd, 13(3),
pp. 332–342. doi: 10.1046/j.1365-2435.1999.00327.x.

Murray, F. W. (1967) 'On the Computation of Saturation Vapor Pressure', *Journal of
Applied Meteorology and Climatology*. Boston MA, USA: American Meteorological
3625 Society, 6(1), pp. 203–204. doi: 10.1175/1520-
0450(1967)006<0203:OTCOSV>2.0.CO;2.

Norby, R. J. et al. (2016) 'Model-data synthesis for the next generation of forest free-
air CO₂ enrichment (FACE) experiments', *New Phytologist*, 209(1), pp. 17–28. doi:
10.1111/nph.13593.

3630 Norby, R. J. and Luo, Y. (2004) 'Evaluating ecosystem responses to rising atmospheric
CO₂ and global warming in a multi-factor world', *New Phytologist*. doi: 10.1111/j.1469-
8137.2004.01047.x.

Oren, R. et al. (2001) 'Soil fertility limits carbon sequestration by forest ecosystems in
a CO₂-enriched atmosphere', *Nature*, 411(6836), pp. 469–472. doi:
3635 10.1038/35078064.

Oren, R. and Pataki, D. E. (2001) 'Transpiration in response to variation in microclimate
and soil moisture in southeastern deciduous forests', *Oecologia*, 127(4), pp. 549–559.
doi: 10.1007/s004420000622.

- Paschalis, A. et al. (2024) 'When do plant hydraulics matter in terrestrial biosphere modelling?', *Global Change Biology*. John Wiley & Sons, Ltd, 30(1), p. e17022. doi: 10.1111/gcb.17022.
- Posit Team (2024) 'RStudio: Integrated Development Environment for R'. Boston, MA: R. Posit Software, PBC. Available at: <http://www.posit.co/>.
- Quick, S. E. (2025) Tree-soil-water relations under elevated CO₂ – following the flow. University of Birmingham. doi: tba.
- Quick, S. E. et al. (2025) 'Water usage of old-growth oak at elevated CO₂ in the FACE (Free-Air CO₂ Enrichment) of climate change', *Biogeosciences*. Copernicus Publications Göttingen, Germany, 22(6), pp. 1557–1581. doi: 10.5194/bg-22-1557-2025.
- R Core Team (2024) 'R version 4.4.1'. Vienna, Austria: R Foundation for Statistical Computing. Available at: <https://www.r-project.org/>.
- Renninger, H. et al. (2015) 'Resource use and efficiency, and stomatal responses to environmental drivers of oak and pine species in an Atlantic Coastal Plain forest ', *Frontiers in Plant Science* . Available at: <https://www.frontiersin.org/articles/10.3389/fpls.2015.00297>.
- Renninger, H. J. et al. (2014) 'Physiological strategies of co-occurring oaks in a water- and nutrient-limited ecosystem', *Tree Physiology*, 34(2), pp. 159–173. doi: 10.1093/treephys/tpt122.
- Schäfer, K. V. R. (2011) 'Canopy Stomatal Conductance Following Drought, Disturbance, and Death in an Upland Oak/Pine Forest of the New Jersey Pine Barrens,

USA', *Frontiers in Plant Science*. Available at:
<https://www.frontiersin.org/articles/10.3389/fpls.2011.00015>.

Shirke, P. A. and Pathre, U. V (2004) 'Influence of leaf-to-air vapour pressure deficit (VPD) on the biochemistry and physiology of photosynthesis in *Prosopis juliflora**,
3665 *Journal of Experimental Botany*, 55(405), pp. 2111–2120. doi: 10.1093/jxb/erh229.

Stokes, V. J., Morecroft, M. D. and Morison, J. I. L. (2010) 'Comparison of leaf water use efficiency of oak and sycamore in the canopy over two growing seasons', *Trees*, 24(2), pp. 297–306. doi: 10.1007/s00468-009-0399-8.

Stoy, P. C. et al. (2006) 'Separating the effects of climate and vegetation on
3670 evapotranspiration along a successional chronosequence in the southeastern US',
Global Change Biology. John Wiley & Sons, Ltd, 12(11), pp. 2115–2135. doi:
10.1111/j.1365-2486.2006.01244.x.

Tang, J. et al. (2006) 'Sap flux–upscaled canopy transpiration, stomatal conductance, and water use efficiency in an old growth forest in the Great Lakes region of the United
3675 States', *Journal of Geophysical Research: Biogeosciences*. John Wiley & Sons, Ltd, 111(G2). doi: 10.1029/2005JG000083.

Tetens, O. (1930) 'Über einige meteorologische Begriffe.', *Z. Geophys* 6, pp. 297–309.

Tor-ngern, P. et al. (2015) 'Increases in atmospheric CO₂ have little influence on transpiration of a temperate forest canopy', *New Phytologist*. John Wiley & Sons, Ltd,
3680 205(2), pp. 518–525. doi: 10.1111/nph.13148.

Valladares, F. et al. (2016) 'Shedding light on shade: ecological perspectives of understorey plant life', *Plant Ecology & Diversity*. Taylor & Francis, 9(3), pp. 237–251. doi: 10.1080/17550874.2016.1210262.

3685 Valladares, F. and Niinemets, Ü. (2008) 'Shade tolerance, a key plant feature of complex nature and consequences', *Annual Review of Ecology, Evolution, and Systematics*, 39, pp. 237–257. doi: 10.1146/annurev.ecolsys.39.110707.173506.

Wang, H., Guan, H. and Simmons, C. T. (2016) 'Modeling the environmental controls on tree water use at different temporal scales', *Agricultural and Forest Meteorology*. Elsevier B.V., 225, pp. 24–35. doi: 10.1016/j.agrformet.2016.04.016.

3690 Wang, W. et al. (2020) 'Tree growth, transpiration, and water-use efficiency between shoreline and upland red maple (*Acer rubrum*) trees in a coastal forest', *Agricultural and Forest Meteorology*, 295, p. 108163. doi: 10.1016/j.agrformet.2020.108163.

3695 Ward, E. J. et al. (2018) 'Evapotranspiration and water yield of a pine-broadleaf forest are not altered by long-term atmospheric [CO₂] enrichment under native or enhanced soil fertility', *Global Change Biology*. Wiley/Blackwell (10.1111), 24(10), pp. 4841–4856. doi: 10.1111/gcb.14363.

Warren, J. M. et al. (2011) 'Ecohydrologic impact of reduced stomatal conductance in forests exposed to elevated CO₂', *Ecohydrology*, 4(2), pp. 196–210. doi: 10.1002/eco.173.

3700 Warren, J. M., Norby, R. J. and Wullschleger, S. D. (2011) 'Elevated CO₂ enhances leaf senescence during extreme drought in a temperate forest', *Tree Physiology*, 31(2), pp. 117–130. doi: 10.1093/treephys/tpr002.

- Wullschleger, S. D. et al. (2002) 'Sensitivity of stomatal and canopy conductance to elevated CO₂ concentration – interacting variables and perspectives of scale', *New Phytologist*. John Wiley & Sons, Ltd, 153(3), pp. 485–496. doi: 10.1046/j.0028-646X.2001.00333.x.
- Xu, Z. et al. (2016) Elevated-CO₂ Response of Stomata and Its Dependence on Environmental Factors , *Frontiers in Plant Science* . doi: 10.3389/fpls.2016.00657.
- Yi, K. et al. (2017) 'Dynamics of stem water uptake among isohydric and anisohydric species experiencing a severe drought', *Tree Physiology*, 37(10), pp. 1379–1392. doi: 10.1093/treephys/tpw126.
- Zhang, M. et al. (2017) 'A global review on hydrological responses to forest change across multiple spatial scales: Importance of scale, climate, forest type and hydrological regime', *Journal of Hydrology*. Elsevier, 546, pp. 44–59. doi: 10.1016/J.JHYDROL.2016.12.040.

Chapter 7 : Tree-Soil-Water relations summary.

In this chapter, the key research findings are summarised and synthesized.

Thesis Summary.

This thesis has recounted the original research undertaken to look at three species of
3720 mature trees which were exposed to eCO_2 or to associated control conditions. The
research generates novel understanding concerning water flow into, through and out
of mature trees in a temperate forest plantation containing dominant oak and
associated subdominant species trees. The overarching aim of this thesis was to
identify any potential changes in the tree hydraulic responses, e.g. water usage, of
3725 trees under eCO_2 concentrations. This thesis gives insight into responses of both
dominant old forest-grown oak (*Q. robur*) trees as well as two subdominant species,
sycamore and hawthorn situated in a high canopy, mixed species, mature forest
ecosystem with exposure to eCO_2 .

This thesis has rested on a large body of previous literature concerning water flow in
3730 trees and at the interface to soil and atmosphere as outlined throughout this thesis.
The research was undertaken against a background of assumptions, aims and
objectives outlined in Ch.1. Plant hydraulics water-energy such as described by Philip,
(1966) in the soil–plant–atmosphere continuum (SPAC), alongside concurrent
assumptions concerning carbon-water and carbon-energy cycle relationships, were
3735 the foundation. That focus of whole-plant hydraulics, functioning in three component
strands, drove measurement, data check and analysis within this thesis in the novel
environment presented by the BIFoR FACE long-term eCO_2 manipulation experiment.

Traditional SPAC studies rarely include consideration of both plant hydraulics and the effects of eCO_2 which can be offered by FACE experiments.

- 3740 The novelty of approach (within the SPAC principles) was the linking of those differing points of interest within the continuum which could be more easily measured, namely the input soil-water, the integral stem xylem sap-water, and the output transpiration (leaf) water vapour characterised by both seasonal (e.g. daylength, light-energy) and meteorological/ environmental (e.g. precipitation, air temperature, RH, VPD) drivers.
- 3745 Outside of the three themes I also considered inputs from precipitation as well as possible groundwater and deep root rehydration effects. Hence I considered the effects of experimentally applied eCO_2 within the whole of the tree water relations paradigm. In the light of these considerations, the project has been ambitious but has achieved most of its broad aims.
- 3750 The research presented in this thesis was motivated by an identified gap in the study of temperate forest ecosystem plant-water changes under eCO_2 . Tree-focused studies concerning mature deciduous high forest species are particularly rare and present challenges in experimental design. Large scale eCO_2 and plant-water forest field experiments, where there are restrictions to tree felling and intrusive instrumentation,
- 3755 have only rarely been studied for long periods of time (greater than a single season). There was a perceived need to rapidly predict how under eCO_2 such forests would adjust, especially since they may in future experience eCO_2 in combination with other climate changes such as drought, flood and increasing temperature (IPCC, 2022).

Drawing together plant water flow themes

- 3760 Here I primarily examined the plant hydraulic responses within an old forest oak plantation FACE experiment alongside more limited examination of larger stem (>

10cm DBH) examples of subdominant species hawthorn and sycamore (Chapters 3 and 5) during years 1 to 6 of the FACE experiment. I also considered the ability of oak to maintain whole tree transpiration under dynamically varying soil moisture status (Chapter 4). In conclusion, I conducted leaf-level measurements enabling the calculation of leaf transpiration for all three tree species studied. This provided an opportunity to consider and contrast middle-day dynamics of leaf-transpiration and whole-tree transpiration (Chapter 6).

By considering two levels of control ($a\text{CO}_2$ and *Ghost*) the study was able to conclude more far-reaching conclusions concerning FACE experiments in semi-natural forest, specifically in respect of the changes to available soil-water and the tree water usage effects of the FACE infrastructure compared with no-infrastructure semi-natural forest trees.

Firstly, I designed an experiment to measure sap flow in 18 oak trees spread equally across array within the FACE experiment treatments. This provided comprehensive cross-treatment data for seasons 3 to 5 of the FACE experiment, enabling examination of the daytime xylem sap flux response of such trees to $e\text{CO}_2$ and to the effects of the FACE infrastructure (Ch. 3). The study incorporated a normalisation process for variation in tree size, specifically stem radius and projected canopy area. This was primarily to quantify the water usage of the trees under $e\text{CO}_2$ treatment but also to determine relative tree water usage effects of the treatments.

Secondly, a soil-water analysis study was conducted based on multi-transducer shallow soil and depth profile (to 1 m depth) VWC measurement in all treatments (Ch. 4). Profile measurements exhibited poor reliability. No transducers had been calibrated

3785 for the soil type in which they sat, such that, in accordance with best practice, I decided
to normalise the data to produce season-by-season relative extractable water (REW)
for treatment comparison. This also had the benefit of overcoming some of the effects
of the differing measurement technology used in different transducer types. Finally,
and as a back check, I compared the limited resulting data from the Enviroscan profile
3790 transducers to the more reliable Delta-T profile VWC transducers installed in year 5.
The shallow raw VWC data was also used to examine responses to early summer dry
periods to measure VWC decrease rates.

Thirdly, and as an extension to the first study to include the stem-flow responses of
sub-dominant species to the treatments, I designed a sap flow study of two species.
3795 Then I extracted and processed sap flux data from these medium-sized hawthorn and
sycamore trees within such arrays as contained them (Ch. 5). There was not an
opportunity to process these data to a full analysis, but some of the results were used
in the leaf-level study.

The Fourth study involved taking in-situ and cut-twigg middle-day measurements of
3800 individual leaves from the three tree species of oak, hawthorn and sycamore over
seasons 3 to 6 of the FACE experiment (Ch. 6). For oak, I either accessed the top
canopy in-situ, or used the leaves of freshly cut twigs where canopy access was not
available to me for these measurements (e.g. in the *Ghost* arrays). For hawthorn and
sycamore the measurements were taken from the bottom tree canopy using ladders,
3805 or deer stands, or directly from the ground. A simple porometer was used for measuring
stomatal conductances in all cases and leaf temperatures were also measured. The
resulting data, in combination with environmental parameter data, enabled calculation
of leaf transpiration and comparisons between these results and those from the xylem

sap flux studies (i.e. whole tree transpiration). The objective was primarily to consider
 3810 how the differing scales of transpiration measurement compared, which also enabled
 speculative exploration of up-scaling of leaf-to-tree and tree-to array patch.

Key findings

The key findings from the research in this thesis were as follows:

Water usage changes in responses to eCO₂ and due to FACE infrastructure

3815 In Chapter 3, the authors report the results of six trees monitored in each of the three
 FACE treatments: infrastructure arrays of (+150 $\mu\text{mol mol}^{-1}$) eCO₂; FACE infrastructure
 control aCO₂ arrays; and control *Ghost* (no-treatment-no-infrastructure) arrays. A
 linear proportionality of *TWU* to tree stem radius, R_b (ca. 3.1 litres d⁻¹ mm⁻¹, 274
 mm $\leq R_b \leq$ 465 mm) was found, which aligned and extended previously derived similar
 3820 relationships for smaller oak trees (e.g. Schmidt, 2007).

R_b was also a very good proxy for projected canopy area, A_c (m²), which was linearly
 proportional to R_b (ca. 617 m² m⁻¹) which implied a mean July water usage of ca. 5
 litres d⁻¹ m⁻² of projected oak canopy in the BIFoR FACE forest. We reported whole-
 season treatment effects following normalisation of *TWU* by individual tree R_b , deriving
 3825 TWU_n (litres d⁻¹ mm⁻¹), differing year on year, alongside July-only results. To our
 knowledge this form of whole tree water usage normalisation has not previously been
 applied.

We found that, in 2019 and 2021 seasons, after correction for repeated-measures by
 individual tree, there was a 13–16%, reduction in eCO₂ TWU_n compared with aCO₂
 3830 TWU_n , with a marginal 4% reduction in 2020. Whilst tests indicated that these results
 were not statistically significant, immediate weather (i.e. sunshine, air temperature/

relative humidity) conditions during those three years differed considerably. There were also legacy effects from a drought in summer 2018 affecting soil-water sources during 2019 and probably 2020. These findings are unlikely to reflect inherent
3835 differences between ambient and elevated trees before switching on the CO₂ as a consistent normalisation relating to stem and xylem size was applied to all oaks.

Control trees exhibited a significant 27% increase in $a\text{CO}_2 \text{ TWU}_n$ compared with *Ghost* tree TWU_n in whole-season 2019, with lesser, non-significant fixed effects in 2020 and 2021. These latter findings have not been fully investigated to determine any direct
3840 causes to explain these effects. Several factors may contribute: the installation or operation of FACE infrastructure; array-specific differences in soil moisture, slope, soil respiration; or the mix of sub-dominant tree species present. These may form a basis for future research and generic validation of FACE study results.

Our results of normalised per-tree water savings under eCO₂ align with sap flow results
3845 for other FACE experiments and greatly extend the duration of observations for oak, elucidating seasonal patterns and interannual differences. Our tree-centred viewpoint complements leaf-level and ground-based measurements to extend our understanding of plant-water usage in old growth oak forest.

Additionally, in Chapter 5, the responses of sycamore and hawthorn trees are reported
3850 with a view to inclusion in a future joint research publication.

Soil-water dynamics.

In Chapter 4, I reported analysis of key parameters associated with the soil, the trees and the environment to determine whether there were any perceived CO₂ effects on soil-water to plant-water dynamics. I compared daily averaged volumetric water

3855 content (VWC, %) and relative extractable water (REW) data between treatment types. I found that normalisation of VWC, by calculation of REW for each sensor type according to depth, using field capacity and wilt point VWC derived from the field data across multiple years, enabled comparison the treatment effects at different profile depths. During the leaf-on treatment seasons 2 to 5 of the experiment, eCO_2 REW
3860 down to 40 cm depth was found to be consistently higher than that of the ambient aCO_2 treatment, with these effects decreasing by depth but there were too few locations of reliable profile data (four locations) to undertake meaningful statistical comparisons. CS sensors (at shallow 12cm depth) provided the most reliable data across the treatments. Seven-year normalisation of shallow VWC data (REW_{7yr}) over seasons 2
3865 to 7 of the experiment, gave better inter-seasonal results. I found that leaf-on season infrastructure data $eCO_2:aCO_2$ analysis showed significantly higher REW_{7yr} (+2.3%, F value 4.9 , $p=0.027$) under eCO_2 . Qualitative correlation of differing transducer technology for shallow (CS) and profile (Enviroscan) sensors gave comparable REW results.

3870 During early summer drought periods shallow VWC decrease slopes were examined across the treatments. Slope varied by starting VWC values with smallest slopes corresponding to low VWC, but no treatment-related effects seemed to be present.

Qualitative correlation of data from Enviroscan and Delta-T profile transducers was undertaken to validate the REW findings within the profile. This visual correlation
3875 demonstrated the heterogeneity of soil conditions across the site reinforcing the decision not to undertake in-situ calibration. VWC and REW at shallow depths decreased similarly across treatments during drought.

Transpiration derived from leaf and stem water flow measurements

In Chapter 6, I report measurement of stomatal conductance and leaf temperature on
3880 in-situ and cut-twig leaves and calculation of leaf transpiration for three tree species
under three experimental conditions, elevated CO₂ (eCO₂) at +150 $\mu\text{mol mol}^{-1}$ and two
ambient controls (aCO₂, ghost). We compared middle-day oak leaf transpiration
patterns across treatment seasons 3 to 5 (2019–2021). For in-situ data in July and
August only, significant reduction in leaf transpiration was found under eCO₂ compared
3885 to aCO₂ conditions in each of the measurement years (median % differences: 2019: ~
-40%, 2020: ~ -23%, 2021: ~ -30%). Whole season in-situ data only showed significant
reduction in 2020 (median % differences: ~ -21%).

Under eCO₂ sycamore and hawthorn subdominant species trees' leaves, respond
differently to dominant oak leaves. This may be due to their position within the canopy
3890 leading to greater shading and to their experiencing of a different subcanopy
microclimate. Comparison between the two subdominant species show that there is a
species-specific response even within very similar subcanopy environments. These
effects are likely to be driven by species leaf size or stomatal density. The opportunity
to compare bottom canopy transpiration of dominant oak trees to these two species'
3895 results was not possible within this study period.

I looked for measurable patterns of peak daily leaf transpiration across each leaf-on
month in the seasonal cycle, but ascertained that, without consistent measurements
on the same days for all species, alongside more localised recording of incident
photosynthetically active radiation (PAR), the patterns were not clearly defined. Data
3900 over the three years combined indicated that peak oak leaf transpiration might occur
between days 202 (20th July) and 232 (20th Aug) in any one year within the middle-day

period studied. A high transpiration day also occurred in September 2020 (Fig 8) for all species, but the drivers for this have not yet been identified. Synchronised field data from all species, collected in a more resource intensive campaign alongside
3905 microclimate data, might facilitate proper interpretation of these results in future.

I looked for synchronisation between peak daily leaf transpiration and peak daytime sap flux within each tree for trees of the same species (especially oak). My aim was to determine if there were delays between the usage of water sourced in the soil and water stored within the tree, possibly at branch level. There were different dynamics
3910 evident in the leaf data due to their collection over a shorter time period. From this experiment, it has not possible to determine if a measurable time delay occurs between reaching the daily peak values of leaf transpiration and time of peak sap flux measured at stem level. Variation of relative humidity and air temperature surrounding the leaf may be more influential in determining VPD than previously thought. There may also
3915 be more complex drivers within the leaf and branch which determine how the water sources are used. The drying effects of air movement at the leaf were not considered in this experiment, leading to an inability to conclude whether synchronisation occurred especially in respect of top oak canopy.

Benefits, Limitations and Opportunities.

Benefits

The experiments summarised above were designed to enable some increased deductions concerning how existing forests will respond to rising CO₂ (Norby *et al.*, 2016). This information now enables improved modelling of water flow and water-usage in mature forest systems under current and future climate conditions.

3925 Trees are individual, with species-specific characteristics and traits. We were fortunate
in being able to focus our study on a relatively uniform-aged plantation of old oak trees
for the FACE experiment, within which this thesis project resided. We have
demonstrated that the use of four thermocouple positions across the sapwood for each
of our oak HPC probe sets has enabled us to successfully capture the position and
3930 size of point sap flux density (derived from sap velocity) giving a more reliable basis to
explore the effects of tree size on both whole-probe-set sap flux and TWU. Two trees
(out of ~six per array) each with two probesets was considered an appropriate sample.

Some of the data so produced is already openly available through repositories and
more will consequently be made available. The data can inform several multifaceted
3935 questions concerning elevated CO₂. As well as further tree hydraulics studies they can
also serve to guide and support studies concerning both carbon assimilation and
extreme weather patterns (e.g. re high temperatures and drought).

There are additional benefits offered by the experiments reported here, especially the
study of the semi-natural conditions within the *Ghost* arrays. This makes this study
3940 exceptionally broad in its impact both for future FACE or other eCO₂ field experiments
and for its contribution of wider knowledge concerning temperate deciduous forests.

Whilst part-time studentship can be viewed as a benefit in long-term research,
timeliness is also an important criteria in climate-related research, such that sharing of
data, even prior to full analysis, has been seen as an essential target of the work.

3945 **Limitations**

With hindsight it could be said that this was a project way beyond the expected remit
of one student researcher. These limitations have meant that the breadth and depth of

research has had to accommodate the expertise and resource limitations which I have presented to the work – a resource balance approach.

3950 Several difficulties emerge in drawing together the results. Efforts were made to tackle full analysis of sap flow data from monitoring of subdominant species for comparison with the dominant plantation oaks. The finalisation of analysis of these data could not be completed within the scope of this study. The data is limited by the presence/absence of uniform spread of these tree species across the experimental arrays. Good
3955 quality data has been captured from successful sap flow, stomatal conductance and leaf temperature monitoring for future exploration.

With respect to sap flux measurement in oaks and subdominant trees there are known limitations in the ability of the HPC system to measure low and reverse sap velocities (Forster, 2017; Quick *et al.*, 2025) and to some extent high sap velocities (Burgess *et al.*, 2001). It would be wise to weigh up the benefits of other types of heat pulse based
3960 sensors if a repeat monitoring exercise takes place.

Wind effects have not been considered as the assumption has been that the experimental forest experiences similar wind speeds and directions across the experimental patches. Outputs affect localised weather and atmospheric conditions
3965 such as air temperature and relative humidity, but we have restricted the boundary of this study to within the forest scale landscape rather than to look at these larger scale interactions.

The interactions tree-to-soil and tree-to-atmosphere posed challenges from the point of view of measurement and scale. Soil-water measurements were both extremely
3970 heterogeneous and not well compartmentalised by soil depth across patches and

across the forest. There were also confounding effects, e.g. from soil microbial activity, underwater stream flow or large root obstructions, which could not be considered (the known-unknowns) despite parallel studies of, for example, soil respiration. Leaf stomatal conductance measurement (for calculation of transpiration) could not always
3975 be undertaken in-situ which altered the ability to interpret results. Whilst there are advantages to using a simpler device such as a porometer (see Ch.6) the device also limits concurrent measurement of external parameters during the campaign.

Opportunities

From the three plant-water strands presented a consistent picture is emerging. The
3980 results from stem sap flux of oak indicate a trend to lower water usage under eCO_2 , but future studies could benefit from a fuller assessment of canopy leaf cover and extent, possibly by use of Terrestrial Laser Scanning (TLS) or drone based LiDAR (Light Detection and Ranging). This would, as a bonus, also support studies which try to assess any changes to carbon allocation under different treatments. For example,
3985 greater immediate season carbon storage in leaves might explain inter-year differences in water usage per tree despite normalisation by stem size. Equally, longer term measurement of changes and preferred depths fine root growth (i.e. enhanced carbon storage or drought resilience) could also affect tree water usage. This type of research is currently in progress and complements the work reported
3990 herein.

Further use of the VWC and REW data within statistical or process models to inform the sap flux and leaf transpiration findings might usefully be undertaken to better elucidate soil-water-plant-water responses to eCO_2 within this FACE experiment. Time

lag analysis could also reveal more fully the effects of drought on subsequent leaf
3995 seasons.

It is likely that even if weather were the main driver of inter-season differences in water
usage, it might take longer than three years to see trends due to the large variation in
single year results. To more fully understand and verify such trends better data
extraction of PAR and wind effects might be required a repeat experiment to remeasure
4000 sap flux over several years, especially in oak, is also recommended.

Future work could offer an opportunity to look more closely at the measurement
limitation of sap flux vav. leaf flux, in particular the apparent speed at which individual
leaf transpiration can change dynamically (at least every minute), as shown from our
porometry results, and other problems of upscaling. Lessons can be learnt from other
4005 studies using sap flux data representing canopy transpiration from trees (Poyatos *et al.*, 2020). Potential application areas for transpiration data and results include: (a)
plant ecophysiology and functional ecology (e.g. see (Kannenber *et al.*, 2022)), where
networked transpiration information can inform, but where a continuously updated
network of open data is still lacking. The enormous variation in the regulation of
4010 transpiration across and within taxa influences both informs and limits the upscaling
from plant based to landscape scale knowledge; (b) ecosystem ecology and
ecohydrology (e.g. see (Wilkening *et al.*, 2024)), where the need to overcome
limitations of 'siloeed' thinking has catalysed closer collaboration between the
ecophysiological and ecohydrological communities resulting in exciting advances in
4015 thinking about SPAC mechanisms and model verification from plant transpiration. The
SAPFLUXNET database could usefully be extended to include more recent
contributions such as are reported here especially as mature oak is poorly represented

in the database currently. In the interim the unique nature of this FACE experimental sap flux data can be utilised stand-alone on an open-access basis in support of the suggested research gaps. One such study concerning higher temperatures has already been completed resulting in a manuscript submitted by Gardner et al. for peer review (submitted 2025).

It is known that each sap flow sensor method is subject to methodological and scaling issues. Most method comparison studies (e.g. (Čermák, Kučera and Nadezhdina, 2004; Vandegehuchte and Steppe, 2012)) only consider the most popular, or most error-prone methods which require calibration. The heat dissipation (HD) method forms 66.4% of the SAPFLUXNET data base (Poyatos *et al.*, 2020). Opportunities to assess the best method will arise for each future project.

When comparing data from differing sources (i.e. differing transducer types having differing maximum sampling rates) I have learnt that comparison needs to be planned to take into account the maximum likely frequency dynamics of the measurement response which is not always known in advance. Rather than assuming that slow and fast processes form a continuum within the plant at differing scales, this revised approach will enable me to design more successful and focused experiments within future field studies.

Finally, in reflecting on the challenges of combining my plant-water related results from soil, stem and leaf, I now recognise the following challenges which emerge from such a medium-term project, namely: achieving appropriate sampling levels to represent measurement of available soil-water for a given tree within a heterogeneous soil environment (present in all forest subsurface environments) and apportionment of

water loss to all above ground evaporative and transpiration-related sinks; linking individual tree characteristics to stem-water measurement so as to normalise and compare different treatment effects and correspondingly identify water sources and sinks; upscaling from leaf to whole tree (and ultimately to patch or forest scale, see
4045 water use efficiency (WUE) in (Walker *et al.*, 2021; Way, Cook and Rogers, 2021); comprehensive baseline and ongoing appreciation of the tree-based and species-based biomass production characteristic dynamics (e.g. see (Walker *et al.*, 2019; Way, Cook and Rogers, 2021; Norby *et al.*, 2024; Norby, 2025)). Design of medium-term projects within long-term manipulation experiments aimed at contributing novelty
4050 and/or more comprehensive quality data to improve mechanistic understanding could, in future, benefit from consideration of these challenges. I would suggest that, in the interest of research efficiency, multiple sources of expertise are combined (Way, Cook and Rogers, 2021) and pooled to inform the project design, research questions (e.g. by updating (Norby and Zak, 2011)) and aims are regularly adapted and updated to
4055 reflect current knowledge and lessons learnt.

References Chapter 7

Burgess, S. S. O. et al. (2001) 'An improved heat pulse method to measure low and reverse rates of sap flow in woody plants†', *Tree Physiology*, 21(9), pp. 589–598. doi: 10.1093/treephys/21.9.589.

4060 Čermák, J., Kučera, J. and Nadezhdina, N. (2004) 'Sap flow measurements with some thermodynamic methods, flow integration within trees and scaling up from sample trees to entire forest stands', *Trees*, 18(5), pp. 529–546. doi: 10.1007/s00468-004-0339-6.

Forster, M. (2017) 'How Reliable Are Heat Pulse Velocity Methods for Estimating Tree Transpiration?', *Forests*. Multidisciplinary Digital Publishing Institute, 8(9), p. 350. doi: 10.3390/f8090350.

IPCC (2014) IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)],
4070 IPCC. Geneva, Switzerland. doi: 10.1016/S0022-0248(00)00575-3.

IPCC (2022) IPCC, 2022: Climate Change 2022: Working Groups II contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.

Kannenbergh, S. A. et al. (2022) 'Opportunities, challenges and pitfalls in characterizing plant water-use strategies', *Functional Ecology*. John Wiley & Sons, Ltd, 36(1), pp. 24–
4075 37. doi: 10.1111/1365-2435.13945.

Norby, R. J. et al. (2016) 'Model-data synthesis for the next generation of forest free-air CO₂ enrichment (FACE) experiments', *New Phytologist*, 209(1), pp. 17–28. doi: 10.1111/nph.13593.

- Norby, R. J. et al. (2024) 'Enhanced woody biomass production in a mature temperate forest under elevated CO₂', *Nature Climate Change*. doi: 10.1038/s41558-024-02090-3.
- Norby, R. J. (2025) 'Forest productivity response to elevated CO₂ in free-air CO₂ enrichment experiments: the 23 percent solution, revisited', *New Phytologist*. John Wiley & Sons, Ltd, n/a(n/a). doi: 10.1111/nph.70162.
- Norby, R. J. and Zak, D. R. (2011) 'Ecological and evolutionary lessons from free air carbon enhancement (FACE) experiments', *Annual Review of Ecology, Evolution, and Systematics*, 42, pp. 181–203. doi: 10.1146/annurev-ecolsys-102209-144647.
- Philip, J. R. (1966) 'Plant Water Relations: Some Physical Aspects', *Annual Review of Plant Physiology. Annual Reviews*, 17(1), pp. 245–268. doi: 10.1146/annurev.pp.17.060166.001333.
- Poyatos, R. et al. (2020) 'Global transpiration data from sap flow measurements: the SAPFLUXNET database', *Earth System Science Data*. doi: 10.5194/essd-2020-227.
- Quick, S. E. et al. (2025) 'Water usage of old-growth oak at elevated CO₂ in the FACE (Free-Air CO₂ Enrichment) of climate change', *Biogeosciences*. Copernicus Publications Göttingen, Germany, 22(6), pp. 1557–1581. doi: 10.5194/bg-22-1557-2025.
- Schmidt, M. (2008) Canopy transpiration of beech forests in Northern Bavaria – Structure and function in pure and mixed stands with oak at colline and montane sites. Bayreuth, . University of Bayreuth. Available at: <https://epub.uni-bayreuth.de/646/>.

4100 Vandegehuchte, M. W. and Steppe, K. (2012) 'Improving sap flux density measurements by correctly determining thermal diffusivity, differentiating between bound and unbound water', *Tree Physiology*, 32(7), pp. 930–942. doi: 10.1093/treephys/tps034.

Walker, A. P. et al. (2019) 'Decadal biomass increment in early secondary succession
4105 woody ecosystems is increased by CO₂ enrichment', *Nature Communications*. Nature Publishing Group UK London, 10(1), p. 454. doi: 10.1038/s41467-019-08348-1.

Walker, A. P. et al. (2021) 'Integrating the evidence for a terrestrial carbon sink caused by increasing atmospheric CO₂', *New Phytologist*. John Wiley & Sons, Ltd, 229(5), pp. 2413–2445. doi: 10.1111/nph.16866.

4110 Way, D. A., Cook, A. and Rogers, A. (2021) 'The effects of rising CO₂ concentrations on terrestrial systems: scaling it up', *New Phytologist*. John Wiley & Sons, Ltd, 229(5), pp. 2383–2385. doi: 10.1111/nph.17096.

Wilkening, J. V et al. (2024) 'Different roads, same destination: The shared future of plant ecophysiology and ecohydrology', *Plant, Cell & Environment*. John Wiley & Sons,
4115 Ltd, 47(9), pp. 3447–3465. doi: 10.1111/pce.14937.

Chapter 8 : Conclusions & suggestions for future work

Future work

Research findings reported here have highlighted how long-term manipulation experiments such as BIFoR FACE offer opportunities to study mature trees' responses to climate changes in a natural setting. Uncertainties are declared as well as those effects deemed significant, to inform future endeavours.

Variation in water usage, by differing species experiencing increases in atmospheric CO₂, has been confirmed to follow the patterns of resilience predicted by previous research. In this instance a wider range of seasonal effects have been considered compared to previous studies as well as a longer duration of monitoring for stem xylem sap flux.

Below I outline five research areas for further investigation or data usage which might enable a more comprehensive understanding of mature forest plant hydraulic responses to increasing rises in CO₂ including how this might affect available soil-water and atmospheric impacts in the future.

Long term effects of eCO₂ on tree responses

Drawing parallels with the related thesis on carbon assimilation (Gardner, 2023), this study indicates that these early results, primarily from years 3 to 5 of the FACE experiment, might usefully be extended and repeated in later years of the manipulation experiment (e.g. years 9 to 11) to embrace seasonal weather perturbations, preferably using as a minimum the same numbers of trees of the same species, and using similar or improved instrumentation types for sap flow and VWC. Oak results obtained here might exhibit a particular response to a drought year such as 2018 (Perkins *et al.*, 2018;

Bose *et al.*, 2021) superimposed on the effects of eCO₂. Additionally, measurements
4140 might be better aligned in time and space with other related measurements being made
within the experiment. This might include repeat dendrochronology sampling (Norby *et al.*, 2024; Popa *et al.*, 2025) and water isotope studies (Fabiani *et al.*, 2022), or
repeating measurements of, for example, stomatal conductance measured by leaf gas
chambers as well as porometers, or extended use of 3D tree canopy measurements
4145 for the selected tree species using portable terrestrial laser scanners.

Inter-species comparisons

Combining the plant and soil hydraulics data I have collected here with that from other
mature deciduous temperate forest sites (e.g. for differing dominant species such as
Ash or Beech) will widen the impact of this current study, even if it is not possible to
4150 treat such forests with eCO₂. As time progresses we know that levels of CO₂ will
continue to rise and be sustained for a considerable period, such that monitoring of
high ambient levels in semi-natural forest will increasingly be of importance, rather than
artificial elevation using, for example, FACE infrastructure.

Cross and inter forest scaling

4155 Between-species comparison of tree water usage has not yet been completed using
trees for the three treatment conditions, but there is now sap flux data available at a
tree level. Such comparisons, albeit specific to this BIFoR forest site, may provide
additional insights into variation of tree species' response to eCO₂.

Whilst some attempts have been made in this project to provide data at leaf- and tree-
4160 scale for three species, it is recognised that more can be done to generalise the results
from the studies reported here. Measurements reported here have made some inter-

year, inter-season comparisons, but could also be scaled up across forest space, using for example leaf area index (LAI, m^2 (leaf) m^{-2} (ground)) alongside species density, and over time in respect of environmental and tree species' response variables (e.g. using links to precipitation, throughfall and/ or air temperature/ RH/ VPD data). For comparison across forests, an update of the β assessment at tree scale (Walker *et al.*, 2021; Way, Cook and Rogers, 2021) to include BIFoR FACE carbon assimilation and water use efficiency results would be desirable.

Development of data: System relations

Building a system relationship or process model aligned to available environmental data and based on selected empirical plant and soil hydraulic data, would be a good next step in using data from this study. Proposed new approaches enabling interface with land surface models are also a consideration bearing in mind the scarcity of tree-based data for climate change prediction (Way, Cook and Rogers, 2021).

Lessons learnt with respect to monitoring plant and soil hydraulic variables

Long-term studies require selections of reliable, durable and sustainable solutions for monitoring of plant and soil variables. I have learnt lessons concerning selection of suitable transducers, specification of data capture capabilities and limitation of application of the transducers used in the project here described. Publication of some of these lessons learnt, alongside offering my experience to others undertaking manipulation experiments, plays a key part in fulfilling my dedication to effectively collaborating with and improving climate and general ecosystem research. From this work I hope that BIFoR, working in UK with such bodies as ECT, Woodland Trust, Forest Research, other academic institutions, and, internationally, with similar parties

4185 involved in forest manipulations experiments, might usefully help to streamline some
of the decision-making and cost-effectiveness of selection of effective measurement
devices.

Concluding remarks

Under climate change the close links between carbon assimilation and transpiration
4190 within plants necessitates a multidisciplinary or transdisciplinary approach. The
research presented in this thesis has expanded the current knowledge concerning
plant hydraulics for mature forest trees experiencing eCO₂. It has also provided new
information which serves to aid our predictions of the effects of elevated CO₂ on whole
tree transpiration and water usage, soil-water dynamics and leaf transpiration under
4195 varying environmental drivers. The significant new knowledge ensuing might now be
used by vegetation modellers, land-surface modellers, forest managers and forest
policy parties in respect of climate change mitigation (e.g. Nature Based solutions).
Land-cover policymakers, considering changes to catchment flows and/ or forest cover
might also use these findings to facilitate practical and timely decision-making
4200 cognisant of the combined effects of eCO₂ and increasing weather extremes (of
temperature and drought for example). Climate change will not wait for lengthy repeat
studies to be completed but researchers might usefully explore and verify these results
more thoroughly and in more depth to extract the maximum benefits and home in on
future targeted research.

4205 **References Chapter 8**

Bose, A. K. et al. (2021) 'Climate sensitivity and drought seasonality determine post-drought growth recovery of *Quercus petraea* and *Quercus robur* in Europe', *Science of The Total Environment*, 784, p. 147222. doi: <https://doi.org/10.1016/j.scitotenv.2021.147222>.

4210 Fabiani, G. et al. (2022) 'Transpiration patterns and water use strategies of beech and oak trees along a hillslope', *Ecohydrology*. John Wiley & Sons, Ltd, 15(2), p. e2382. doi: 10.1002/eco.2382.

Gardner, A. (2023) The effect of elevated CO₂ on the ecophysiology of mature *Quercus robur* in a temperate deciduous UK woodland. University of Birmingham.

4215 Available at: <http://etheses.bham.ac.uk/id/eprint/13420>.

Norby, R. J. et al. (2024) 'Enhanced woody biomass production in a mature temperate forest under elevated CO₂', *Nature Climate Change*. doi: 10.1038/s41558-024-02090-3.

Perkins, D. et al. (2018) 'Impact of Climate Trends and Drought Events on the Growth
4220 of Oaks (*Quercus robur* L. and *Quercus petraea* (Matt.) Liebl.) within and beyond Their Natural Range', *Forests*, 9(3), p. 108. doi: 10.3390/f9030108.

Popa, A. et al. (2025) 'Spatiotemporal Variability of Dendroecological Indicators in Pedunculate Oak (*Quercus robur* L.) Tree-Rings Across Europe in Relation to Species Distribution Models', *Global Change Biology*. John Wiley & Sons, Ltd, 31(11), p.
4225 e70567. doi: <https://doi.org/10.1111/gcb.70567>.

Walker, A. P. et al. (2021) 'Integrating the evidence for a terrestrial carbon sink caused by increasing atmospheric CO₂', *New Phytologist*. John Wiley & Sons, Ltd, 229(5), pp. 2413–2445. doi: 10.1111/nph.16866.

Way, D. A., Cook, A. and Rogers, A. (2021) 'The effects of rising CO₂ concentrations
4230 on terrestrial systems: scaling it up', *New Phytologist*. John Wiley & Sons, Ltd, 229(5), pp. 2383–2385. doi: 10.1111/nph.17096.

Chapter 9 : References

4235 Note: References to the publication in Chapter 3 are only contained in that Chapter, not listed here.

Ainsworth, E. A., & Rogers, A. (2007) 'The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions.', *Plant, Cell & Environment*, 30(3), pp. 258–270. doi: 10.1111/j.1365-3040.2007.01641.x.

4240 Ainsworth, E. A. and Long, S. P. (2005) 'What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂', *New Phytologist*, 165(2), pp. 351–372. doi: 10.1111/j.1469-8137.2004.01224.x.

Ainsworth, E. A. and Long, S. P. (2021) '30 years of free-air carbon dioxide
4245 enrichment (FACE): What have we learned about future crop productivity and its potential for adaptation?', *Global Change Biology*, 27(1), pp. 27–49. doi: 10.1111/gcb.15375.

Ainsworth, E. A. and Rogers, A. (2007) 'The response of photosynthesis and stomatal conductance to rising [CO₂]: Mechanisms and environmental interactions',
4250 *Plant, Cell and Environment*, pp. 258–270. doi: 10.1111/j.1365-3040.2007.01641.x.

Akhmatov, M. K., Salaš, P. (2005) 'The daytime woody plants water output by transpiration.', *Acta univ. agric. et silvic. Mendel. Brun.*, LIII(No. 2.), pp. 163–176.

Allen, L. H. et al. (2020) 'Fluctuations of CO₂ in Free-Air CO₂ Enrichment (FACE) depress plant photosynthesis, growth, and yield', *Agricultural and Forest
4255 Meteorology*, 284, p. 107899. doi: <https://doi.org/10.1016/j.agrformet.2020.107899>.

Amatya, D. (Ed.). (2016) Forest hydrology : processes, management and assessment. GEN, Boston, MA: CAB International,. doi: 10.1079/9781780646602.0000.

Anderegg, W. R. L. et al. (2015) 'Pervasive drought legacies in forest ecosystems and their implications for carbon cycle models', Science. American Association for the Advancement of Science, 349(6247), pp. 528–532. doi: 10.1126/science.aab1833.

Aranda, I. et al. (2012) 'Species-specific water use by forest tree species: From the tree to the stand', Agricultural Water Management. Elsevier, 114, pp. 67–77. doi: 10.1016/J.AGWAT.2012.06.024.

Ávila-Lovera, E., Winter, K. and Goldsmith, G. R. (2023) 'Evidence for phylogenetic signal and correlated evolution in plant–water relation traits', New Phytologist. John Wiley & Sons, Ltd, 237(2), pp. 392–407. doi: 10.1111/nph.18565.

Bachofen, C. et al. (2023) 'Stand structure of Central European forests matters more than climate for transpiration sensitivity to VPD', Journal of Applied Ecology. John Wiley & Sons, Ltd, 60(5), pp. 886–897. doi: 10.1111/1365-2664.14383.

Bachofen, C. et al. (2024) 'Tree water uptake patterns across the globe', New Phytologist. John Wiley & Sons, Ltd, 242(5), pp. 1891–1910. doi: 10.1111/nph.19762.

Bader, M. K. F., & Körner, C. (2010) 'No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO₂ enrichment', Global Change Biology. John Wiley & Sons, Ltd, 16(10), pp. 2830–2843. doi: <https://doi.org/10.1111/j.1365-2486.2010.02159.x>.

- 4280 Bader, M., Hiltbrunner, E. and Körner, C. (2009) 'Fine root responses of mature deciduous forest trees to free air carbon dioxide enrichment (FACE)', *Functional Ecology*. John Wiley & Sons, Ltd, 23(5), pp. 913–921. doi: <https://doi.org/10.1111/j.1365-2435.2009.01574.x>.
- 4285 Bader, M. K.-F. et al. (2013) 'Central European hardwood trees in a high-CO₂ future: synthesis of an 8-year forest canopy CO₂ enrichment project', *Journal of Ecology*. John Wiley & Sons, Ltd, 101(6), pp. 1509–1519. doi: 10.1111/1365-2745.12149.
- Bader, M. K.-F. et al. (2022) 'Less pronounced drought responses in ring-porous than in diffuse-porous temperate tree species', *Agricultural and Forest Meteorology*, 327, p. 109184. doi: 10.1016/j.agrformet.2022.109184.
- 4290 Baert, A. et al. (2015) 'Variable hydraulic resistances and their impact on plant drought response modelling', *Tree Physiology*, 35(4). doi: 10.1093/treephys/tpu078.
- Barataud, F. et al. (1995) 'Soil water dynamics in an oak stand. II. A model of the soil-root network compared with experimental data', *Plant and Soil*, 172, pp. 29–43. doi: 10.1007/BF00020857.
- 4295 Barton, C. V. M. et al. (2012) 'Effects of elevated atmospheric [CO₂] on instantaneous transpiration efficiency at leaf and canopy scales in *Eucalyptus saligna*', *Global Change Biology*. John Wiley & Sons, Ltd, 18(2), pp. 585–595. doi: 10.1111/j.1365-2486.2011.02526.x.
- 4300 Batke, S. P. et al. (2020) 'Plant responses to decadal scale increments in atmospheric CO₂ concentration: comparing two stomatal conductance sampling methods', *Planta*, 251(2), p. 52. doi: 10.1007/s00425-020-03343-z.

Beer, C. et al. (2009) 'Temporal and among-site variability of inherent water use efficiency at the ecosystem level', *Global Biogeochemical Cycles*. John Wiley & Sons, Ltd, 23(2). doi: 10.1029/2008GB003233.

Bennett, A. C. et al. (2015) 'Larger trees suffer most during drought in forests worldwide', *Nature Plants*, 1(10), p. 15139. doi: 10.1038/nplants.2015.139.

Bernacchi, C. J. and VanLoocke, A. (2015) 'Terrestrial Ecosystems in a Changing Environment: A Dominant Role for Water', *Annual Review of Plant Biology*. Annual Reviews, 66(1), pp. 599–622. doi: 10.1146/annurev-arplant-043014-114834.

BIFoR. (2014) WSP Phase 1 Botanical survey A9RCB9B 2014.

BIFoR (2024) 'The Forest Time-Machine', Old Joe, Autumn. Available at: <https://www.oldjoe.co.uk/article/forest-time-machine>.

Birkel, C. et al. (2024) 'Importance of measured transpiration fluxes for modelled ecohydrological partitioning in a tropical agroforestry system', *Agricultural and Forest Meteorology*, 346, p. 109870. doi: 10.1016/j.agrformet.2023.109870.

Black, T. A. (1979) 'Evapotranspiration from Douglas fir stands exposed to soil water deficits', *Water Resources Research*. John Wiley & Sons, Ltd, 15(1), pp. 164–170. doi: 10.1029/WR015i001p00164.

Blaen, P. J. et al. (2017) 'High-frequency monitoring of catchment nutrient exports reveals highly variable storm event responses and dynamic source zone activation', *Journal of Geophysical Research: Biogeosciences*. John Wiley & Sons, Ltd, 122(9), pp. 2265–2281. doi: 10.1002/2017JG003904.

Bonan, G. B. (2008) 'Forests and climate change: Forcings, feedbacks, and the climate benefits of forests', *Science*, 320(5882), pp. 1444–1449. doi: 10.1126/science.1155121.

- 4325 Bose, A. K. et al. (2021) 'Climate sensitivity and drought seasonality determine post-drought growth recovery of *Quercus petraea* and *Quercus robur* in Europe', *Science of The Total Environment*, 784, p. 147222. doi: <https://doi.org/10.1016/j.scitotenv.2021.147222>.

- Bréda, N. et al. (1995) 'Soil water dynamics in an oak stand: I. Soil moisture, water
4330 potentials and water uptake by roots', *Plant and Soil*. Springer, 172(1), pp. 17–27.
Available at: <http://www.jstor.org/stable/42947459>.

- Bréda, N. et al. (2006) 'Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences', *Ann. For. Sci.*, 63(6), pp. 625–644. Available at:
4335 <https://doi.org/10.1051/forest:2006042>.

Britannica (no date). Available at: <https://www.britannica.com/biography/Julius-von-Sachs#ref34690> (Accessed: 2 June 2025).

- Brodribb, T. J. (2009) 'Xylem hydraulic physiology: The functional backbone of terrestrial plant productivity', *Plant Science*, 177(4), pp. 245–251. doi:
4340 <https://doi.org/10.1016/j.plantsci.2009.06.001>.

Brown, H. R. (2013) 'The theory of the rise of sap in trees: some historical and conceptual remarks', *Physics in Perspective*. Springer, 15, pp. 320–358.

- Brown, H. T. and Escombe, F. (1900) 'Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants.', *Philosophical Transactions of Royal Society of London*, B 193:, pp. 223–291. doi: 10.1093/oxfordjournals.aob.a088791.
- Buck, A. L. (1981) "New Equations for Computing Vapor Pressure and Enhancement Factor.", *Journal of Applied Meteorology* (1962-1982), 20(12), pp. 1527–32. Available at: www.jstor.org/stable/26180379.
- Buckley, T. N. (2019) 'How do stomata respond to water status?', *New Phytologist*. John Wiley & Sons, Ltd, 224(1), pp. 21–36. doi: 10.1111/nph.15899.
- Burgess, S. S. O. et al. (2001) 'An improved heat pulse method to measure low and reverse rates of sap flow in woody plants†', *Tree Physiology*, 21(9), pp. 589–598. doi: 10.1093/treephys/21.9.589.
- Čermák, J., Kučera, J. and Nadezhdina, N. (2004) 'Sap flow measurements with some thermodynamic methods, flow integration within trees and scaling up from sample trees to entire forest stands', *Trees*, 18(5), pp. 529–546. doi: 10.1007/s00468-004-0339-6.
- Cowan, I. R. and Farquhar, G. D. (1977) 'Stomatal function in relation to leaf metabolism and environment', *Symposia of the society for experimental biology*, (February 1977).
- Delpierre, N. et al. (2024) 'Phenology Across Scales: An Intercontinental Analysis of Leaf-Out Dates in Temperate Deciduous Tree Communities', *Global Ecology and Biogeography*. John Wiley & Sons, Ltd, 33(12), p. e13910. doi: 10.1111/geb.13910.

- 4365 DOE., U. S. (2020) U.S. Department of Energy Free-Air CO₂ Enrichment Experiments: FACE Results, Lessons, and Legacy, No Title. doi: 10.2172/1615612.
- Donohue, R. J. et al. (2017) 'A simple hypothesis of how leaf and canopy-level transpiration and assimilation respond to elevated CO₂ reveals distinct response patterns between disturbed and undisturbed vegetation', *Journal of Geophysical Research: Biogeosciences*, 122(1), pp. 168–184. doi: 10.1002/2016JG003505.
- 4370 Donovan, L. A. and Ehleringer, J. R. (1994) 'Water stress and use of summer precipitation in a Great Basin shrub community', *Functional Ecology*. JSTOR, pp. 289–297. doi: 10.2307/2389821.
- Dorigo, W. A. et al. (2013) 'Global Automated Quality Control of In Situ Soil Moisture Data from the International Soil Moisture Network', *Vadose Zone Journal*. John Wiley & Sons, Ltd, 12(3), p. vzj2012.0097. doi: 10.2136/vzj2012.0097.
- Drake, B. G., Gonzalez-Meler, M. A. and Long, S. P. (1997) 'More Efficient Plants: A Consequence of Rising Atmospheric CO₂?', *Annual Review of Plant Physiology and Plant Molecular Biology*, 48, pp. 609–639. doi: 10.1146/annurev.arplant.48.1.609.
- 4380 Edwards, W. R. N. and Warwick, N. W. M. (1984) 'Transpiration from a kiwifruit vine as estimated by the heat pulse technique and the penman-monteith equation', *New Zealand Journal of Agricultural Research*, 27:4, pp. 537–543,. doi: 10.1080/00288233.1984.10418016.
- Ehleringer, J. R. Hall, A. E. Farquhar, G. D. (1993) "Introduction: Water use in relation to productivity", in *Stable Isotopes and Plant Carbon-water Relations*, pp. 3–8.
- 4385

Ellison, D. et al. (2017) 'Trees, forests and water: Cool insights for a hot world',
Global Environmental Change. doi: 10.1016/j.gloenvcha.2017.01.002.

4390 Ellison, D., Pokorný, J. and Wild, M. (2024) 'Even cooler insights: On the power of
forests to (water the Earth and) cool the planet', Global Change Biology. John Wiley
& Sons, Ltd, 30(2), p. e17195. doi: 10.1111/gcb.17195.

Ellsworth, D. S. (1999) 'CO₂ enrichment in a maturing pine forest: Are CO₂
exchange and water status in the canopy affected?', Plant, Cell and Environment,
22(5), pp. 461–472. doi: 10.1046/j.1365-3040.1999.00433.x.

4395 Fabiani, G. et al. (2022) 'Transpiration patterns and water use strategies of beech
and oak trees along a hillslope', Ecohydrology. John Wiley & Sons, Ltd, 15(2), p.
e2382. doi: 10.1002/eco.2382.

Fan, Y. et al. (2017) 'Hydrologic regulation of plant rooting depth', Proceedings of the
National Academy of Sciences of the United States of America, 114(40), pp. 10572–
4400 10577. doi: 10.1073/pnas.1712381114.

Fares, A. and Alva, A. K. (2000) 'Soil Water Components Based on Capacitance
Probes in a Sandy Soil', Soil Science Society of America Journal. John Wiley &
Sons, Ltd, 64(1), pp. 311–318. doi: 10.2136/sssaj2000.641311x.

Flo, V. et al. (2021) 'Climate and functional traits jointly mediate tree water-use
4405 strategies', New Phytologist. John Wiley & Sons, Ltd, 231(2), pp. 617–630. doi:
10.1111/nph.17404.

Flo, V. et al. (2022) 'Vapour pressure deficit is the main driver of tree canopy conductance across biomes', *Agricultural and Forest Meteorology*, 322, p. 109029. doi: 10.1016/j.agrformet.2022.109029.

4410 Forster, A. M. (2019) 'The Dual Method Approach (DMA) Resolves Measurement Range Limitations of Heat Pulse Velocity Sap Flow Sensors', *Forests* . doi: 10.3390/f10010046.

Forster, M. (2017) 'How Reliable Are Heat Pulse Velocity Methods for Estimating Tree Transpiration?', *Forests*. Multidisciplinary Digital Publishing Institute, 8(9), p. 350. doi: 10.3390/f8090350.

De Frenne, P. et al. (2021) 'Forest microclimates and climate change: Importance, drivers and future research agenda', *Global Change Biology*. John Wiley & Sons, Ltd, 27(11), pp. 2279–2297. doi: 10.1111/gcb.15569.

Friedlingstein, P. et al. (2023) 'Global Carbon Budget 2023', *Earth System Science Data*, 15(12), pp. 5301–5369. doi: 10.5194/essd-15-5301-2023.

Fu, Y. H. et al. (2019) 'Daylength helps temperate deciduous trees to leaf-out at the optimal time', *Global Change Biology*. Wiley Online Library. doi: 10.1111/gcb.14633.

Gao, X. et al. (2019) 'Testing of observation operators designed to estimate profile soil moisture from surface measurements', *Hydrological Processes*. John Wiley & Sons, Ltd, 33(4), pp. 575–584. doi: 10.1002/hyp.13344.

Gardner, A. et al. (2022) 'Is photosynthetic enhancement sustained through three years of elevated CO₂ exposure in 175-year-old *Quercus robur*?', *Tree Physiology*, 42(1), pp. 130–144. doi: 10.1093/treephys/tpab090.

- 4430 Gardner, A. et al. (2023) 'Optimal stomatal theory predicts CO₂ responses of stomatal conductance in both gymnosperm and angiosperm trees', *New Phytologist*. John Wiley & Sons, Ltd, 237(4), pp. 1229–1241. doi: 10.1111/nph.18618.
- Gardner, A. (2023) The effect of elevated CO₂ on the ecophysiology of mature *Quercus robur* in a temperate deciduous UK woodland. University of Birmingham. Available at: <http://etheses.bham.ac.uk/id/eprint/13420>.
- 4435 Gardner, A. et al. (2025) 'Elevated CO₂ buffers negative impacts of heat stress on growth of 180 years-old *Quercus robur*', *Plant Physiology*, (in review).
- Gauthey, A. et al. (2024) 'High heat tolerance, evaporative cooling, and stomatal decoupling regulate canopy temperature and their safety margins in three European oak species', *Global Change Biology*. John Wiley & Sons, Ltd, 30(8), p. e17439. doi: 10.1111/gcb.17439.
- 4440 Gimeno, T. E. et al. (2016) 'Conserved stomatal behaviour under elevated CO₂ and varying water availability in a mature woodland', *Functional Ecology*, 30(5), pp. 700–709. doi: 10.1111/1365-2435.12532.
- Givnish, T. J. (ed. . (1986) *On the Economy of Plant Form and Function*. Cambridge/New York: Cambridge Univ. Press.
- 4445 Goldstein, G. et al. (1998) 'Stem water storage and diurnal patterns of water use in tropical forest canopy trees', *Plant, Cell & Environment*. John Wiley & Sons, Ltd, 21(4), pp. 397–406. doi: 10.1046/j.1365-3040.1998.00273.x.

- Granda, E. et al. (2020) 'Day length regulates seasonal patterns of stomatal
4450 conductance in *Quercus* species', *Plant, Cell & Environment*. John Wiley & Sons,
Ltd, 43(1), pp. 28–39. doi: 10.1111/pce.13665.
- Green, S. R. and Clothier, B. E. (1988) 'Water use of kiwifruit vines and apple trees
by the heat-pulse technique', *Journal of Experimental Botany*, 39(1), pp. 115–123.
doi: 10.1093/jxb/39.1.115.
- 4455 Guerrieri, R. et al. (2016) 'Evapotranspiration and water use efficiency in relation to
climate and canopy nitrogen in U.S. forests', *Journal of Geophysical Research:
Biogeosciences*, 121(10), pp. 2610–2629. doi: 10.1002/2016JG003415.
- Hagan Brown, W. et al. (2025) 'Elevated CO₂ Increases the Canopy Temperature of
Mature *Quercus robur* (Pedunculate Oak)', *Global Change Biology*. John Wiley &
4460 Sons, Ltd, 31(11), p. e70565. doi: 10.1111/gcb.70565.
- Handy, G. et al. (no date) 'Changed Fine Root Biomass and Morphology in a Mature
Temperate Forest Under Elevated CO₂', (in draft).
- Hanson, P. J. and Walker, A. P. (2020) 'Advancing global change biology through
experimental manipulations: Where have we been and where might we go?', *Global
4465 Change Biology*. John Wiley & Sons, Ltd (10.1111), 26(1), pp. 287–299. doi:
10.1111/gcb.14894.
- Harley, P. C. and Baldocchi, D. D. (1995) 'Scaling carbon dioxide and water vapour
exchange from leaf to canopy in a deciduous forest. I. Leaf model parametrization',
Plant, Cell & Environment. John Wiley & Sons, Ltd, 18(10), pp. 1146–1156. doi:
4470 10.1111/j.1365-3040.1995.tb00625.x.

Harper, N. J. (2023) Are the baseline control spaces at the BIFoR Free Air Carbon Enrichment experiment being polluted? University of Birmingham.

Harper, N. J. (2024) Are the baseline control spaces at the BIFoR Free Air Carbon Enrichment experiment being polluted? University of Birmingham.

4475 Hart, K. M. et al. (2020) 'Characteristics of free air carbon dioxide enrichment of a northern temperate mature forest', *Global Change Biology*, 26(2), pp. 1023–1037. doi: 10.1111/gcb.14786.

Hassler, S. K., Weiler, M. and Blume, T. (2018) 'Tree-, stand- and site-specific controls on landscape-scale patterns of transpiration', *Hydrology and Earth System*
4480 *Sciences*, 22(1), pp. 13–30. doi: 10.5194/hess-22-13-2018.

Hatton, T. J., Catchpole, E. A. and Vertessy, R. A. (1990) 'Integration of sapflow velocity to estimate plant water use', *Tree Physiology*, 6(2), pp. 201–209. doi: 10.1093/treephys/6.2.201.

Haworth, M., Elliott-Kingston, C. and McElwain, J. (2013) 'Haworth, M., Elliott-
4485 Kingston, C., McElwain, J.C. (2013) Co-ordination of physiological and morphological responses of stomata to elevated [CO₂] in vascular plants. *Oecologia* 171(1):71-82', *Oecologia*, 171, pp. 71–82.

He, B. et al. (2017) 'A new indicator of ecosystem water use efficiency based on surface soil moisture retrieved from remote sensing', *Ecological Indicators*, 75, pp.
4490 10–16. doi: 10.1016/j.ecolind.2016.12.017.

Hemery, G. E., Savill, P. S. and Pryor, S. N. (2005) 'Applications of the crown diameter–stem diameter relationship for different species of broadleaved trees',

Forest Ecology and Management, 215(1), pp. 285–294. doi:
<https://doi.org/10.1016/j.foreco.2005.05.016>.

- 4495 Hendrey, G. et al. (1999) 'A free-air enrichment system for exposing tall forest
vegetation to elevated atmospheric CO₂', *Global Change Biology*, 5, pp. 293–309.
doi: 10.1046/j.1365-2486.1999.00228.x.

- Herbst, M. et al. (2007) 'Edge effects and forest water use: A field study in a mixed
deciduous woodland', *Forest Ecology and Management*, 250(3), pp. 176–186. doi:
4500 10.1016/j.foreco.2007.05.013.

Herbst, M. et al. (2008) 'Comparative measurements of transpiration and canopy
conductance in two mixed deciduous woodlands differing in structure and species
composition', *Tree Physiology*, 28(6), pp. 959–970. doi: 10.1093/treephys/28.6.959.

- Holtum, J. A. M. and Winter, K. (2003) 'Photosynthetic CO₂ uptake in seedlings of
4505 two tropical tree species exposed to oscillating elevated concentrations of CO₂.',
Planta, Nov(218(1)), pp. 152–8. doi: 10.1007/s00425-003-1089-1.

- Hu, Y. et al. (2023) 'Tree-level stomatal regulation is more closely related to xylem
hydraulic traits than to leaf photosynthetic traits across diverse tree species',
Agricultural and Forest Meteorology, 329, p. 109291. doi:
4510 10.1016/j.agrformet.2022.109291.

Huang, X. et al. (2016) 'Soil moisture dynamics within soil profiles and associated
environmental controls', *CATENA*, 136, pp. 189–196. doi:
<https://doi.org/10.1016/j.catena.2015.01.014>.

- Hubick, K. and Farquhar, G. (1989) 'Carbon isotope discrimination and
4515 photosynthesis', Annual review of plant physiology and plant molecular biology.
- IPCC (2014) IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of
Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental
Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)],
IPCC. Geneva, Switzerland. doi: 10.1016/S0022-0248(00)00575-3.
- 4520 IPCC (2022) IPCC, 2022: Climate Change 2022: Working Groups II contribution to
the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.
- IPCC (2023) 'Technical Summary', in Intergovernmental Panel on Climate Change
(IPCC) (ed.) Climate Change 2021 – The Physical Science Basis: Working Group I
Contribution to the Sixth Assessment Report of the Intergovernmental Panel on
4525 Climate Change. Cambridge: Cambridge University Press, pp. 35–144. doi: DOI:
10.1017/9781009157896.002.
- Iqbal, S. et al. (2021) 'Interannual variation in sap flow response in three xeric shrub
species to periodic drought', Agricultural and Forest Meteorology, 297, p. 108276.
doi: 10.1016/j.agrformet.2020.108276.
- 4530 Kannenberg, S. A. et al. (2022) 'Opportunities, challenges and pitfalls in
characterizing plant water-use strategies', Functional Ecology. John Wiley & Sons,
Ltd, 36(1), pp. 24–37. doi: 10.1111/1365-2435.13945.
- De Kauwe, M. G., Medlyn, B. E., Zaehle, S., Walker, A. P., Dietze, M. C., Hickler, T.,
Jain, A. K., Luo, Y., Parton, W. J., Prentice, I. C., Smith, B., Thornton, P. E., Wang,
4535 S., Wang, Y.-P. P., et al. (2013) 'Forest water use and water use efficiency at
elevated CO₂: a model-data intercomparison at two contrasting temperate forest

FACE sites', *Global Change Biology*. John Wiley & Sons, Ltd, 19(6), pp. 1759–1779. doi: 10.1111/gcb.12164.

4540 De Kauwe, M. G., Medlyn, B. E., Zaehle, S., Walker, A. P., Dietze, M. C., Hickler, T., Jain, A. K., Luo, Y., Parton, W. J., Prentice, I. C., Smith, B., Thornton, P. E., Wang, S., Wang, Y. P., et al. (2013) 'Forest water use and water use efficiency at elevated CO₂: A model-data intercomparison at two contrasting temperate forest FACE sites', *Global Change Biology*, 19(6), pp. 1759–1779. doi: 10.1111/gcb.12164.

4545 De Kauwe, M. G. et al. (2017) 'Ideas and perspectives: how coupled is the vegetation to the boundary layer?', *Biogeosciences*. Copernicus Publications, 14(19), pp. 4435–4453. doi: 10.5194/bg-14-4435-2017.

Keel, S. G. et al. (2007) 'Stomatal conductance in mature deciduous forest trees exposed to elevated CO₂', *Trees - Structure and Function*, 21(2), pp. 151–159. doi: 10.1007/s00468-006-0106-y.

4550 Keenan, T. F. et al. (2013) 'Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise', *Nature*, 499(7458), pp. 324–327. doi: 10.1038/nature12291.

4555 Keenan, T., Sabaté, S. and Gracia, C. (2008) 'Forest Eco-physiological Models and Carbon Sequestration BT - Managing Forest Ecosystems: The Challenge of Climate Change', in Bravo, F. et al. (eds). Dordrecht: Springer Netherlands, pp. 83–102. doi: 10.1007/978-1-4020-8343-3_5.

Kemppinen, J. et al. (2024) 'Microclimate, an important part of ecology and biogeography', *Global Ecology and Biogeography*. John Wiley & Sons, Ltd, n/a(n/a), p. e13834. doi: 10.1111/geb.13834.

- 4560 King, J. (2011) *Reaching for the Sun: How Plants Work*. 2nd edn. Cambridge: Cambridge University Press. doi: 10.1017/CBO9780511973895.
- Köcher, P., Horna, V. and Leuschner, C. (2013) 'Stem water storage in five coexisting temperate broad-leaved tree species: significance, temporal dynamics and dependence on tree functional traits', *Tree Physiology*, 33(8), pp. 817–832. doi: 4565 10.1093/treephys/tpt055.
- Kourmouli, A. (2022) *Soil respiration and nutrient dynamics at a mature temperate woodland - the BIFoR FACE experiment*. University of Birmingham. Available at: <https://etheses.bham.ac.uk/id/eprint/11835/>.
- Kourmouli, A. et al. (2025) 'Rapid Increase in Soil Respiration and Reduction in Soil Nitrate Availability Following CO₂ Enrichment in a Mature Oak Forest', *ACS Omega*. 4570 American Chemical Society, 10(1), pp. 1624–1634. doi: 10.1021/acsomega.4c09495.
- Landsberg, J., Waring, R. and Ryan, M. (2017) 'Water relations in tree physiology: where to from here?', *Tree Physiology*, 37(1), pp. 18–32. doi: 10.1093/treephys/tpw102.
- 4575 Lavergne, A. et al. (2019) 'Observed and modelled historical trends in the water use efficiency of plants and ecosystems', *Global Change Biology*. John Wiley & Sons, Ltd (10.1111), 0(ja). doi: 10.1111/gcb.14634.
- Lavergne, A. et al. (2020) 'Impacts of soil water stress on the acclimated stomatal limitation of photosynthesis: Insights from stable carbon isotope data', *Global Change* 4580 *Biology*. John Wiley & Sons, Ltd, 26(12)(7158–7172). doi: <https://doi.org/10.1111/gcb.15364>.

Law, B. E. et al. (2002) 'Environmental controls over carbon dioxide and water vapor exchange of terrestrial vegetation', *Agricultural and Forest Meteorology*, 113(1), pp. 97–120. doi: 10.1016/S0168-1923(02)00104-1.

4585 Lawes, J. B. (1850) *Experimental Investigation Into the Amount of Water Given Off by Plants During Their Growth: Especially in Relation to the Fixation and Source of Their Various Constituents*. Clowes.

Leakey, A. D. B. et al. (2009) 'Elevated CO₂ effects on plant carbon, nitrogen, and water relations: Six important lessons from FACE', *Journal of Experimental Botany*,
4590 60(10), pp. 2859–2876. doi: 10.1093/jxb/erp096.

Lembrechts, J. J. et al. (2020) 'SoilTemp: A global database of near-surface temperature', *Global Change Biology*. John Wiley & Sons, Ltd, 26(11), pp. 6616–6629. doi: 10.1111/gcb.15123.

Leuning, R. et al. (1995) 'Leaf nitrogen, photosynthesis, conductance and
4595 transpiration: scaling from leaves to canopies', *Plant, Cell & Environment*. John Wiley & Sons, Ltd, 18(10), pp. 1183–1200. doi: 10.1111/j.1365-3040.1995.tb00628.x.

Leuzinger, S. et al. (2005) 'Responses of deciduous forest trees to severe drought in Central Europe', *Tree Physiology*, 25(6), pp. 641–650. doi:
10.1093/treephys/25.6.641.

4600 Leuzinger, S. and Körner, C. (2007) 'Water savings in mature deciduous forest trees under elevated CO₂', *Global Change Biology*. John Wiley & Sons, Ltd, 13(12), pp. 2498–2508. doi: 10.1111/j.1365-2486.2007.01467.x.

- Li, Y. (2024) 'Climate feedback from plant physiological responses to increasing atmospheric CO in Earth system models', *New Phytologist*. John Wiley & Sons, Ltd, n/a(n/a). doi: 10.1111/nph.20184.
- Liang, J. et al. (2023) 'Linking water use efficiency with water use strategy from leaves to communities', *New Phytologist*, 240. doi: 10.1111/nph.19308.
- MacKenzie, A. R. et al. (2021) 'BIFoR FACE: Water–soil–vegetation–atmosphere data from a temperate deciduous forest catchment, including under elevated CO₂', *Hydrological Processes*. John Wiley & Sons, Ltd, 35(3), p. e14096. doi: 10.1002/hyp.14096.
- MacKenzie, R. et al. (2021) 'BIFoR FACE: Water-soil-vegetation-atmosphere research in a temperate deciduous forest catchment, including under elevated CO₂', *Hydrological Processes*, pp. 1–9.
- Marshall, D. C. (1958) 'Measurement of Sap Flow in Conifers by Heat Transport.', *Plant Physiology*, 33(6), pp. 385 LP – 396. doi: 10.1104/pp.33.6.385.
- Martínez-Sancho, E. et al. (2022) 'Drought impacts on tree carbon sequestration and water use – evidence from intra-annual tree-ring characteristics', *New Phytologist*. John Wiley & Sons, Ltd, n/a(n/a). doi: 10.1111/nph.18224.
- McDonnell, J. J. (2014) 'The two water worlds hypothesis: ecohydrological separation of water between streams and trees?', *Wiley Interdisciplinary Reviews: Water*. doi: 10.1002/wat2.1027.
- McElrone, A. J. et al. (2013) *Water Uptake and Transport in Vascular Plants.*, Nature Education Knowledge. Available at:

- 4625 <https://www.scirp.org/reference/referencespapers?referenceid=3416499> (Accessed: 17 November 2025).
- McGill, R., Tukey, J. W. and Larsen, W. A. (1978) 'Variations of Box Plots', *The American Statistician*. [American Statistical Association, Taylor & Francis, Ltd.], 32(1), pp. 12–16. doi: 10.2307/2683468.
- 4630 Medlyn, B. E. et al. (2001) 'Stomatal conductance of forest species after long-term exposure to elevated CO₂ concentration: a synthesis', *New Phytologist*, 149, pp. 247–264.
- Medlyn, B. E. et al. (2015) 'Using ecosystem experiments to improve vegetation models', *Nature Clim. Change*. Nature Publishing Group, a division of Macmillan
- 4635 Publishers Limited. All Rights Reserved., 5(6), pp. 528–534. doi: 10.1038/nclimate2621.
- Meinzer, F. C., James, S. A. and Goldstein, G. (2004) 'Dynamics of transpiration, sap flow and use of stored water in tropical forest canopy trees', in *Tree Physiology*. doi: 10.1093/treephys/24.8.901.
- 4640 Mencuccini, M. (2003) 'The ecological significance of long-distance water transport: short-term regulation, long-term acclimation and the hydraulic costs of stature across plant life forms', *Plant, Cell & Environment*. John Wiley & Sons, Ltd, 26(1), pp. 163–182. doi: 10.1046/j.1365-3040.2003.00991.x.
- Mishra, V. et al. (2020) 'Performance evaluation of soil moisture profile estimation
- 4645 through entropy-based and exponential filter models', *Hydrological Sciences Journal*. Taylor & Francis, 65(6), pp. 1036–1048. doi: 10.1080/02626667.2020.1730846.

Monteith, J. L. and Unsworth, M. H. (2013) 'Principles of Environmental Physics: Plants, Animals, and the Atmosphere', in. Available at:
<https://api.semanticscholar.org/CorpusID:126432457>.

4650 Morecroft, M. D. and Roberts, J. M. (1999) 'Photosynthesis and stomatal conductance of mature canopy Oak (*Quercus robur*) and Sycamore (*Acer pseudoplatanus*) trees throughout the growing season', *Functional Ecology*. John Wiley & Sons, Ltd, 13(3), pp. 332–342. doi: 10.1046/j.1365-2435.1999.00327.x.

Murray, F. W. (1967) 'On the Computation of Saturation Vapor Pressure', *Journal of Applied Meteorology and Climatology*. Boston MA, USA: American Meteorological Society, 6(1), pp. 203–204. doi: 10.1175/1520-0450(1967)006<0203:OTCOSV>2.0.CO;2.

Murray, M. et al. (2019) 'Convergence in Maximum Stomatal Conductance of C3 Woody Angiosperms in Natural Ecosystems Across Bioclimatic Zones ', *Frontiers in Plant Science* , p. 558. Available at:
<https://www.frontiersin.org/article/10.3389/fpls.2019.00558>.

Nehemy, M. F. et al. (2021) 'Tree water deficit and dynamic source water partitioning', *Hydrological Processes*. John Wiley & Sons, Ltd, 35(1), p. e14004. doi: 10.1002/hyp.14004.

4665 NOAA ESRL (2024) at Mauna Loa, Hawaii, United States (MLO). Available at:
<https://gml.noaa.gov/ccgg/trends/mlo.html> (Accessed: 10 December 2024).

Norby, R. J. et al. (1999) 'Tree responses to rising CO₂ in field experiments : implications for the future forest', *Plant, Cell & Environment*, (22), pp. 683–714.

- Norby, R. J. et al. (2016) 'Model-data synthesis for the next generation of forest free-air CO₂ enrichment (FACE) experiments', *New Phytologist*, 209(1), pp. 17–28. doi: 10.1111/nph.13593.
- Norby, R. J. et al. (2024) 'Enhanced woody biomass production in a mature temperate forest under elevated CO₂', *Nature Climate Change*. doi: 10.1038/s41558-024-02090-3.
- 4675 Norby, R. J. (2025) 'Forest productivity response to elevated CO₂ in free-air CO₂ enrichment experiments: the 23 percent solution, revisited', *New Phytologist*. John Wiley & Sons, Ltd, n/a(n/a). doi: 10.1111/nph.70162.
- Norby, R. J. and Luo, Y. (2004) 'Evaluating ecosystem responses to rising atmospheric CO₂ and global warming in a multi-factor world', *New Phytologist*. doi: 4680 10.1111/j.1469-8137.2004.01047.x.
- Norby, R. J. and Zak, D. R. (2011) 'Ecological and evolutionary lessons from free air carbon enhancement (FACE) experiments', *Annual Review of Ecology, Evolution, and Systematics*, 42, pp. 181–203. doi: 10.1146/annurev-ecolsys-102209-144647.
- Novick, K. et al. (2009) 'The relationship between reference canopy conductance and 4685 simplified hydraulic architecture', *Advances in Water Resources*. doi: 10.1016/j.advwatres.2009.02.004.
- Oishi, A. C. et al. (2013) 'Spatial and temporal variability of soil CO₂ efflux in three proximate temperate forest ecosystems', *Agricultural and Forest Meteorology*, 171–172, pp. 256–269. doi: 10.1016/j.agrformet.2012.12.007.

- 4690 Oishi, A. C. et al. (2014) 'Sustained effects of atmospheric [CO₂] and nitrogen availability on forest soil CO₂ efflux', *Global Change Biology*. John Wiley & Sons, Ltd, 20(4), pp. 1146–1160. doi: 10.1111/gcb.12414.
- Oren, R. et al. (1999) 'Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit', *Plant, Cell & Environment*. John Wiley & Sons, Ltd, 22(12), pp. 1515–1526. doi: 10.1046/j.1365-3040.1999.00513.x.
- 4695 Oren, R. et al. (2001) 'Soil fertility limits carbon sequestration by forest ecosystems in a CO₂-enriched atmosphere', *Nature*, 411(6836), pp. 469–472. doi: 10.1038/35078064.
- Oren, R. and Pataki, D. E. (2001) 'Transpiration in response to variation in microclimate and soil moisture in southeastern deciduous forests', *Oecologia*, 127(4), pp. 549–559. doi: 10.1007/s004420000622.
- 4700 Orlowski, N. et al. (2023) 'Challenges in studying water fluxes within the soil-plant-atmosphere continuum: A tracer-based perspective on pathways to progress', *Science of The Total Environment*, 881, p. 163510. doi: 10.1016/j.scitotenv.2023.163510.
- 4705 Paschalis, A. et al. (2024) 'When do plant hydraulics matter in terrestrial biosphere modelling?', *Global Change Biology*. John Wiley & Sons, Ltd, 30(1), p. e17022. doi: 10.1111/gcb.17022.
- Pedersen, B. S. (1999) 'THE MORTALITY OF MIDWESTERN OVERSTORY OAKS AS A BIOINDICATOR OF ENVIRONMENTAL STRESS', *Ecological Applications*. John Wiley & Sons, Ltd, 9(3), pp. 1017–1027. doi: 10.1890/1051-0761(1999)009[1017:TMOMOO]2.0.CO;2.

- Perkins, D. et al. (2018) 'Impact of Climate Trends and Drought Events on the Growth of Oaks (*Quercus robur* L. and *Quercus petraea* (Matt.) Liebl.) within and
4715 beyond Their Natural Range', *Forests*, 9(3), p. 108. doi: 10.3390/f9030108.
- Peters, R. L. et al. (2023) 'Daytime stomatal regulation in mature temperate trees prioritizes stem rehydration at night', *New Phytologist*. John Wiley & Sons, Ltd, n/a(n/a). doi: <https://doi.org/10.1111/nph.18964>.
- Philip, J. R. (1966) 'Plant Water Relations: Some Physical Aspects', *Annual Review of Plant Physiology*. Annual Reviews, 17(1), pp. 245–268. doi:
4720 10.1146/annurev.pp.17.060166.001333.
- Popa, A. et al. (2025) 'Spatiotemporal Variability of Dendroecological Indicators in Pedunculate Oak (*Quercus robur* L.) Tree-Rings Across Europe in Relation to Species Distribution Models', *Global Change Biology*. John Wiley & Sons, Ltd,
4725 31(11), p. e70567. doi: <https://doi.org/10.1111/gcb.70567>.
- Posit Team (2024) 'RStudio: Integrated Development Environment for R'. Boston, MA: R. Posit Software, PBC. Available at: <http://www.posit.co/>.
- Poyatos, R. et al. (2016) 'SAPFLUXNET: towards a global database of sap flow measurements', *Tree Physiology*, 36(12), pp. 1449–1455. doi:
4730 10.1093/treephys/tpw110.
- Poyatos, R. et al. (2020) 'Global transpiration data from sap flow measurements: the SAPFLUXNET database', *Earth System Science Data*. doi: 10.5194/essd-2020-227.

- Quick, S. E., Curioni, G., Harper, N. J., Krause, S., and MacKenzie, A. R. (2025) 'Water usage of old growth oak at elevated CO₂ in the FACE of climate change',
4735 EGU sphere Biogeosciences. doi: 10.5194/bg-22-1557-2025.
- Quick, S. E. (2025) Tree-soil-water relations under elevated CO₂ – following the flow. University of Birmingham. doi: tba.
- Quick, S. E. et al. (2025) 'Water usage of old-growth oak at elevated CO₂ in the FACE (Free-Air CO₂ Enrichment) of climate change', Biogeosciences. Copernicus
4740 Publications Göttingen, Germany, 22(6), pp. 1557–1581. doi: 10.5194/bg-22-1557-2025.
- R Core Team (2024) 'R version 4.4.1'. Vienna, Austria: R Foundation for Statistical Computing. Available at: <https://www.r-project.org/>.
- Rabbai, A. et al. (2023) 'Soil moisture and temperature dynamics in juvenile and
4745 mature forest as a result of tree growth, hydrometeorological forcings, and drought', Hydrological Processes. John Wiley & Sons, Ltd, 37(6), p. e14919. doi:
10.1002/hyp.14919.
- Reich, P. B. et al. (2018) 'Effects of climate warming on photosynthesis in boreal tree species depend on soil moisture', Nature. Springer US, 562(7726), pp. 263–267. doi:
4750 10.1038/s41586-018-0582-4.
- Renninger, H. et al. (2015) 'Resource use and efficiency, and stomatal responses to environmental drivers of oak and pine species in an Atlantic Coastal Plain forest ', Frontiers in Plant Science . Available at:
<https://www.frontiersin.org/articles/10.3389/fpls.2015.00297>.

- 4755 Renninger, H. J. et al. (2014) 'Physiological strategies of co-occurring oaks in a water- and nutrient-limited ecosystem', *Tree Physiology*, 34(2), pp. 159–173. doi: 10.1093/treephys/tpt122.
- Roberts, A. M. I. et al. (2015) 'Predicting a change in the order of spring phenology in temperate forests', *Global Change Biology*. doi: 10.1111/gcb.12896.
- 4760 Robinson, J. L., Slater, L. D. and Schäfer, K. V. R. (2012) 'Evidence for spatial variability in hydraulic redistribution within an oak–pine forest from resistivity imaging', *Journal of Hydrology*, 430–431, pp. 69–79. doi: 10.1016/j.jhydrol.2012.02.002.
- Salomón, R. L. et al. (2022) 'The 2018 European heatwave led to stem dehydration but not to consistent growth reductions in forests', *Nature Communications*, 13(1), p. 28. doi: 10.1038/s41467-021-27579-9.
- 4765 Sánchez-Pérez, J. M. et al. (2008) 'Water uptake by trees in a riparian hardwood forest (Rhine floodplain, France)', *Hydrological Processes*, 22(3), pp. 366–375. doi: 10.1002/hyp.6604.
- Saxton, K. E. and Rawls, W. J. (2006) 'Soil Water Characteristic Estimates by Texture and Organic Matter for Hydrologic Solutions', *Soil Science Society of America Journal*. John Wiley & Sons, Ltd, 70(5), pp. 1569–1578. doi: 10.2136/sssaj2005.0117.
- 4770 Schäfer, K. V. R. (2011) 'Canopy Stomatal Conductance Following Drought, Disturbance, and Death in an Upland Oak/Pine Forest of the New Jersey Pine Barrens, USA', *Frontiers in Plant Science*. Available at: <https://www.frontiersin.org/articles/10.3389/fpls.2011.00015>.
- 4775

Schäfer, Karina V R et al. (2002) 'Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO₂ concentration', *Global Change Biology*, 8(9), pp. 895–911.

4780 Schmidt, M. (2008) Canopy transpiration of beech forests in Northern Bavaria – Structure and function in pure and mixed stands with oak at colline and montane sites. Bayreuth, University of Bayreuth. Available at: <https://epub.uni-bayreuth.de/646/>.

Seneviratne, S. I. et al. (2010) 'Investigating soil moisture–climate interactions in a changing climate: A review', *Earth-Science Reviews*, 99(3), pp. 125–161. doi: 10.1016/j.earscirev.2010.02.004.

Sentek (2011) Calibration of Sentek soil moisture sensors. V2.0. Sentek Sensor Technologies. doi: info:doi/.

Shirke, P. A. and Pathre, U. V (2004) 'Influence of leaf-to-air vapour pressure deficit (VPD) on the biochemistry and physiology of photosynthesis in *Prosopis juliflora**, *Journal of Experimental Botany*, 55(405), pp. 2111–2120. doi: 10.1093/jxb/erh229.

Smith, A. R. et al. (2013) 'Tree species diversity interacts with elevated CO₂ to induce a greater root system response', *Global Change Biology*, 19(1), pp. 217–228. doi: 10.1111/gcb.12039.

4795 Stagge, J. H. et al. (2017) 'Observed drought indices show increasing divergence across Europe', *Scientific Reports*, 7(1), p. 14045. doi: 10.1038/s41598-017-14283-2.

Steger, D. N. et al. (2024) 'Site matters - Canopy conductance regulation in mature temperate trees diverges at two sites with contrasting soil water availability',

- Agricultural and Forest Meteorology, 345, p. 109850. doi:
4800 <https://doi.org/10.1016/j.agrformet.2023.109850>.
- Steppe, K. et al. (2015) 'Sap flow as a key trait in the understanding of plant hydraulic functioning', *Tree Physiology*, 35(4), pp. 341–345. doi: 10.1093/treephys/tpv033.
- Stevenson, J. L. et al. (2023) 'Quantifying heterogeneity in ecohydrological partitioning in urban green spaces through the integration of empirical and modelling
4805 approaches', *Environmental Monitoring and Assessment*, 195(4), p. 468. doi:
10.1007/s10661-023-11055-6.
- Stokes, V. J., Morecroft, M. D. and Morison, J. I. L. (2010) 'Comparison of leaf water use efficiency of oak and sycamore in the canopy over two growing seasons', *Trees*, 24(2), pp. 297–306. doi: 10.1007/s00468-009-0399-8.
- 4810 Stoy, P. C. et al. (2006) 'Separating the effects of climate and vegetation on evapotranspiration along a successional chronosequence in the southeastern US', *Global Change Biology*. John Wiley & Sons, Ltd, 12(11), pp. 2115–2135. doi:
10.1111/j.1365-2486.2006.01244.x.
- Tang, J. et al. (2006) 'Sap flux–upscaled canopy transpiration, stomatal conductance,
4815 and water use efficiency in an old growth forest in the Great Lakes region of the United States', *Journal of Geophysical Research: Biogeosciences*. John Wiley & Sons, Ltd, 111(G2). doi: 10.1029/2005JG000083.
- Tansey, C. J., Hadfield, J. D. and Phillimore, A. B. (2017) 'Estimating the ability of plants to plastically track temperature-mediated shifts in the spring phenological
4820 optimum', *Global Change Biology*. Wiley Online Library. doi: 10.1111/gcb.13624.

Teepe, R., Dilling, H. and Beese, F. (2003) 'Estimating water retention curves of forest soils from soil texture and bulk density', *Journal of Plant Nutrition and Soil Science*. John Wiley & Sons, Ltd, 166(1), pp. 111–119. doi: 10.1002/jpln.200390001.

4825 Tetens, O. (1930) 'Über einige meteorologische Begriffe.', *Z. Geophys* 6, pp. 297–309.

Thomas, F. (2000) 'Vertical rooting patterns of mature *Quercus* trees growing on different soil types in northern Germany', *Plant Ecology*, 147, pp. 95–103. doi: 10.1023/A:1009841921261.

4830 Tor-ngern, P. et al. (2015) 'Increases in atmospheric CO₂ have little influence on transpiration of a temperate forest canopy', *New Phytologist*. John Wiley & Sons, Ltd, 205(2), pp. 518–525. doi: 10.1111/nph.13148.

Toro, G., Flexas, J. and Escalona, J. M. (2019) 'Contrasting leaf porometer and infrared gas analyser methodologies: an old paradigm about the stomatal conductance measurement', *Theoretical and Experimental Plant Physiology*, 31(4), pp. 483–492.
4835 doi: 10.1007/s40626-019-00161-x.

Tumber-Dávila, S. J. et al. (2022) 'Plant sizes and shapes above and belowground and their interactions with climate', *New Phytologist*. John Wiley & Sons, Ltd, 235(3), pp. 1032–1056. doi: 10.1111/nph.18031.

Valladares, F. et al. (2016) 'Shedding light on shade: ecological perspectives of
4840 understorey plant life', *Plant Ecology & Diversity*. Taylor & Francis, 9(3), pp. 237–251.
doi: 10.1080/17550874.2016.1210262.

Valladares, F. and Niinemets, Ü. (2008) 'Shade tolerance, a key plant feature of complex nature and consequences', *Annual Review of Ecology, Evolution, and Systematics*, 39, pp. 237–257. doi: 10.1146/annurev.ecolsys.39.110707.173506.

4845 Vandegehuchte, M. W. and Steppe, K. (2012) 'Improving sap flux density measurements by correctly determining thermal diffusivity, differentiating between bound and unbound water', *Tree Physiology*, 32(7), pp. 930–942. doi: 10.1093/treephys/tps034.

Venturas, M. D., Sperry, J. S. and Hacke, U. G. (2017) 'Plant xylem hydraulics: What
4850 we understand, current research, and future challenges', *Journal of Integrative Plant Biology*. Blackwell Publishing Ltd., pp. 356–389. doi: 10.1111/jipb.12534.

Verstraeten, W. W., Veroustraete, F. and Feyen, J. (2008) 'Assessment of Evapotranspiration and Soil Moisture Content Across Different Scales of Observation', *Sensors*, 8(1), pp. 70–117. doi: 10.3390/s8010070.

4855 Vicca, S. et al. (2012) 'Urgent need for a common metric to make precipitation manipulation experiments comparable', *New Phytologist*. John Wiley & Sons, Ltd, 195(3), pp. 518–522. doi: 10.1111/j.1469-8137.2012.04224.x.

Vico, G. et al. (2011) 'Effects of stomatal delays on the economics of leaf gas exchange under intermittent light regimes', *New Phytologist*. John Wiley & Sons, Ltd,
4860 192(3), pp. 640–652. doi: 10.1111/j.1469-8137.2011.03847.x.

Vitasse, Y. et al. (2019) 'Contrasting resistance and resilience to extreme drought and late spring frost in five major European tree species', *Global Change Biology*. John Wiley & Sons, Ltd, 25(11), pp. 3781–3792. doi: 10.1111/gcb.14803.

- Vodnik, D. et al. (2013) 'Interspecific variability of plant stomatal response to step
4865 changes of [CO₂]', *Environmental and Experimental Botany*, 88, pp. 107–112. doi:
10.1016/j.envexpbot.2011.12.029.
- Walker, A. P. et al. (2015) 'Predicting long-term carbon sequestration in response to
CO₂ enrichment: How and why do current ecosystem models differ?', *Global
Biogeochemical Cycles*. John Wiley & Sons, Ltd, 29(4), pp. 476–495. doi:
4870 10.1002/2014GB004995.
- Walker, A. P. et al. (2019) 'Decadal biomass increment in early secondary
succession woody ecosystems is increased by CO₂ enrichment', *Nature
Communications*. Nature Publishing Group UK London, 10(1), p. 454. doi:
10.1038/s41467-019-08348-1.
- 4875 Walker, A. P. et al. (2021) 'Integrating the evidence for a terrestrial carbon sink
caused by increasing atmospheric CO₂', *New Phytologist*. John Wiley & Sons, Ltd,
229(5), pp. 2413–2445. doi: 10.1111/nph.16866.
- Walker, J. P., Willgoose, G. R. and Kalma, J. D. (2004) 'In situ measurement of soil
moisture: a comparison of techniques', *Journal of Hydrology*, 293(1), pp. 85–99. doi:
4880 10.1016/j.jhydrol.2004.01.008.
- Wang, H., Guan, H. and Simmons, C. T. (2016) 'Modeling the environmental controls
on tree water use at different temporal scales', *Agricultural and Forest Meteorology*.
Elsevier B.V., 225, pp. 24–35. doi: 10.1016/j.agrformet.2016.04.016.
- Wang, W. et al. (2020) 'Tree growth, transpiration, and water-use efficiency between
4885 shoreline and upland red maple (*Acer rubrum*) trees in a coastal forest', *Agricultural
and Forest Meteorology*, 295, p. 108163. doi: 10.1016/j.agrformet.2020.108163.

- Wang, Z. and Wang, C. (2021) 'Responses of tree leaf gas exchange to elevated CO₂ combined with changes in temperature and water availability: A global synthesis', *Global Ecology and Biogeography*, (July), pp. 1–13. doi: 10.1111/geb.13394.
- 4890 10.1111/geb.13394.
- Ward, E. J. et al. (2018) 'Evapotranspiration and water yield of a pine-broadleaf forest are not altered by long-term atmospheric [CO₂] enrichment under native or enhanced soil fertility', *Global Change Biology*. Wiley/Blackwell (10.1111), 24(10), pp. 4841–4856. doi: 10.1111/gcb.14363.
- 4895 Warren, J. M. et al. (2011) 'Ecohydrologic impact of reduced stomatal conductance in forests exposed to elevated CO₂', *Ecohydrology*, 4(2), pp. 196–210. doi: doi:10.1002/eco.173.
- Warren, J. M., Norby, R. J. and Wullschleger, S. D. (2011) 'Elevated CO₂ enhances leaf senescence during extreme drought in a temperate forest', *Tree Physiology*, 31(2), pp. 117–130. doi: 10.1093/treephys/tpr002.
- 4900 31(2), pp. 117–130. doi: 10.1093/treephys/tpr002.
- Way, D. A., Cook, A. and Rogers, A. (2021) 'The effects of rising CO₂ concentrations on terrestrial systems: scaling it up', *New Phytologist*. John Wiley & Sons, Ltd, 229(5), pp. 2383–2385. doi: 10.1111/nph.17096.
- Whalley, W. R., Ober, E. S. and Jenkins, M. (2013) 'Measurement of the matric potential of soil water in the rhizosphere', *Journal of Experimental Botany*, 64(13), pp. 3951–3963. doi: 10.1093/jxb/ert044.
- 4905 3951–3963. doi: 10.1093/jxb/ert044.
- Wickham, H. (2016) 'ggplot2: Elegant Graphics for Data Analysis.' New York: Springer-Verlag .

- Wilkening, J. V et al. (2024) 'Different roads, same destination: The shared future of
4910 plant ecophysiology and ecohydrology', *Plant, Cell & Environment*. John Wiley &
Sons, Ltd, 47(9), pp. 3447–3465. doi: 10.1111/pce.14937.
- Wolkovich, E. M. et al. (2025) 'Why longer seasons with climate change may not
increase tree growth', *Nature Climate Change*, 15(12), pp. 1283–1292. doi:
10.1038/s41558-025-02476-x.
- 4915 Woods, D. B. and Turner, N. C. (1971) 'Stomatal response to changing light by four
tree species of varying shade tolerance', *New Phytologist*. John Wiley & Sons, Ltd,
70(1), pp. 77–84. doi: 10.1111/j.1469-8137.1971.tb02512.x.
- Wright, I. J. et al. (2004) 'The worldwide leaf economics spectrum', *Nature*,
428(6985), pp. 821–827. doi: 10.1038/nature02403.
- 4920 Wullschleger, S. D. et al. (2002) 'Sensitivity of stomatal and canopy conductance to
elevated CO₂ concentration – interacting variables and perspectives of scale', *New
Phytologist*. John Wiley & Sons, Ltd, 153(3), pp. 485–496. doi: 10.1046/j.0028-
646X.2001.00333.x.
- Wullschleger, S. D. and Norby, R. J. (2001) 'Sap velocity and canopy transpiration in
4925 a sweetgum stand exposed to free-air CO₂ enrichment (FACE)', *New Phytologist*.
John Wiley & Sons, Ltd, 150(2), pp. 489–498. doi: 10.1046/j.1469-
8137.2001.00094.x.
- Wullschleger, S. D., Tschaplinski, T. J. and Norby, R. J. (2002) 'Plant water relations
at elevated CO₂ - Implications for water-limited environments', *Plant, Cell and
4930 Environment*, 25(2), pp. 319–331. doi: 10.1046/j.1365-3040.2002.00796.x.

Xu, Z. et al. (2016) Elevated-CO₂ Response of Stomata and Its Dependence on Environmental Factors , *Frontiers in Plant Science* . doi: 10.3389/fpls.2016.00657.

Yi, K. et al. (2017) 'Dynamics of stem water uptake among isohydric and anisohydric species experiencing a severe drought', *Tree Physiology*, 37(10), pp. 1379–1392.

4935 doi: 10.1093/treephys/tpw126.

Zhang, L., Dawes, W. R. and Walker, G. R. (2001) 'Response of mean annual evapotranspiration to vegetation changes at catchment scale', *Water Resources Research*. John Wiley & Sons, Ltd, 37(3), pp. 701–708. doi: 10.1029/2000WR900325.

4940 Zhang, L. and Zhang, S. (2019) 'The quantitative impact of different leaf temperature determination on computed values of stomatal conductance and internal CO₂ concentrations', *Agricultural and Forest Meteorology*, 279, p. 107700. doi: 10.1016/j.agrformet.2019.107700.

Zhang, M. et al. (2017) 'A global review on hydrological responses to forest change
4945 across multiple spatial scales: Importance of scale, climate, forest type and hydrological regime', *Journal of Hydrology*. Elsevier, 546, pp. 44–59. doi: 10.1016/J.JHYDROL.2016.12.040.

Ziegler, C. et al. (2023) 'Quantification and uncertainty of root growth stimulation by elevated CO₂ in a mature temperate deciduous forest', *Science of The Total
4950 Environment*, 854, p. 158661. doi: 10.1016/j.scitotenv.2022.158661.

Ziegler, C. (2023) Quantitative modelling of root growth and carbon allocation: bridging theory and experiment. University of Birmingham.

- Zweifel, R., Zimmermann, L. and Newbery, D. M. (2005) 'Modeling tree water deficit from microclimate: an approach to quantifying drought stress', *Tree Physiology*, 25(2), pp. 147–156. doi: 10.1093/treephys/25.2.147.

Chapter 10 : Appendices

10. Appendices:

Appendix 1: Chapter 1 Supplementary Material.	1
First page extracts of coauthor publications.	1
Appendix 2: Chapter Two Supplementary Material.	6
2-A: Maps of Arrays showing details of precipitation, throughfall, soil moisture/ temperature measurement transducer positioning and of soil sampling areas.....	6
2-B: Detailed method for sap flow probeset installations.....	11
2-C: Detailed method for Tree core sampling and processing.	15
2-D: Detailed method for stomatal conductance and leaf temperature measurement using porometry.	35
2-E: Report justifying drilling of mature trees.....	46
Appendix 3: Chapter 3 Supplementary Material.....	58
Appendix 4: Chapter 4 Supplementary Material.....	85
Appendix 5: Chapter 6 Supplementary Material.....	104

Appendix 1: Chapter 1 Supplementary Material.

First page extracts of coauthor publications.

RESEARCH ARTICLE OPEN ACCESS

Elevated CO₂ Increases the Canopy Temperature of Mature *Quercus robur* (Pedunculate Oak)

William Hagan Brown^{1,2}  | Emanuel Glöer³ | Ralph Fyfe³ | A. Rob MacKenzie⁴ | Nicholas J. Harper⁵ | Peter Gandonerton¹ | Kris Hart⁴  | Giulio Carlini^{4,5} | Susan Quick⁴  | Scott J. Davidson¹  | Emily Yetton³ | Jen L. Diehl^{6,7} | Sophie Fauset¹

¹School of Geography, Earth and Environmental Sciences, University of Plymouth, Plymouth, UK | ²CSIR-Forestry Research Institute of Ghana, Kumasi, Ghana | ³School of Geography, Faculty of Environment, University of Leeds, Leeds, UK | ⁴Birmingham Institute of Forest Research, and School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, UK | ⁵Forest Research, Climate Change Group, Northern Research Station, Rotham, Gloucestershire, UK | ⁶Center for Ecosystem Science and Society (ECOSS), Northern Arizona University, Flagstaff, Arizona, USA | ⁷School of Informatics, Computing, and Cyber Systems (FCCS), Northern Arizona University, Flagstaff, Arizona, USA

Correspondence: William Hagan Brown (william.haganbrown@plymouth.ac.uk) | Sophie Fauset (sophie.fauset@plymouth.ac.uk)

Received: 1 May 2023 | Revised: 6 September 2023 | Accepted: 16 September 2023

Funding: Financial support was provided by the JARRS Foundation, the University of Birmingham, University of Plymouth, the John Horsman Trust, and the UK Natural Environment Research Council through grants NE/S015833/1 (to A.R.M.) and NE/V004366/1 (to S.F.), the ARIES DTP studentship (to W.H.B.), and the Biological Continuity Trust and the Royal Forestry Society for fieldwork grants.

Keywords: carbon dioxide | climate change | free-air CO₂ enrichment (FACE) | leaf traits | microclimate | stomata | stomatal conductance | temperate forests

ABSTRACT

The canopy thermal response of natural forests to elevated CO₂ (eCO₂) is an understudied biophysical feedback in the global climate system. We investigated the effects of eCO₂ (150 μmol mol⁻¹ above ambient) on canopy temperature (T_{can}) dynamics of mature (> 175 years) *Quercus robur* (oak) at the Birmingham Institute for Forest Research Free Air CO₂ Enrichment (BIFOR-FACE) facility in Staffordshire, England, during the growing seasons of 2021, 2022 and 2023. We employed long-term, high-frequency thermal infrared (TIR) imaging to measure T_{can} . Our results show that daily maximum oak T_{can} under eCO₂ was, on average, approximately 1.3°C higher than under ambient (aCO₂) conditions (21.5°C ± 4.4°C for aCO₂ vs. 22.8°C ± 5.2°C for eCO₂ oaks). Moreover, daily maximum T_{can} -air temperature (T_{air}) differences were significantly higher under eCO₂, resulting from more frequent extreme temperature excursions. These differences appear primarily to be driven by reduced stomatal conductance under eCO₂, which limits transpirational cooling and alters the surface energy balance. This effect was evident in the different relationship between T_{can} - T_{air} and vapour pressure deficit (VPD) for eCO₂ compared to aCO₂, showing a reduction in transpirational cooling under high VPD. Also, CO₂-induced leaf structural and anatomical modifications, such as increased leaf mass per area, may have enhanced solar radiation absorption, thereby enabling greater canopy warming under high radiation conditions. Thus, eCO₂ could likely cause a reduction in leaf transpiration in oaks, reducing its contribution to processes such as humidification of the lower atmosphere and precipitation in local and regional climates. Our findings highlight how high CO₂ conditions may intensify thermal stress in temperate forests, influencing water and carbon cycles and potentially impacting forest resilience. Furthermore, T_{can} will be essential for refining global Earth system models, which often use T_{air} as a proxy for T_{can} , despite the latter's direct influence on carbon and hydrological cycles.

This is an open access article under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2023 The Author(s). Global Change Biology published by John Wiley & Sons Ltd.

Global Change Biology. 2023; 31:e73565.
<https://doi.org/10.1111/gcb.70645>

1 of 15

Microclimate, an important part of ecology and biogeography

Julia Kemppinen¹ | Jonas J. Lembrechts² | Koenraad Van Meerbeek³ |
Jofre Carnicer⁴ | Nathalie Isabelle Chardon⁵ | Paul Kardol⁶ | Jonathan Lenoir⁷ |
Daijun Liu⁸ | Ilya Maclean⁹ | Jan Pergl¹⁰ | Patrick Saccone¹¹ |
Rebecca A. Senior¹² | Ting Shen^{13,14} | Sandra Stowińska¹⁵ | Vigdis Vandvik¹⁶ |
Jonathan von Oppen¹⁷ | Juha Aalto¹⁸ | Biruk Ayalew¹⁹ | Olivia Bates²⁰ |
Cleo Bertelsmeier²⁰ | Romain Bertrand²¹ | Rémy Beugnon^{22,23,24} |
Jeremy Borderieux²⁵ | Josef Bruna¹⁰ | Lauren Buckley²⁶ | Jelena Bujan²⁰ |
Angelica Casanova-Katny²⁷ | Ditte Marie Christiansen²⁸ | Flavien Collart²⁰ |
Emiel De Lombaerde²⁹ | Karen De Pauw²⁹ | Leen Depauw²⁹ |
Michele Di Musciano³⁰ | Raquel Díaz Borrego³¹ | Joan Díaz-Calafat³² |
Diego Ellis-Soto³³ | Raquel Esteban³⁴ | Geerte Fálthammar de Jong³⁵ |
Elise Gallois³⁶ | Maria Begoña García³⁷ | Loïc Gillerot²⁹ | Caroline Greiser³⁸ |
Eva Grii³⁹ | Stef Haesen³ | Arndt Hampe⁴⁰ | Per-Ola Hedwall³² |
Gabriel Hes²¹ | Helena Hespanhol⁴¹ | Raúl Hoffrén⁴² | Kristoffer Hylander¹⁹ |
Borja Jiménez-Alfaro⁴³ | Tommaso Jucker⁴⁴ | David Klimes⁴⁵ |
Joonas Kolstela¹⁸ | Martin Kopecký⁴⁶ | Bence Kovács^{47,48} | Eduardo Eiji Maeda⁴⁹ |
František Máliš⁵⁰ | Matěj Man¹⁰ | Corrie Mathiak⁵¹ | Eric Meiner⁵² |
Ilona Naujokaitis-Lewis⁵³ | Ivan Nijs⁵⁴ | Signe Normand⁵⁵ | Martin Nuñez⁵⁶ |
Anna Orczewska⁵⁷ | Pablo Peña-Aguilera⁵⁸ | Sylvain Pincebourde⁵⁹ |
Roman Plichta⁶⁰ | Susan Quick⁶¹ | David Renault⁶² | Lorenzo Ricci³⁰ |
Tuuli Rissanen⁴⁹ | Laura Segura-Hernández⁶³ | Federico Selvi⁶⁴ |
Josep M. Serra-Díaz⁶⁵ | Lydia Soifer⁴⁵ | Fabien Spicher⁶⁶ | Jens-Christian Svenning⁶⁷ |
Anouch Tamian⁶⁸ | Arno Thomaes⁶⁹ | Marijke Thoonen⁶⁹ | Brittany Trew⁹ |
Stijn Van de Vondel⁷⁰ | Liesbeth van den Brink^{71,72} | Pieter Vangansbeke^{29,73} |
Sanne Verdonck⁷⁴ | Michaela Vitkova⁷⁵ | Maria Vives-Inglá^{31,76} |
Loke von Schmalensee⁷⁷ | Runxi Wang⁷⁸ | Jan Wild¹⁰ | Joseph Williamson⁷⁹ |
Florian Zellweger⁸⁰ | Xiaqu Zhou⁸¹ | Emmanuel Junior Zuza⁸² |
Pieter De Frenne²⁹

Julia Kemppinen, Jonas Lembrechts and Koenraad Van Meerbeek should be considered joint first authors.

For affiliations refer to page 13.

This is an open access article under the terms of the [Creative Commons Attribution License](#), which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Authors. *Global Ecology and Biogeography* published by John Wiley & Sons Ltd.

Global Ecol Biogeogr. 2024;33:e13834.
<https://doi.org/10.1111/geb.13834>

wileyonlinelibrary.com/journal/geb | 1 of 23

Received: 2 November 2020 | Revised: 8 February 2021 | Accepted: 10 February 2021
DOI: 10.1002/hyp.14096

RESEARCH AND OBSERVATORY CATCHMENTS: THE
LEGACY AND THE FUTURE

WILEY

BIFoR FACE: Water–soil–vegetation–atmosphere data from a temperate deciduous forest catchment, including under elevated CO₂

A. Rob MacKenzie^{1,2} | Stefan Krause^{1,2} | Kris M. Hart¹ |
Richard M. Thomas^{1,3} | Phillip J. Blaes^{1,4} | R. Liz Hamilton^{1,2} |
Giulio Curioni^{1,2} | Susan E. Quick^{1,2} | Angeliki Kourmouli^{1,2} |
David M. Hannah^{1,2} | Sophie A. Comer-Warner^{1,2} | Nicolai Brekenfeld^{1,2} |
Sami Ullah^{1,2} | Malcolm C. Press^{1,5}

¹Birmingham Institute of Forest Research,
University of Birmingham, Birmingham, UK

²School of Geography, Earth and
Environmental Science, University of
Birmingham, Birmingham, UK

³Now at Big Sky Science Ltd, West
Midlands, UK

⁴Now at Sustainability Dept., Yorkshire Water,
Leeds, UK

⁵Now at Manchester Metropolitan University,
Manchester, UK

Correspondence

A. Rob MacKenzie, Birmingham Institute of
Forest Research, University of Birmingham,
Birmingham B15 2TT, UK.
Email: a.r.mackenzie@bham.ac.uk

Funding information

Natural Environment Research Council, Grant/
Award Numbers: NE/N020502/1, NE/
P003486/1, NE/S015833/1, NE/T000449/1,
NE/T012323/1; University of Birmingham;
The JABBS Trust; Norbury Park Estate; The
John Horseman Trust; Ecological Continuity
Trust

Abstract

The ecosystem services provided by forests modulate runoff generation processes, nutrient cycling and water and energy exchange between soils, vegetation and atmosphere. Increasing atmospheric CO₂ affects many linked aspects of forest and catchment function in ways we do not adequately understand. Global levels of atmospheric CO₂ will be around 40% higher in 2050 than current levels, yet estimates of how water and solute fluxes in forested catchments will respond to increased CO₂ are highly uncertain. The Free Air CO₂ Enrichment (FACE) facility of the University of Birmingham's Institute of Forest Research (BIFoR) is the only FACE in mature deciduous forest. The site specializes in fundamental studies of the response of whole ecosystem patches of mature, deciduous, temperate woodland to elevated CO₂ (eCO₂). Here, we describe a dataset of hydrological parameters – seven weather parameters at each of three heights and four locations, shallow soil moisture and temperature, stream hydrology and CO₂ enrichment – retrieved at high frequency from the BIFoR FACE catchment.

KEYWORDS

climate change, elevated carbon dioxide, long-term monitoring, soil moisture, soil–vegetation–atmosphere transfer, stream metabolism

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. *Hydrological Processes* published by John Wiley & Sons Ltd.

Hydrological Processes. 2021;35:e14096.
<https://doi.org/10.1002/hyp.14096>

wileyonlinelibrary.com/journal/hyp | 1 of 8

Soil moisture and temperature dynamics in juvenile and mature forest as a result of tree growth, hydrometeorological forcings, and drought

Andrea Rabbai^{1,2} | Doris E. Wendt³ | Giulio Curioni^{1,2} | Susan E. Quick^{1,2} |
A. Robert MacKenzie^{1,2} | David M. Hannah^{1,2} | Nicholas Kettridge^{1,2} |
Sami Ullah^{1,2} | Kris M. Hart^{1,2} | Stefan Krause^{1,2,4}

¹School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, UK

²Birmingham Institute of Forest Research, University of Birmingham, Edgbaston, UK

³Department of Engineering, University of Bristol, Bristol, UK

⁴LEHNA - Laboratoire d'écologie des hydrosystèmes naturels et anthropisés, University Claude Bernard, Lyon, France

Correspondence

Andrea Rabbai, University of Birmingham, Birmingham, Edgbaston, B15 2TT, UK.
Email: axr1049@student.bham.ac.uk

Abstract

Afforestation, as one of the major drivers of land cover change, has the potential to provide a wide range of ecosystem services. Aside from carbon sequestration, afforestation can improve hydrological regulation by increasing soil water storage capacity and reducing surface water runoff. However, afforested areas are rarely studied over time scales appropriate to determine when changes in soil hydrological processes occur as the planted (mixed) forests establish and grow. This study investigates the seasonal soil moisture and temperature dynamics, as well as the event-based responses to precipitation and dry periods, for a mature and a juvenile forest ecosystem over a 5-year time period. Generally, soil moisture was higher in the juvenile forest than in the mature forest, suggesting a lower physiological water demand. Following the 2018 drought, soil moisture dynamics in the growing juvenile plantation began to match those of the mature forest, owing to canopy development and possibly also to internal resilience mechanisms of the young forest to these external hot weather perturbations. Soil temperature dynamics in the juvenile plantation followed air temperature patterns closely, indicating lower thermal regulation capacity compared to the mature forest. While our findings show that an aggrading juvenile plantation achieves mature forest shallow soil moisture storage dynamics at an early stage, well before physiological maturity, this was not the case for soil temperature. Our results shed light on long-term trends of seasonal and event-based responses of soil moisture and temperatures in different-aged forest systems, which can be used to inform future assessments of hydrological and ecosystem responses to disturbances and forest management.

KEYWORDS

afforestation, BIFoR, drought, forest hydrology, micro-climate, soil moisture

This is an open access article under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2023 The Authors. *Hydrological Processes* published by John Wiley & Sons Ltd.

Hydrological Processes. 2023;37:e14919.
<https://doi.org/10.1002/hyp.14919>

[wileyonlinelibrary.com/journal/hyp](https://onlinelibrary.wiley.com/doi/10.1002/hyp.14919) | 1 of 15

Appendix 2: Chapter Two Supplementary Material.

2-A: Maps of Arrays showing details of precipitation, throughfall, soil moisture/temperature measurement transducer positioning and of soil sampling areas.

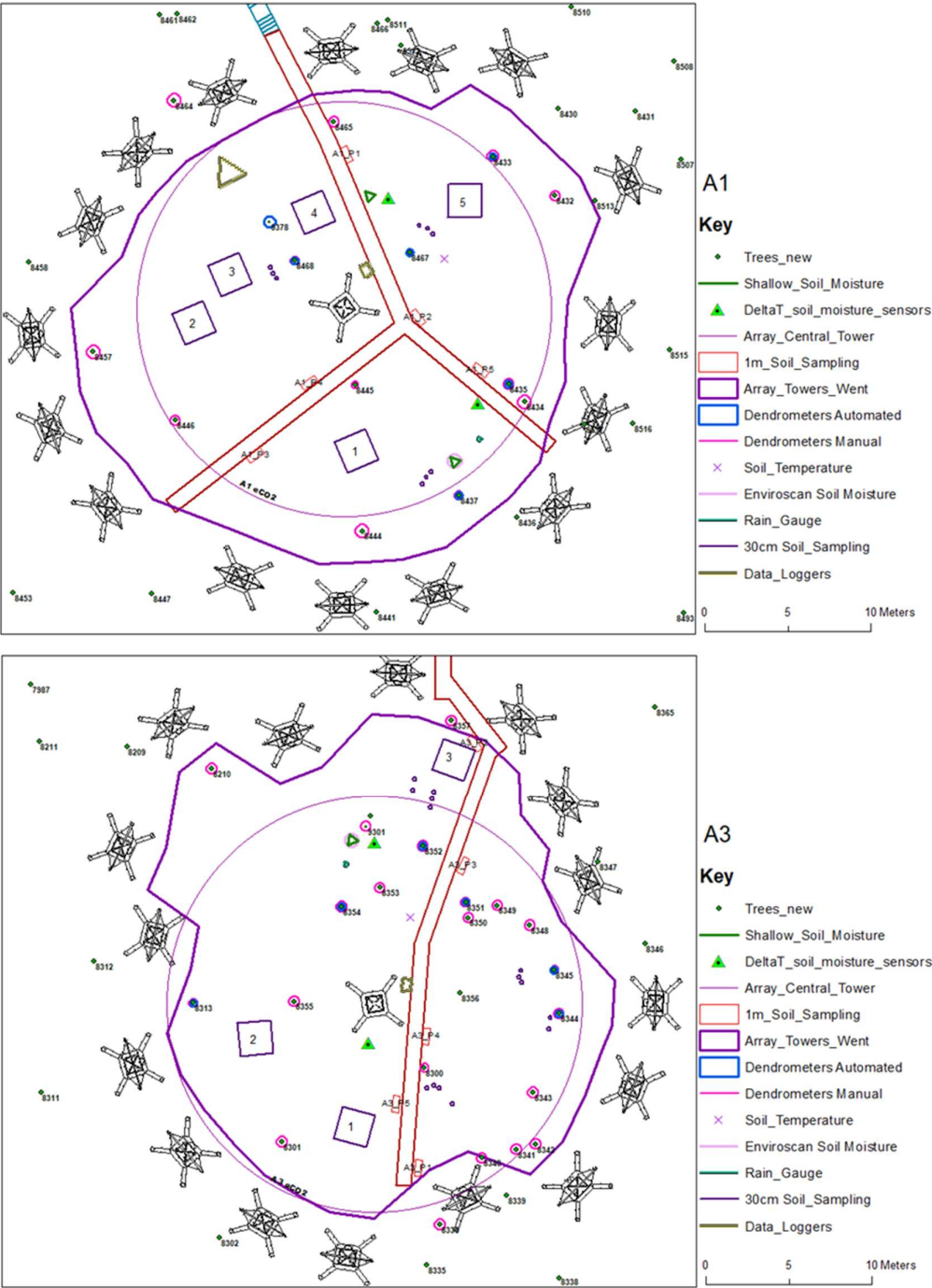


Figure App2A- 1: Array 1 (eCO₂) and Array 3 (aCO₂) maps.

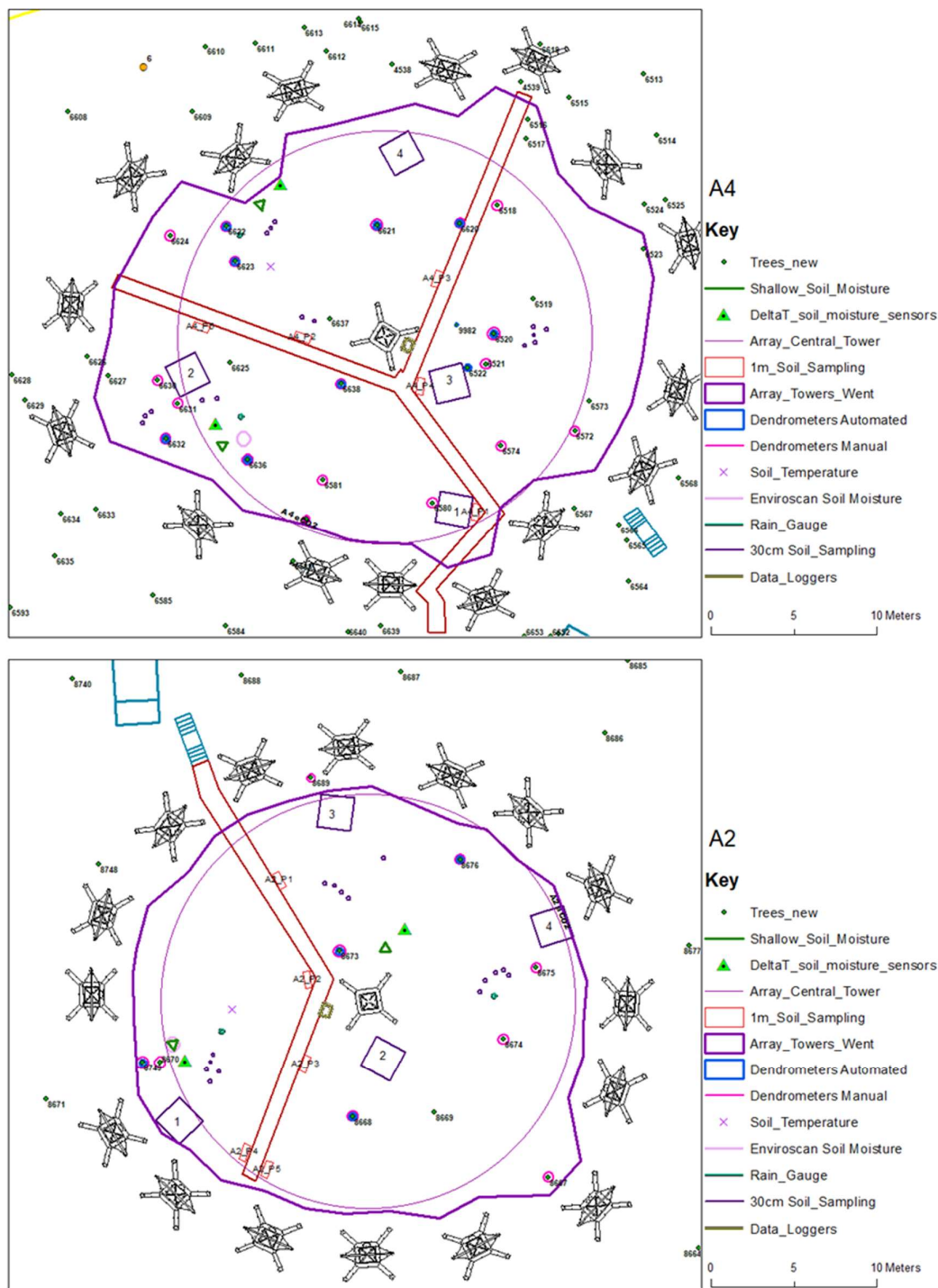


Figure App2A- 2: Array 4 (eCO₂) and Array 2 (aCO₂) maps.

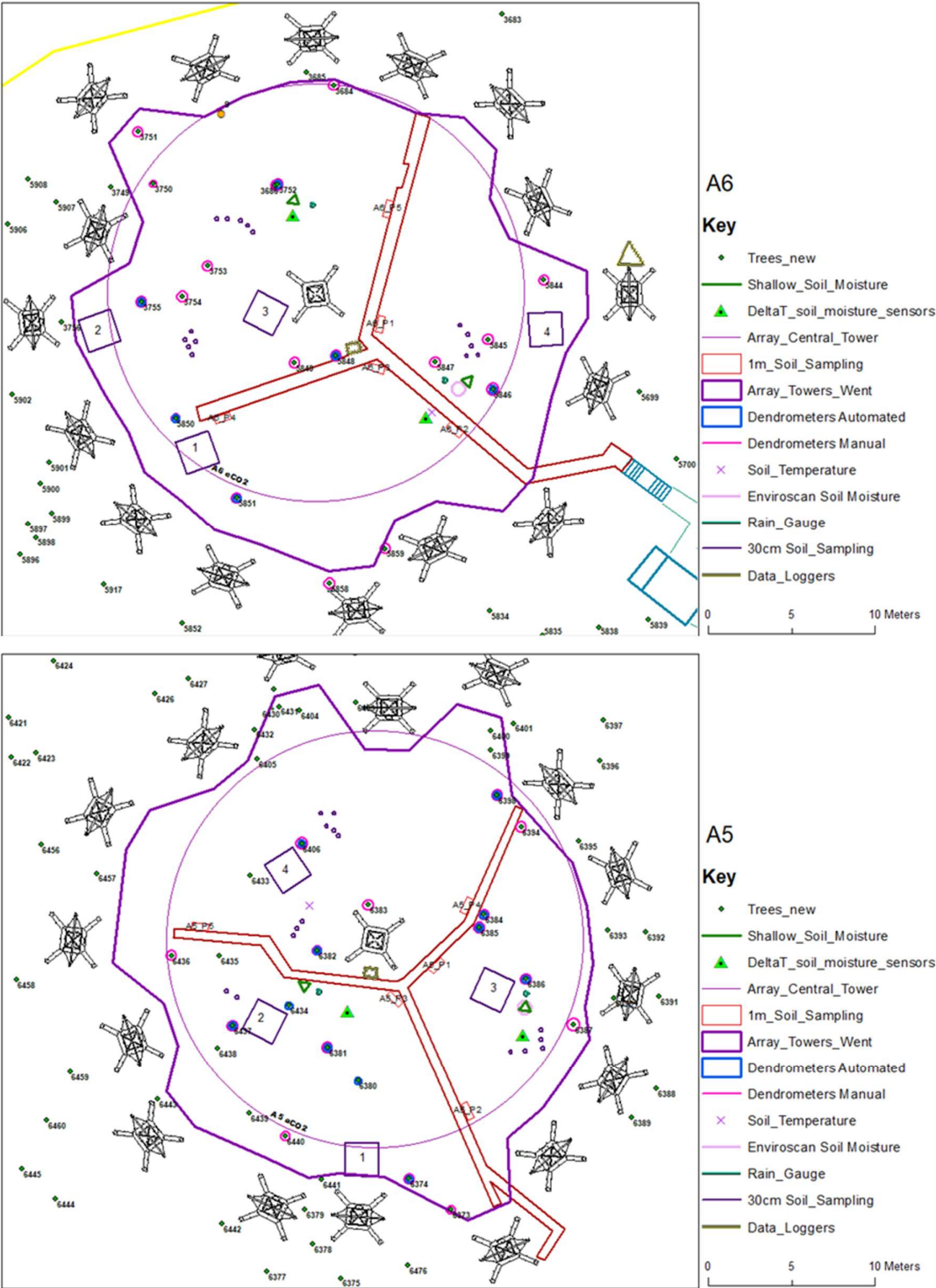


Figure App2A- 3: Array 6 (eCO₂) and Array 5 (aCO₂) maps.

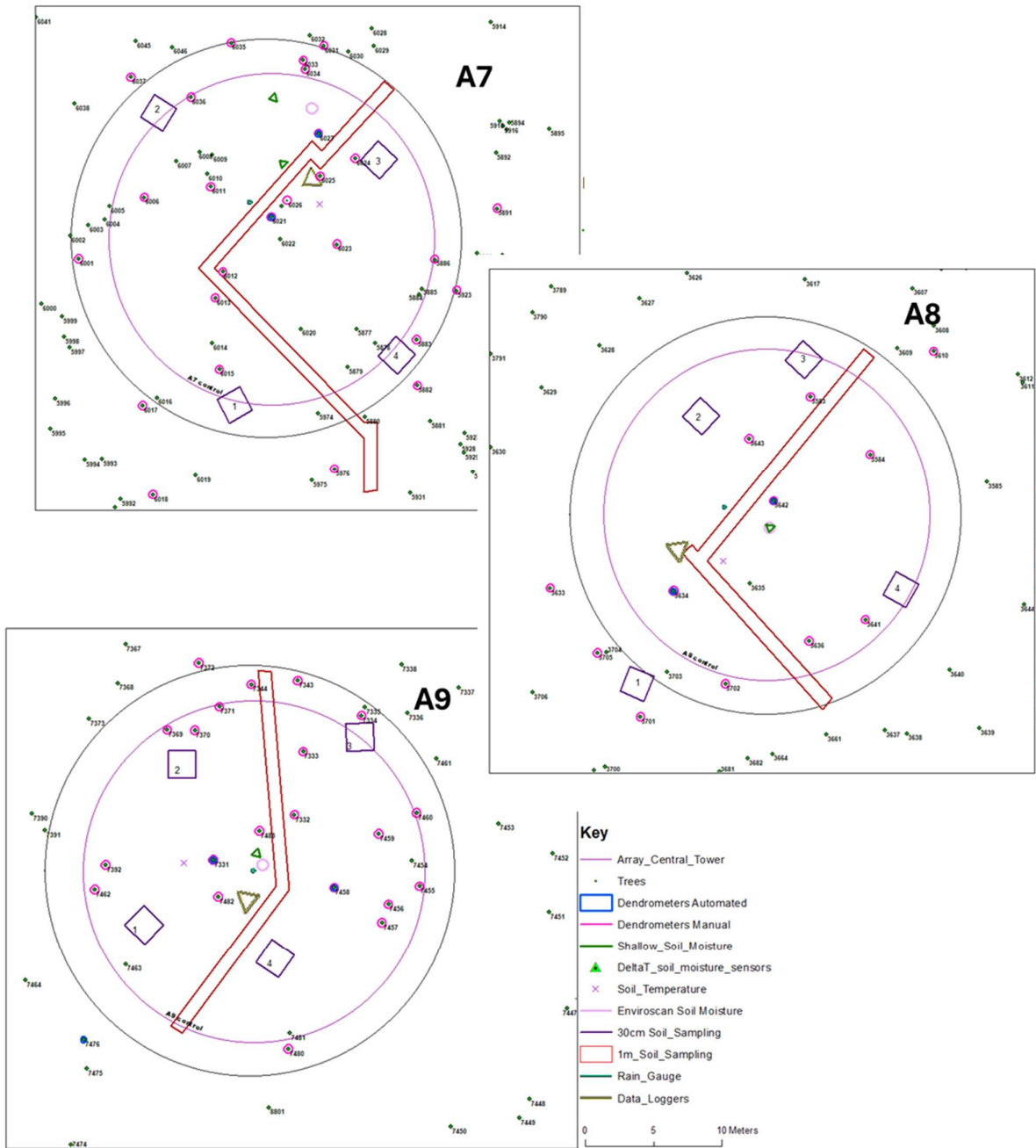


Figure App2A- 4: Array 7, Array 8 and Array 9 (Ghost) maps.

2-B: Detailed method for sap flow probeset installations

DETAILED DESCRIPTION OF ACTIVITY BEING ASSESSED

● Please include proposed schedule ●

Task A Trial drilling of selected sites to demonstrate sapflow measurement/ monitor water usage. This activity covers process of preparation and drilling of tree for probe insertion:

- 1) Assemble all tools including: flexible tape measure/chalk/ string to measure circumference at probe set (heater probe + two thermistor probes) positions. Height is just below standards DBH avoiding other e.g. dendrometry heights. Laptop with LoggerNet software, sapflow program, battery drill, chisel, gauge to test hole depth (if needed), callipers for bark depth, Vaseline, meter to check continuity of connections/ printed labels for probe sets (usually completed before going to target tree), screwdrivers/ crimping for wiring, pre-made leads.
- 2) Assemble box of probe equipment (including special drill, cooling water) and (if no data recorder in position) data recorder on trolley or suitable tripod
- 3) Pre-test probes & label (also test data recorder if needed) in workshop
- 4) Ensure Batteries for portable drill, laptop are fully charged including any spares. Ensure data recorder battery is fully charged and charging system operational. Check Heater battery system is ready to deploy (see separate method statement for this electrical installation) and take a research cabinet key to enable isolation/ connection. handheld data recorder control also.
- 5) Biosecurity measures for all equipment to be used on tree (e.g. alcohol based Propellar) prior to installation on each tree. Gloves/ bags for collection of cores (if corer used) and for inserting Vaseline/ pre-sterilised probes. Use plant nappy if re-sterilisation of any equipment (e.g. drills) is required



Photo 1

1) Follow instructions in Tranzflo Manual for chiselling bark & drilling holes (extract attached) summary (+photos of 7cm probes):

a) Measure and record circumference at height of installation (between 1m and 1.5 m height) to nearest mm. Mark nominal positions with chalk and attach drilling template with strap. Record ID, location and species of Tree to enable update of GIS map of (nearest) research array on recording form. (photo 1)

b) Wearing gloves, drill holes 20mm depth at a time, horizontally through template, using high speed drill until hole is 20mm longer than probe (100mm for 80mm probes), or as deep as drill can achieve. Chalk around drilling template to ensure position of holes can be seen. Remove template. (Photo2)



Photo 2

Re-drill holes to achieve maximum depth with longest drill. Temporarily plug hole with clean Vaseline using gloves. (Photo 3)

- c) (Optional) Carefully chisel out a small section of bark just above the drilled positions in one tree per research patch (if required) without damaging the cambium. Retain bark in labelled zip bag for later bark depth measurement check.
 - d) Check hole depths from cambium into sapwood and re-drill to obtain correct depths if necessary; N.B. use spacers on probes to ensure probe does not go too deep.
- 2) (Optional) Use corer and callipers to test depth of bark/ cambium at each heat sensor probe position. Record for each position = upper and lower.



Photo 3



Photo 4

3) Insert two heat sensor probes (unmarked probe at upper position, red or white dot is on lower probe) with spacers carefully using more Vaseline if needed. Protect connector from contamination. (Photo 4)

4) Insert heat pulse heater probe to full depth (no spacer) using more Vaseline, checking the probe is fully into the tree at correct depth.

5) Tape all probes gently to tree with reflective waterproof tape (don't bend). Add supporting band (e.g. plastic

washing line) and secure/ support cables routing them towards the heaterbox enclosure or logger with spiralwrap/ cables ties. (Photo 5). Insulation may be added if available and needed.

- 6) If connectors are not to be immediately installed bag cables for future connection and tie to tree.

Task B connect all probes to datalogger (if necessary via a waterproof enclosure for extension leads) and set up using procedures programs pre-tested in workshop.



Photo 5

Appendices

See separate documents for schedule 2018-19 and shorter probe installation to sub-dominant trees in 2020 as agreed Ops manager.

Schedule (2016-2017):

Date	Activity	Who	Authorised by
17-22Nov'16	Check and prepare all equipment required. Agree site with KH	SQ, PB + assistant	KH <i>Complete</i>
23Nov'16 & Jan 5 th onwards	Select and check trial site – one tree. Identify specific hazards on day re weather (wind rain, understorey trip, standing water). Agree safe to install probes without attaching power. Complete installation task A	SQ, PB + assistant	KH (23 Nov 16) <i>Complete</i> KH/deputy staff (5 th Jan 17 on)
5-31 Jan'17	Complete installation of datalogger (Task B) with dummy collection of data with or without multiplexing for up to 4 probes sets (12 drill holes) in 1 tree (oak Tree No. tbc, adjacent Array 9), which may be live pulsed.	SQ/ PB + assistant	NH or PB
23Nov'16 & 5 Jan 2017 onwards & on completion of wiring	Ensure site is left in safe condition so no trip hazards or loose cables present. Review and adjust method and risk assessment in preparation for future installations. Monitor tree health and continuing data condition.	SQ	KH + PB
5 th Jan-28 Feb 17	Refine and justify method, order additional equipment and agree selection of oak trees in all Arrays for monitoring.	SQ	KH + Science team
1 st Mar 2017 onwards	Install up to 4 probes sets/tree in 2 oak trees per Array (Arrays 1-9). Commission to give live data.	SQ + volunteers	KH + Science team
31 st Mar 17	Consider which other species trees to monitor and design method for species comparison. Plan equipment needs, installation and agree.	SQ	KH + Science team

Appendices

*Items requiring biosecurity measures

Tranzflo Items per sap flow position – (max 2 positions /tree)	Y/N	Tools and equipment (bold for on-site initial drilling etc)	Onsite Y/N
2x heat pulse temp sensor probes	*	Tranzflo Metal drilling template	* Y
1x Heater pulse probe + power lead		Tranzflo Drill bits	* Y
1 Heater box with flying leads (power + control)	*		
2x short leads for connection to multiplexer		Multimeter – checking probes	Yes
		Flexible tape measure or string + metal tape for measuring tree circumference & height of probesets +/- 1mm	Yes
		Campbell Datalogger <i>CR1000 + multiplexer</i>	Yes
		external 12V (>70 AHr) battery	Yes
		Laptop with SC32 interface and Campbell Software	SQ (or NH) provides
		Strap to attach drilling template	Yes
		Thick sticky tape for fixing probes to tree	Yes
		High speed Battery drill + spare battery (charged)	Yes
		Low Platform/ box to stand on (if required for height of person/ holes)	Yes (optional)
		Vaseline	Yes
		Bark Borer	
		Chisel for bark removal	* KH or SQ to supply chisels
		Disposable Gloves	KH or SQ to supply
		Plant Nappy/Blue roll/ tissue/ plastic bags – for keeping items clean during installation	Yes
		Propellar spray (or equivalent) alcohol based for probe/ tool biosecurity	Yes
		Small zip bags – retaining tree bark core for testing/ analysis (if required)	KH or SQ to supply
		(electronic preferably) Callipers for tree core	SQ to supply
		Feeler prong marked with target hole depth	* Yes
		Metal rule	Yes
		Bottle of clean water -for cooling drill bit	Yes
		Clipboard + recording sheet, pen/pencil	SQ to supply
		Plastic spacers (Tranzflo supplied) or Clean corks* drilled out with small hole to probe dia.	* SQ to supply
		Bollard/ tape to indicate work area (if others are working in vicinity)	Yes
		Cable tie and plastic washing line (to secure cables and enclosure) as required.	Yes
		Research cabinet key	Yes

2-C: Detailed method for Tree core sampling and processing.

This Appendix contains the following documents:

1. Mini Report containing sampling method, weighing/drying method and calculation methods for obtaining woody matrix parameters for oak trees.
2. Photos of sampling, measuring and inspection of cores for woody matrix calculation.

Document 1: following 18 pages

Methods for calculating actual sapflow following wound correction.

Background:

In order to study tree ring growth and measure density of xylem during seasonal xylem sapflow, it is necessary to take incremental cores from those trees being monitored. As it is not possible to sample full incremental cores during the long-term experiment, we took small mini cores of wood adjacent to the position of the sapflow monitoring instrumentation. Mini cores may also be taken at other positions in future if it is desired to look at the variability of xylem growth around the tree at breast height. By taking cores close to xylem sapflow probe set positions it is hoped that variations in ring width due to tree growth asymmetry, numbers of rings within measurement core and differences in annual growth increment due to maturity and decline, might be averaged out enabling comparison between those trees being monitored.

To test the method in winter 2018-2109, a 10mm long Trephor micro-coring device was borrowed from Swansea University (Prof. Neil Loader). Whilst growth cores are usually taken during Spring and Summer we took preliminary cores to see the growth over the past 1-2 years of sapflow installation.

The other and primary purpose for coring was to confirm the wood density parameters of the combined woody matrix to enable calculation of a conversion factor between compensated heat pulse velocity obtained from the thermocouple transducer probes and sap flux density along the heat pulse path to the measurement point. This is to be used to post-process the wound compensated heat velocity data.(see Appendix A)

To this end two additional full incremental cores were taken from similar sized trees outside of the experimental arrays. These cores were expected to more closely reflect the wood characteristics of oak as they would contain a better cross-section of xylem tissue.

Appendices

We speculate that, as bark tissue contains less voids, the mini core samples will have a higher greenwood woody matrix density which distorts the calculation towards a higher conversion factor. In comparison the full incremental core samples will more accurately reflect the wood characteristics experienced around the sapflow probesets.

Methods

Sequence for collecting cores

The following briefly describes the staged sample collection procedures carried out on oak trees BIFoR FACE. Twenty-one mini cores were taken from two trees in each of the following arrays: A1 (eCO₂), A3 (ambient), A7,8&9 (natural). In addition two full incremental cores (0.1m radial depth) were taken from two oak trees outside of the experimental arrays.

(following to be converted into written paragraphs if needed)

Procedure 1. (in woodland)

Equipment Proc. 1 (woodland):

- Propellar?, de-Ionised water? Trephor (small corer + clearing device) + wooden + rubber mallets.
- Containers (microcentrifuge tubes) and re-sealable bags (10cm x?) for samples.
- Sharpie pen. Sapflow Tree number plan. Recording sheet/clipboard. Camera. Hand lenses.
- Plant nappy & footprint mats. Nitrile gloves. Waste beaker + empty water container for rinsing water. Blue roll to dry the corer.
- Coolbox/bag to contain the samples at ambient temperature.
- Thermometer to measure bark/ cambium surface temperature. Protective bag or box for sharp ends.

Appendices

- Balance accurate to (?? +/- g). Beaker, pipette (size?) to measure water.

Method Proc.1A (prep + woodland + weighing & drying onsite at LongBarn)

N.B. a photographic or microscope record of the samples is desirable and was captured for most of the samples. (Figures)

1. (wetlab) Clean (propeller?) and dry equipment which will be used for sampling (lab sterilisation of corer at start, then use de-ionised water with blue roll on site) i.e. coring equipment before taking the first core, and between cores. Place sterilised equipment in resealable bag/ resealable box. Have additional containers/ bags available (see below). Wear gloves to prevent contamination.
2. Choose a time to take the sample which is between sapflow heat pulses (or stop logger program for period of sampling).
3. Take a core sample near to the position of the sapflow probes on the East side of the tree. Repeat for West side.
4. N.B. take the sample through bark crack positions if possible. Alternatively, first remove small piece of the bark (if loose) and then re-core through the cambium to the depth of the corer.
5. Carefully remove the core.
6. Place samples in containers or bags (see below). Label each container/bag with date, time of day, tree number, species and core position (East or West) and height above ground level, and temperature.
7. Take sample to lab. Record its fresh weight, *MF* [kg] using an accurate mass balance. This weight is equal to the mass of water and the mass of dry woody matrix since the mass of the gas content is considered negligible (see Edwards and Warwick (1984), Marshall (1958)).

Appendices

8. Measure the dimensions of the sample i.e. height of the cylindrical core sample, dia of cylinder using an electronic calliper or micrometer. Examine and Photograph the sample preferably using a microscope.
9. Weigh a narrow tall measuring cylinder containing enough deionised water and spare capacity to float the sample. Submerge the core sample in the cylinder. Encourage it to float away from the sides of the cylinder Record weight water displaced M_{water} which equals the displacement of water, D_T [kg]. V_T is then equal D_T / ρ_L N.B. See below for calculations carried out
10. Remove sample from water, allow to drain and reweigh (to determine if any extra water has been absorbed) – record new weight if it differs from fresh weight, M_F [kg]. Consider the implications. N.B. See below
11. The core sample is then oven-dried (temp ambient <30 degrees) for at least 5 days. Sample is reweighed to determine the mass of dry wood, M_D [kg].
12. On completion of measurements store samples in appropriately dimensioned Eppendorf tubes or paper bags, away from direct light and at ambient cool conditions.

Method Procedure 1B (assessed separately) for taking incremental cores outside the research arrays

Carry out steps 1-5 of Method 1A, but use a 4mm incremental corer instead of the Trephor device to about 10cm depth, at an appropriate height of the stem (no lower than 1.1m) preferably on the West side of the tree, being careful to keep corer horizontal and radial to the stem.

Appendices

6B Place samples in pre-weighed micro Eppendorf tubes or in bags. Label each container/bag with date, time of day, tree number, species and core position (East or West) and height above ground level, and temperature.

Carry out steps 7-12 of Method 1A

Place samples in pre-weighed micro Eppendorf tubes or in bags. Label each container/bag with date, time of day, tree number, species and core position (East or West) and height above ground level, and temperature.

Carry out steps 7-12 of Method 1A

13. Immediate Calculations: The difference between the fresh weight MF and the dry weight MD , ($MF-MD$) is equal to the mass of water, ML [kg], contained in the fresh core sample.

Limitations of the methods

The method 1A described above was followed for all the mini-cores over two sampling dates (03 Jan and 16 Jan 2019). Due to having to complete work within one day on 03 Jan, all the first set of fresh samples were weighed to obtain MF , but the volume displacement and calliper measurements could not be completed until 8th Jan. by which time some natural drying of these first cores will have taken place whilst stored in the lab. All first phase mini cores were then dried in a temperature controlled oven until 14th Jan and samples kept ready for dry weight measurement on 16th Jan.

In the case of the second set of mini-cores all necessary measurements of the green samples were made on the day of collection and drying followed sequentially to determine dry weight.

Appendices

Similarly for the two full incremental cores the correct timing and sequence of measurements and processes was followed. Only one orientation (West-facing) of incremental core was taken from each tree.

We discuss the limitations of this on the reliability of heat pulse velocity to sap flux density conversion factor calculation in the discussion section.

Comparison of calculation methods.

Due to uncertainty of the reliability of outcomes from different measurement techniques and calculation methods, the data collected from the core samples was subjected to some comparative calculations (see Edwards and Warwick (1984), Marshall (1958)).

This comparison was considered due to it being pointed out by an independent observer (Steve Green of Tranzflo NZ Ltd, Palmerston, New Zealand) that the sum of the wet and dry volume fractions exceeded 1, a physical impossibility. Double checks were therefore made on all the raw data. Three calculation methods were used: Calculation 1A using displacement method for calculation of green sample volume, Calculation 1B using callipers to measure the green sample dimensions, and Calculation 2 using the displacement method for calculation of volume. They are described as follows.

Calculation 1 reference: Edwards and Warwick (1984)

This method was that recommended by the sap flow transducer manufacturer ((Tranzflo NZ Ltd, Palmerston, New Zealand) in their user manual.

Edwards and Warwick developed the working equation to calculate point sapflux density J (units here are $(m^3 m^{-2} s^{-1})$), the volume of fluid (**sap**) that is passing, or flowing, across a given area of sapwood per **unit** of time, using Equation (4) from the reference:

Appendices

$$J = (0.505 F_M + F_L) v_c (4) \text{ [conversion factor } c_1 = (0.505 F_M + F_L) \text{)]}$$

where F_M and F_L are the volume fractions of wood and water, respectively based on Archimedes' principle. The factor 0.505 is related to the thermal properties of the woody matrix and is assumed to be constant within and between species. v_c is the wound corrected heat-pulse velocity in mks units $m s^{-1}$.

The volume fraction of water is

$$F_L = M_L / (\rho_L V_T)$$

where V_T (m^3) is the total volume of the green sample, ρ_L the density of sap is assumed very close to water (1000 kg m^{-3}), and M_L [kg] is equal to the mass of water in the fresh core sample calculated from the difference between the fresh weight and the dry weight, $(M_F - M_D)$.

Similarly, the volume fraction of wood

$$F_M = M_D / (\rho_M V_T)$$

where the **density of dry wood**, ρ_M equals **1530 kg m^{-3}** . For the purposes of this calculation we adopt the assumption of Edwards and Warwick that this is uniform across species.

There are two simple options available for measuring V_T . Firstly the water displacement method described in the sequence above, and by the manufacturer which is reputedly preferred for small values of mass (Calculation 1A), or secondly by taking the physical dimensions of the cylindrical sample (Calculation 1B). The disadvantage of the second method, in addition to the inaccuracies with small samples is that unless the core sample is first squared off, there will be asymmetry in the

Appendices

cylinder length. The cylinder diameter will also vary due to the method of forcing the corer into a woody matrix under tension. (see photos below in Fig 1. And Fig 2)



Fig.1 To show typical mini cores. Left image: Approx. one third (circa 3-4mm) xylem tissue. Right image: Distortion and cylindrical asymmetry during drying process

Calculation 2 reference: Marshall (1958)

Marshall's (1958) analysis showed that if the sap and woody matrix is considered to form a homogeneous medium, then the sap flux density, J [$\text{cm}^3 \text{cm}^{-2} \text{s}^{-1}$], can be calculated from his equation:

$$J = P(0.33 + M)V_c \quad (3) \text{ or}$$

$$J = c_2 V_c$$

Where conversion factor $c_2 = P(0.33 + M)$

In Eqn (3) P [g cm^{-3}] is the wood density (oven dry weight of wood/green volume), The factor 0.33 is the specific heat (*dimensionless*) of dry wood and M (*dimensionless*) is the moisture content ratio $(MF - MD)/MD$ ((wet weight - oven dry weight)/oven dry weight) of sapwood and V_c wound corrected heat-pulse velocity [cm s^{-1}]. We adopt Marshall's assumption that the specific heat is constant for all species.

To convert this equation to mks units therefore we need only convert the conversion factor c_2 where P [g cm^{-3}] wood density has a dimension. So to convert the wood density from mks to cgs units it is necessary to use the factor $10^3 / 10^6 = 10^{-3}$. The

Appendices

density and moisture content of the sapwood are both physical properties of the woody matrix, and they can be determined easily from the green and dried trunk cores. Indeed Edwards and Warwick used Marshall's principles to develop their own volume fraction approach.

Results

Table 1 & Fig 3 show the comparative findings of the various methods.

Appendices

Tree number	Facing direction	Array	Circum. (m) at sample ht	Average tree radius when sampled	Date & time of sampling	C1 Edwards and Warwick Conversion factor (0.505 FM + FL)	C2 conversion factor using measured Vol	Marshall conversion factor P (0.33 + M)	Ratio displacement volume / measured volume
7476	N	9	2.17	0.345	03/01/2019	1.400E+00	1.318E+00	1.400E+00	9.417E-01
7476	SW	9	2.17	0.345	03/01/2019	1.663E+00	9.862E-01	1.663E+00	5.929E-01
7476	SE	9	2.17	0.345	03/01/2019	1.537E+00	1.247E+00	1.537E+00	8.116E-01
7331	W	9	2.54	0.404	03/01/2019 11.40 GMT	1.203E+00	8.728E-01	1.203E+00	7.256E-01
7331	E	9	2.54	0.404	03/01/2019	1.417E+00	9.525E-01	1.417E+00	6.720E-01
6021	W	7	2.66	0.423	03/01/2019 c12.40hrs	6.180E-01	2.028E+00	6.180E-01	3.282E+00
6021	E	7	2.66	0.423	03/01/2019 c12.40hrs	1.552E+00	7.985E-01	1.552E+00	5.144E-01
6027	W	7	2.08	0.331	03/01/2019 c 12.50hrs	1.406E+00	8.641E-01	1.405E+00	6.148E-01
6027	E	7	2.08	0.331	03/01/2019 c 12.50hrs	1.256E+00	1.111E+00	1.256E+00	8.842E-01
3634	W	8	2.716	0.432	03/01/2019	1.394E+00	9.047E-01	1.394E+00	6.488E-01
3634	E	8	2.716	0.432	03/01/2019	9.757E-01	4.103E-01	9.756E-01	4.205E-01
3642	W	8	1.77	0.282	03/01/2019	1.204E+00	7.378E-01	1.203E+00	6.130E-01
3642	E	8	1.77	0.282	03/01/2019	1.169E+00	7.891E-01	1.169E+00	6.750E-01
9301	W	3	2.58	0.411	16/01/2019 11.05hrs	7.518E-01	6.500E-01	7.518E-01	8.645E-01
9301	E	3	2.58	0.411	16/01/2019 11.05hrs	7.808E-01	5.638E-01	7.808E-01	7.221E-01
8351	W	3	1.72	0.274	16/01/2019 10.20hrs	7.398E-01	5.988E-01	7.398E-01	8.094E-01
8351	E	3	1.72	0.274	16/01/2019 10.20hrs	4.910E-01	4.910E-01	4.909E-01	NA
8468	W	1	1.73	0.275	16/01/2019 12.15hrs	6.390E-01	5.061E-01	6.390E-01	7.920E-01
8468	E	1	1.73	0.275	16/01/2019 12.15hrs	1.089E+00	1.270E+00	1.088E+00	1.167E+00
8467	W	1	1.66	0.264	16/01/2019 11.55hrs	5.309E-01	5.701E-01	5.309E-01	1.074E+00
8467	E	1	1.66	0.264	16/01/2019 11.55hrs	5.682E-01	4.643E-01	5.682E-01	8.172E-01
SUMs						2.238E+01	1.813E+01	2.238E+01	1.764E+01
Averages (kms)						1.066E+00	8.635E-01	1.066E+00	8.821E-01
Incremental cores taken from outside the Arrays.									
8606-W		path near to A1&A3	2.215	0.353	15-03-2019 12.00hrsGMT	8.671E-01	8.939E-01	8.671E-01	1.031E+00
3176 -W		near Gate 5	1.93	0.307	15-03-2019 12.20hrs GMT	6.110E-01	5.160E-01	6.109E-01	8.445E-01
Average of full cores						7.391E-01	7.049E-01	7.390E-01	9.377E-01
Mean all cores						1.038E+00	8.498E-01	1.037E+00	8.486E-01
weighted mean						8.480E-01	7.578E-01	8.480E-01	9.192E-01

Table 1. To show results of various calculation methods for correction factor between wound compensated heat pulse velocity and point sap flux density. (note to ARM – weighted average was an experiment allowing for volume of mini core relative to volume of full cores.)

Appendices

Calculation methods 1A and 2 gave identical results. In both cases there were anomalies with the conversion factors calculated for the mini cores.

Calculation method 1B gave a consistently larger variance in measurements of green volume (to be analysed in R later if so required) relative to method 1A.

This finding leads us to consider possible causes of the green volume differences.

These could be due to:

- a) The differing proportion of bark to xylem tissue
- b) the impact of delayed measurement of raw samples on the conversion factor calculation.
- c) The possibility that due to the season of sampling gas void were present in the samples
- d) Other reasons caused the anomaly.

It is interesting to note (see Fig.1) that, due to insufficient depth of sampling, only a very small part of the mini-core was actually sampling the xylem wood, the remainder being phloem, cambium and inner bark wood which is very different in its moisture content to xylem wood (ref to be sought). For the full incremental cores, the xylem wood made up the largest portion of the sample but was across the equivalent of the whole depth of the sapflow probe which includes differing density of lumen fractions (the moisture containing part of the wood sample).

The results of methods 1a and 2 were plotted (Fig 4) against average tree radius at measurement point, derived from measuring tree circumference at time of sampling of the core (Table 1).

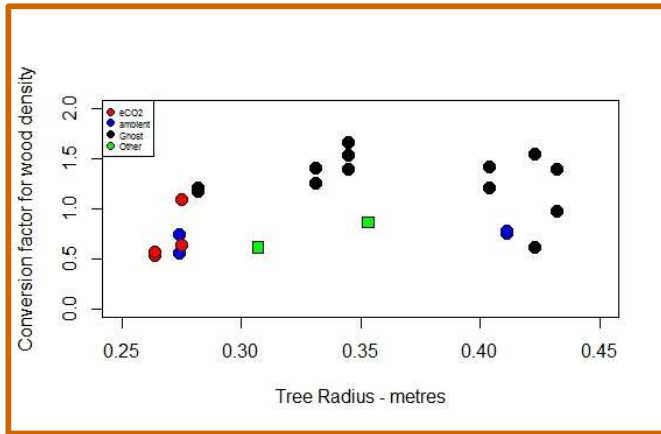


Fig 3. Results of conversion factor C1 against tree radius

Discussion

For mini cores our calculation is based on mass/ volume of a reasonable quantity of green (and subsequently dried) samples of current/ previous season xylem wood matrix from the outer xylem. As explained in the results, these samples contain a large proportion of bark tissue which influences the calculation in respect of xylem wood characteristics. It is estimated that two seasons' growth is sampled using a 10mm depth mini corer. It is recommended that a longer corer be obtained (e.g. 15mm) to give better information about season growth when sampling oaks in experimental arrays.

Only two full incremental cores, from positions outside the experimental arrays, could be taken to characterise the wood characteristics for the full probe depth. The full incremental core samples, as sampled to approximately 10cm depth, gave a more representative results for woody characteristics and sap flux density correction factor calculation.

Conclusion.

Wood core sampling is a useful method for examining xylem growth, especially if used consistently with the same tree at more than one sampling point to represent an

Appendices

averaging of xylem growth around the tree circumference. Mini cores cause less damage to trees being monitored within a long-term experiment. More detailed measurement of green mini cores, taken from consistent positions of selected trees, should be taken at annual or two-yearly intervals. Adjustments can be made using circumference measurements/ dendrometer records to adjust for xylem cross-sectional area increase in any one year. By using a scale with a microscope to measure growth shown by the mini cores, we should in future be able to compare differing effects of treatments.

We found that incremental cores, taken to the depth of sap flow monitoring probesets, gave a representative result for wood characteristics, but should best be taken at two positions in each tree to reflect the asymmetry of xylem growth.

It is recommended that further incremental cores be taken to give a more representative sample of wood characteristics, both across the woodland, in terms of orientation West and East, and across the range of radii of trees being monitored. This will enable calculation of a more representative correction factor for the heat pulse sap flux calculations and hence enable better estimates of sap flux density across the xylem of each sapflow monitored tree.

References

- Edwards, W. R. N., & Warwick, N. W. M. (1984). Transpiration from a kiwifruit vine as estimated by the heat pulse technique and the penman-monteith equation. *New Zealand Journal of Agricultural Research*.
<https://doi.org/10.1080/00288233.1984.10418016>
- Marshall, D. C. (1958). Measurement of Sap Flow in Conifers by Heat Transport. *Plant Physiology*, 33(6), 385 LP – 396. <https://doi.org/10.1104/pp.33.6.385>

Appendices

Appendix from original methods document

Method 2

Extracts from Barrett et al (1995)

Barrett, D. J. *et al.* (1995) 'Evaluation of the heat pulse velocity technique for measurement of sap flow in rainforest and eucalypt forest species of south-eastern Australia', *Plant, Cell & Environment*. Wiley/Blackwell (10.1111), 18(4), pp. 463–469. doi: 10.1111/j.1365-

ment. Consequently, the heat pulse velocity (not sap velocity as in Edwards & Warwick 1984 nor sap flux as in Green & Clothier 1988) must be corrected for wounding:

$$V_h' = a + bV_h + cV_h^2, \quad (2)$$

where V_h' is the corrected heat pulse velocity (m s^{-1}) and the coefficients a , b and c were derived by Swanson & Whitfield (1981) from numerical solutions of Marshall's (1958) equations for various wound sizes. Sap flux density (V_s ; m s^{-1}) is calculated from the corrected heat pulse velocity as

$$V_s = \frac{V_h' \rho_b (C_w + mC_s)}{\rho_s C_s}, \quad (3)$$

where C_w and C_s are the specific heat capacities of dry wood ($1.38 \times 10^6 \text{ J g}^{-1} \text{ }^\circ\text{C}^{-1}$; Skaar 1972) and sap (water = $4.19 \times 10^6 \text{ J g}^{-1} \text{ }^\circ\text{C}^{-1}$), respectively, ρ_b (kg m^{-3}) is the basic density of dry sapwood, ρ_s (kg m^{-3}) is the density of sap (water = $1 \times 10^3 \text{ kg m}^{-3}$), and m (kg water kg^{-1} wood dry weight) is the moisture content of sapwood (Closs 1958; Marshall 1958; Swanson & Whitfield 1981). Note that Eqn 3 is the correct expression for sap flux in Système International units rather than the older form which has persisted since Marshall (1958).

Sap flow (Q ; $\text{m}^3 \text{ s}^{-1}$) may be calculated by integration around the tree bole of a parabolic function fitted to the radial sap velocity profile (Swanson 1975; Cohen, Fuchs & Green 1981; Edwards & Warwick 1984; Green & Clothier 1988). While such a relationship has been observed in some conifers (Swanson 1975), it cannot be assumed for other species, particularly ring-porous species

(e.g. Miller, Vavrina & Christensen 1980; Dye, Olbrich & Poulter 1991). Alternatively, sap flow may be calculated from the sap velocity weighted by the area of sapwood most closely associated with each point temperature measurement (Hatton, Catchpole & Vertessy 1990) as

$$Q = \sum \pi(r_i^2 - r_{i+1}^2)V_s, \quad (4)$$

where r_i and r_{i+1} are the outer and inner radii of an annulus of sapwood most closely associated with the i th temperature sensor. This method makes no assumption about the profile of sap velocity through the sapwood but does assume that sap velocity is constant within each annulus around the tree bole. Consequently, multiple sampling points dispersed through the sapwood are required for robust estimation of sap flux (Dye *et al.* 1991).

.....

The wound width for each species was measured from hand sections of sapwood taken from each sapling 2 d after probe insertion. Sections were examined under both light microscopy, at $40\times$ magnification, and scanning electron microscopy using the procedures outlined in Exley, Butterfield & Meylan (1974), Exley, Meylan & Butterfield (1977) and Kucera (1981). Cubes (approximately $3\times 3\times 5$ mm) of xylem tissue were sectioned from samples of sapwood taken adjacent to drill holes in trees in the field. Sectioned material was mounted on specimen stubs prior to coating with a gold-paladium conducting layer and stored in a desiccation container.

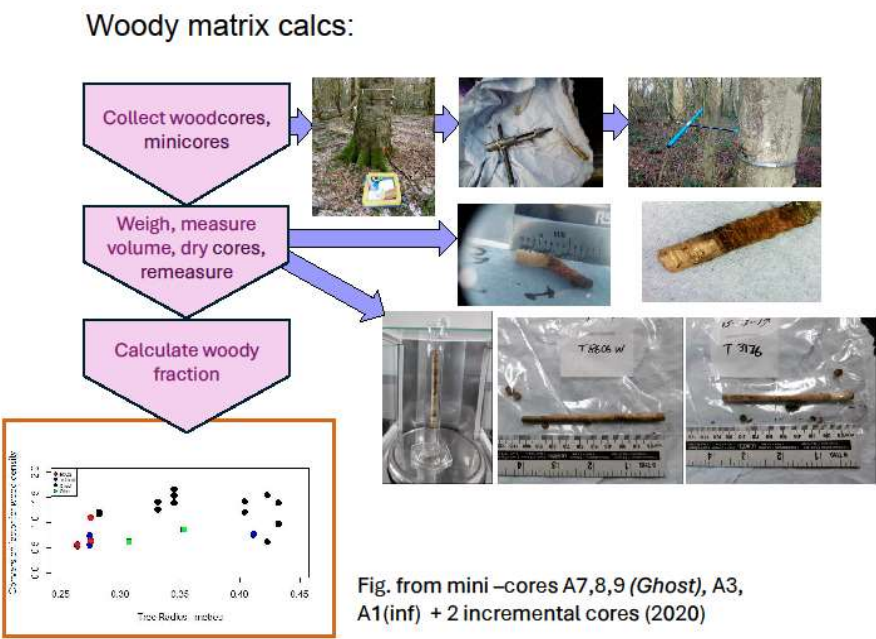
Wood density and moisture content of the sapwood were measured in samples collected with a 4-mm-diameter increment corer and placed in pre-weighed micro-Eppendorf tubes. Wood volume was determined by water displacement of fresh samples. Wet and dry weights were determined before and after drying samples for 24 h at 110°C . Wood density, ρ_b , was calculated from the ratio of dry weight to wood volume. Wood moisture fraction, m , was calculated from the ratio of weight of water in the wood sample to wood dry weight. Sap flow was calculated every 15 min using the above variables in Eqns 1–4 and converted to units of kg h^{-1} for comparison with other studies.

Appendices

Method 3

Requirements for retaining sample for ^{13}C isotope purposes (message N. Loader).

If you sample a core from any of the trees, and do not need them for your own research, please can you keep the microcore in a microcentrifuge tube (labelled) and then freeze it as it might be possible to test if we can get a ^{13}C from the sample early- and late-wood as a way of non-invasive sampling.



2-D: Detailed method for stomatal conductance and leaf temperature measurement using porometry.

This Appendix contains the following documents:

1. Brief method statement for porometry of cut-twig and by arborists in-situ in canopy.
2. Brief method statement for porometry and leaf temperature measurement at height using Canopy Access system (CAS).

Document 1: following 5 pages

Method Statement for porometry measurements through canopy and at ground level.

A: Calibration and Measurement by research staff/ students or operations personnel.

1. Prior to taking measurement ensure porometer (e.g. Decagon device) is in good clean condition with fresh desiccant and calibrated/ equilibrated (use **Calibration card** procedure).
- N.B. Check battery condition before calibration; after battery change it is necessary to reset the date and time on the device. Store used desiccant in a suitable bag or bottle for recycling/ disposal.
2. For ground level measurements use standard measurement process (Use **Quick Start Guide** procedure) as shown on Decagon card. All measurements should normally be of the bottom of the leaf and use the **Auto** process (30secs). Unclip from leaf on completion. Each measurement should be labelled (use **Annotate**) indicating the species and position of the sample (e.g. nearest/ actual tree number), or else timings recorded (then use **Save**) so reading can be annotated when downloaded.
3. When **readings** in a particular position are complete switch off the device using the **Power** button.
4. Note: If the air temperature is very low or humidity levels are high it may be necessary to **equilibrate** between readings – the instrument prompts this. Equilibrate without clamping to leaf and with clip closed.
5. If the instrument is not to be used for a while, remove the batteries and store in the case.

B: Measurements by Tree Climbers/ Contractors in canopy under direction of researchers/ ops. Staff.

1. Prior to taking measurements in the canopy, calibrate and check the instrument in line with **process A.1** above.
2. Show the contractor how to turn on the instrument, start measurement, what the screen looks like during measurement and how to save the reading by undertaking a reading at ground level of, for example, bramble leaf. **See Contractor Canopy Readings sheet below.**
3. Follow advice of climbing experts as to where to stand and how to communicate during the climbing operations.
4. During the canopy leaf sampling, at least one reading will be attempted at each oak (or other sampling point) e.g. the top/ middle/base of canopy for oaks and at an appropriate height for sub-dominant species to be sampled. If first reading is successful, two repeats will be done on the same leaf. If equilibration is needed between samples these repeated readings will only be completed if equilibration takes less than a minute.
5. The leaf/ twig sampled will be dropped to the ground for the researcher/ technician to put in water and re-measured (using process **A.2 to A.3**) once the climber has completed their work and brought the porometer to the ground. Leaflets/ twigs will be placed immediately in pots of rain water or de-ionised water will be labelled to show species, height, tree number and array number. Measurement will be done at edge of/ near to Array (but in full light conditions) unless it is raining (see Process C).
6. On completion of the ground level measurements leaflets/twigs will be placed in the labelled sample bags and in the shipper for transportation.

C: Process during or after rain for measuring leaf samples collected from canopy

1. Use **process B.5** to preserve water in samples and transport to Welfare or other sheltered outdoor position.
2. Dry leaf to be measured with absorbent material (e.g. cotton cloth or paper towel).
3. Carry out measurements as per **process A** in outside but sheltered conditions (e.g. balcony at Welfare). If rain stops, then a sunny/ light position near welfare can be used for all measurements.



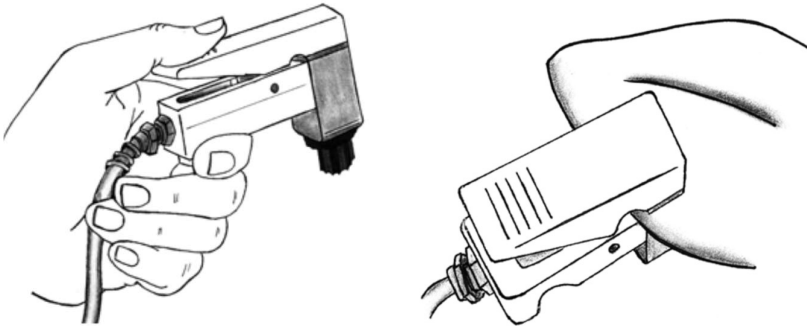
Press Power button to turn ON

Check Measurement screen is shown and press ENTER

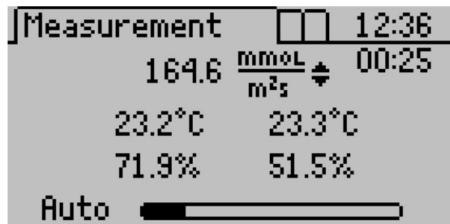


Follow prompts to attach clip to leaf holding clip as follows, black sensor towards ground, leaf horizontal:

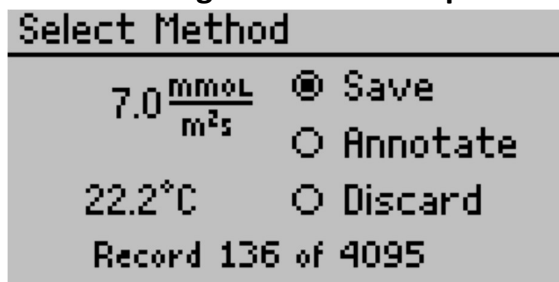
Correct



Automatic reading will take about 30secs with following screen:

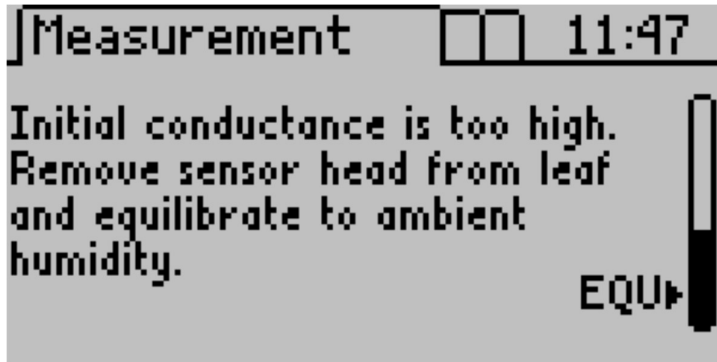


When reading is finished unclip and SAVE the reading by pressing ENTER:



To take another reading clip on leaf again and press ENTER. If necessary wait to EQUILIBRATE.

To EQUILIBRATE ensure clip is removed from leaf and closed. Occasionally shake the measurement head vertically to speed up the process.



Then continue with measurements by pressing ENTER.

If you press the MENU switch by mistake the measurement in progress will be cancelled. Keep pressing the MENU switch until you see the Measurement screen again. Restart your measurement by unclipping and then reclipping to the leaf.



To save Batteries press Power switch to turn OFF at end of

readings.



Appendices

Document 2 – following 3 pages

Appendices

Method Statement for in-situ porometry related measurements using the canopy access system (CAS).

Note: measurements cannot be taken during rain or when leaves are wet.

Canopy Access system (CAS) measurement of Oak/ sub-dominants near central tower:

Note CAS point of contact, Peter Miles (PM), will authorise scheduling of any work and advise on how to carry equipment to height.

Any equipment brought up to height will require a lanyard to prevent any damage or harm caused by a falling item. These will be provided by operations staff and secured and checked during pre-hoisting checks.

A tool bag can also be attached to the harness or bosuns chair to enclose any loose lanyards and equipment to prevent tangling in the branches or hoisting/rescue equipment.

Re-rigging of the system for 2020 will be checked by SEQ and will target only those trees reachable from the central CAS system which have sapflow measurement devices fitted.

Measurements to be taken on each occasion

1. Leaf temperature of bottom of leaf (non-contact thermometer)
2. Stomatal conductance (using porometer)

Leaves will be labelled with masking tape or coloured cord on first access per treatment season to enable future repeat measurements.

Three sets of measurements will be taken on each leaf sampled (total measurement time range/ array 25- 40 minutes). The person measuring will target a minimum of three leaves per tree measured, as near the top of the canopy as possible and within reach of the person safely. The person on the ground will record the leaf temperature measurements for the person aloft, using radio. Movement up and down the CAS system will occur after each of the Array measurements, working in matched pairs of Arrays (4/2, 1/3, 6/5). if shadowing of Licor measurements possible in future, then timing will be synchronised to ensure movement between arrays is possible (e.g. Licor in Array 4, Porometry in Array2)

Appendices

Other measurements at height relating to use of CAS.

Height of target leaves of oaks and sub-dominants (e.g. Sycamore and hawthorn) will be estimated either by reference to a height marked on the central tower (refer to PM), or else using the laser distance measurement device.

Overall tower heights are known from design plans/drawings. The height can be calculated by measuring distance from the sampling position and the bottom of the cantilever arm using a laser measurement device and then subtracted from the overall tower height.

Porometry

Useful Links and documents:

SC-1 Manual + instructions card (hardcopy in instrument case, soft copy on memory stick at back of manual and available at these links

Manual: file (Leaf Porometer – Manual.pdf) & hardcopy available

[http://library.metergroup.com/Manuals/20773%20 SC-1 Manual Web.pdf](http://library.metergroup.com/Manuals/20773%20SC-1_Manual_Web.pdf)

Calibration: file (Leaf Porometer – Manual.pdf) & hardcopy available + video

<https://www.metergroup.com/environment/articles/quick-guide-calibrating-sc-1-porometer>

<https://www.youtube.com/watch?v=EScH84UTEGc>

Cleaning and repair: : file (Leaf Porometer – Manual.pdf) & hardcopy available + video

<https://www.metergroup.com/environment/articles/sc1-leaf-porometer-repair/>

Method Statement for porometry measurements using CAS.

1) Annually at start of treatment season (see useful links above):

Clean and maintain porometer in line with the Manual and online guidance, to prepare instrument for measurement season.

Appendices

Use water, or dry lint-free cloth/ cotton bud as indicated in the guidance and re-assemble, replace seals if need be. If isopropyl is to be used, ensure the part is rinsed with water after cleaning.

Take special care not to contaminate sensor.

2) Porometry readings of top/mid-canopy *in-situ*:

Introduction

Tree number	Array	Species	Sapflow tree – in reach of central tower CAS rigging	July 2020 Contingency Plan for COVID lockdown –limited CAS
8468	1	Oak	Y	Y top canopy
9301	3	Oak	Y	Y top canopy
6621	4	Oak	Y	Y top canopy
8673	2	Oak	Y	Y top canopy
6406	5	Oak	Y	May be omitted July
5846	6	Oak	?	May be omitted July
5848	6	Sycamore	?	Any reachable. Or use Mid-canopy ladder *
9982	4	Hawthorn	?	Any reachable. Or use Mid-canopy ladder *

N.B. *See RAMS 180.

Priority is for trial to target just those oaks/ sub-dominant trees in reach of the central tower with sap flow probes installed as shown on the following list. Equipment required is shown in Appendix 1

Plan the day of month by looking at the weather forecast for coming week and choosing the sunniest possible day(s) along with CAS support availability. Do not start campaign if it is raining or the porometer will not calibrate due to high humidity.

Calibration takes between 20 minutes and 40 minutes depending on the relative Humidity on the day. This is carried out each day before CAS measurements

Appendices

A: Calibration and Measurement by research staff/ students or operations personnel.

1. (at least daily) Prior to taking measurement ensure porometer (e.g. Decagon device) is in good clean condition with fresh desiccant and calibrated/ equilibrated (use **Calibration** procedure - see card, Manual and on-line video www.decagon.com/porocal).

N.B. Check battery condition before calibration; after battery change it is necessary to reset the date and time on the device. Store used desiccant in a suitable bag or bottle for recycling/ disposal.

2. Carry porometer in case whenever possible until target tree is reached. Access top or mid canopy in line with standard CAS procedure.
3. In each Array record time readings were commenced in Field notebook or on the standard form (to be prepared).
4. Before using porometer, measure the temperature of centre of the underside of the leaf using an infrared thermometer (see equipment list) held quite close to the leaf but not touching it. First measure the leaf temperature, radio the measurement to the person on the ground along with reference e.g. leaf 1, reading 1. The person on the ground will Record this on a standard form (to be prepared) or notebook.
5. Using Porometer use standard measurement process (Use **Quick Start Guide** procedure) as shown on Decagon card and below. All measurements should normally be of the bottom of the leaf and use the **Auto** process (30secs). Unclip from leaf on completion. Take three successive readings of stomatal conductance of the chosen leaf, choosing a slightly different position each time and save/ annotate the readings:

Where possible each measurement should be labelled (use **Annotate**) with simple letters and numbers

Ax= Array(number)

Tx=Tree (number)

Lx = Leaf(number)

Vx= Value(number)

so reading can be identified when downloaded.

6. Note: If the air temperature is very low or humidity levels are high it may be necessary to **equilibrate** between readings – the instrument prompts this. Equilibrate without clamping to leaf and with clip closed.
7. Ensure porometer is switched off using the **Power** button. and contained in clean carrying bag after the readings are complete before coming down from CAS. Place in case before proceeding to another array.
8. If readings are interrupted due to rain/ high humidity, reschedule and if necessary repeat measurements for the pair of arrays which were interrupted on another day (e.g. 1/3)

Appendices

2-E: Report justifying drilling of mature trees

N.B. 11 pages follow – excludes Appendices

UK BIFoR FACE Start-up and way forward for measurement of water/ sap flow in trees and plants.

Discussion paper for Science team. Copy of draft was available from author (unpublished note)
Prepared by Sue Quick Dec 2016- 3rd Apr 2017. Finalised for thesis June 2025.

Background

There is a need to measure sap flow in trees at Mill Haft as part of the research schedule. This measurement will form a part of proposed research into Tree-Soil-Water relations in eCO₂, helping to populate the water cycle budget and derive Water Use Efficiency (WUE) figures for each tree measured and consequently extrapolate per each array. Also needed for other projects.

Some heat pulse (heater plus thermocouple sap probes type TranzFlo) transducers have already been purchased (see Table 1) and are planned to be used Spring 2017 for measurements of sap flow in the stems of the dominant trees *Quercus robur* as well as > 10cm circumference sub dominant species if they are suitable.

HP4TC	HP probes with sensors @ 5, 15, 30 & 55 mm (stainless) – 70mm length	four copper-constantan junctions (42 swg)	thermo-couple
HTR8	Heater probe (1.9 mm dia., 70 mm length)	19 swg stainless steel tube, containing a central nichrome resistance wire (5 Ω m ⁻¹) which is insulated inside a fine Teflon tube.	

Table 1: Heat pulse probes and heater types.

This paper looks at a justification for use of this method, as it is marginally intrusive. It selects the approach which will, whilst minimising risk to the trees, help to characterise the oaks and different species of trees selected and give reliable comparative data for Control and Treatment Arrays. It may also be deployed in Ghost arrays if so agreed. A likely duration of this experiment is considered.

The paper also looks at whether other methods may be adapted for use at Mill Haft as the project progresses. This may be in addition to or instead of the heat pulse method, but the emphasis would be on reducing intrusiveness and hence exposure to tree health risk.

Water Use efficiency context

There are three scales at which Water Use Efficiency is currently studied: the first is the global/ large scale and is estimated from satellite observational data such as gross primary production (GPP) and averaged surface soil moisture (He, 2016); the second is localised to stand scale land use e.g. agricultural, forestry and based on locally measured GPP and Evapotranspiration deduced from precipitation and other water measurements (ET) (Amatya (2016 pp.6)); thirdly tree/vegetation experimental studies consider carbon uptake change per unit of water loss (Hopmans and van Genuchten, 2005) usually at tree or leaf scale. The latter method has traditionally been undertaken in controlled plantation experiments which can control watering and evaporation. Our study lies nearest to the second method and is also at or below the spatial scale at which flux towers traditionally operate (e.g. see eddy covariance (EC) methodology Verstraeten et al. (2008, p86) but will need to consider the errors introduced by smaller areas studied in arrays.

Appendices

Definitions	References
General: Water use efficiency (WUE, also called transpiration efficiency - TE) indicates the ability of plants to limit transpiration loss by control of stomata opening & closing. It is defined at different scales: a) leaf, b) whole plant, c) stand/ landscape area.	
a) Leaf i) the ratio between net CO ₂ assimilation (A) and stomatal conductance to H ₂ O (g _s), known as intrinsic water use efficiency (iWUE), ii) the ratio between A and transpiration (E), (i.e. ratio of water loss to carbon gain (rate of carbon uptake per unit of water lost A/E)) known as instantaneous WUE	a) i) (Guerrieri <i>et al.</i> , 2016); ii) Farquhar <i>et al.</i> , 1989 (see field limitations pp.522-523); (Donovan and Ehleringer, 1994)
b) from flux towers: GPP (gross primary productivity/ET(evapotranspiration) defined as ecosystem water use efficiency, WUE _e	b) (Amatya, 2016); (Law <i>et al.</i> , 2002) (need for longer term studies); (Beer <i>et al.</i> , 2009); (Keenan <i>et al.</i> , 2013)
c) soil water use efficiency (SWUE), based on gross primary production and surface soil moisture derived from remote sensing products (ECV-SM) '	c) (He <i>et al.</i> , 2017)

Fig.1. Key definitions re Water Use Efficiency

Data required to determine water budget

A separate table (Appendix C) has been drafted showing the measurement points which may be required to determine WUE in the study proposed by SQ (supervisor documents). A key factor is sapflow (that is, water flow from root intake up through the xylem to the leaves).

Justification of Heat Pulse method for initial measurements of sapflow.

The Science team (especially Stefan Krause and Phil Blaen) have already considered advice from the research community as to the best method of measurement. It is recognised that , for all proven sap flow methods, installation is problematic and may only result in 50% success. Strengths and Weaknesses, Opportunities and Threats of the differing approaches have been considered and are listed in Table 2 below.

Suggested Approach to enable optimisation

A literature search is in progress and the results from this study with highlight the various practical methods of measurement, current applications and advantages/ disadvantages of application in respect of plant health and timber value effects. The heat based measurement methods are most commonly used giving repeatable results as reported in the literature. There are a number of advantages and disadvantages of the methods according to the aim of the measurement. For our purpose, where exact values of sapflow are less important than comparative measurements over time, both within and between species, in respect of their treatment with eCO₂, we have considered the literature on suitability to measure vertical xylem flow in main stem as the determining factor. Another analysis may be required were the measures to be extended to root, branch xylem flow, or to radial xylem flow and phloem flow (e.g. in respect of Carbon storage mechanisms during the dormant period).

Tatarinov *et al* (2005) have undertaken a comparative study of the heat pulse compensation method (HPC), heat dissipation technique (HDT) and tissue heat balance (THB) and method to assess looking at uncertainties due to both wood heterogeneity (which varies with tree species type), temperature gradient in measurement zone (which varies with wood densities for example)

Appendices

and radial sap flow (which again is variable with species type). References for these three methods are given in Table 3. The conclusions of this study are extracted and presented in Appendix D. Steppe(2015) has also undertaken a review of current methods, including non heat based techniques (see also Table 4).

	Strength	Weakness	Opportunity	Threat	Notes
Heat Pulse method	Well tested and used in variety of tree-soil-water experiments	3 Probes cause small wounds in tree giving disease vulnerability	Can share experience and data with other users if relevant. Can sample sap for testing disease.	Calculations to determine tree water usage are complex and depend on xylem wood structure	To be reviewed for alternative methods following initial trials + 1 st year
Length of Probe/ Nos. of thermocouples	4 thermocouples/ probe gives better likelihood of measuring peak sap flow position	8cm x0.2cm wound may enable introduction of pathogens	A horizontal flow profile may be measurable. Fine tuning of position available (start with shallower depth and increase)	Long drill holes may not be horizontally aligned, drills may snap in tree. Probes may get bent	Review length/ depth requirements after initial year's trial.
Duration of Experiment	Probes are durable s.steel & can be reused if carefully extracted	Manufacturer recommends reliability of readings for 3months minimum. We need 12-24months of data preferably so redrilling might be req'd	Initial data may supply sufficient evidence for deducing flow/ flow density from other instrumentation (e.g. radial dendrometry)	Species comparison is best done with similar probes in all trees – the healing may differ in different species causing truncated comparison experiment	Acquire additional shorter probe sets (2x thermocouples) for use in 10-20cm stems
Species application	Existing Probes suitable for stem dias. >20cm	Not many larger dia. trees of non-oak species.	Multi-thermocouple probes enable lateral flow & tuning of position	Using different probe types or depths of probe means comparison experiment may not be repeatable.	Prepare to repeat experiment with different probe length/ depths if necessary. (especially for smaller trees)
Other data requirements: a) tree diameter/ radius, b) bark depth c) heartwood radius, d) stomatal conductance	a) & b) easily measurable	c) traditionally measured from incremental core – may need to be estimated from other tree data, but often tree-specific shape	Use of averaged values scaled to be tree specific gives less intrusiveness. d) available from other projects?	Heartwood estimate error could influence usefulness of sapflow measurement	Research best current methods of heartwood size measurement and recommend best options
Other possible data requirements: a) ambient temperature, b) relative humidity, c) water availability (soil, precipitation etc)	a) b) c) can all be measured near or at tree	Current instrumentation does not cover these within each array	Data is available at met stations to give good estimate for a) and b). c) Extend soil moisture measurement to all arrays	Stem respiration not currently considered when measuring sapflow	CO2 is main influence on experiment design but other factors (e.g. water storage) need consideration.

Table 2: Strengths, weaknesses, opportunities and threats of Tranzflo methods

Measurement method	Description	Refs	Notes
--------------------	-------------	------	-------

Appendices

Heat pulse - compensation (HPC)	Uses two thermocouple probes & one heat pulse probe (or?. an active downstream sensor @ - 10mm and a passive upstream at 40mm from the heater)	Swanson (1962) cited Green (1998)x, (Green and Clothier, 1988), Hernandez-Santana, V. et al (2016) Wang (2016) 'Sap flow was monitored at 30-min intervals in the tree trunks at 1.3 m above ground using the compensation heat-pulse technique'	xTranzflo Manual. Other manufacturers include:Czechglobe(Czech research institute) Tranzflo system uses down stream at 40mm & upstream at 10mm. Others (e.g. Wang) use common probe spacing 10 mm above the heater and the other 5 mm below Most Sensitive to position depth – see Tatarinov (2005) & Appendix D- especially for low flow rates.
Heater – Tmax (also called heat dissipation technique HDT)	Method of sap movement measurement based on a heater and downstream sensor 'simple sap flow calculations, high accuracy and temporal resolution' (Yan,2016)	Granier method, based on convective heat transport (Granier 1985). See also Herbst et al(2007)+. Green et al(2009)x, Hannukainen (online 2010) and comparison study by Tatarinov (2005) Yan M-J et al (2016) & see Wang et al (2016) & Zhang <i>et al.</i> (2012)	x Tranzflo website + 'thermal dissipation probe (TDP) sets (Dynamax Inc., Houston TX, USA). Each TDP set comprised of two metal probes with a diameter of 1.2 mm and a length of 30 mm. In field maple 80 mm long probes were used.'
Tissue heat balance (THB) method	3D heating of stem segment.	(Cermak <i>et al</i> 1973, 1982, 1991, Kučera <i>et al</i> 1977) as cited Tatarinov et al (2005)	
Heat Ratio Method (HRM)	Uses multiple thermocouples or calibrated high precision thermistors per probe (2 to 4)	Burgess et al (2001); Eller et al (no date); Vandegehuchte et al. (2015), all cited Steppe (2015)	Developed from HP compensation method ICT (who have also developed PSY1 Stem Psychrometer to give hydraulic conductivity.)
Heat Field Deformation (HFD) principle	Uses multiple needle sensors to measure change of temp (ΔT) below, above, and to the side of a line heater. Suited to measurement of extended radial sap flow and hydraulic stress	Developed by Nadezhdina, Čermák and Nadezhdin (1998).as cited by Kučerová et al (2010) and Nadezhdina et al (2007) cited ICT	ICT – up to 8 heat transducers per probe
Non intrusive heat pulse system	Heat (pulse) is applied to surface of sapling with a near-infrared laser. Sap heat propagation measured externally by infrared camera.	Helfter et al (2007). Also Bauerle et al. (2002) as cited Helfter.	Xylem & Phloem flow with one instrument. Experimental equipment – not commercially available. Laboratory/ nursery conditions. Calibraton by weighing lysimetry (WL) data.
species-specific calibrations for heat pulse methods		Steppe et al. 2010 cited Steppe (2015)	
Wound effect adjustments	Necessary for needle probes, especially when using HPC method or HDT	for heat pulse method: Burgess et al. (2001); Green et al. (2009); Vandegehuchte and Steppe (2013), all cited Steppe (2015) for HDI : Green(2009), cited Steppe(2015) and Wiedermann (2016)	See also Tranzflo manual.
further improvement in the upscaling of sap flow measurements	Translation of point measurements into stand or ecosystem wide measures	'upscaled estimates often affected by the sensor-to-stand scaling procedure' (Oishi et al. 2008 as cited in Steppe(2015)).	Further research in progress.

Table 3: Various methods based on heat dissipation through xylem and phloem along with

Appendices

The literature review has revealed two more heat based techniques. One from manufacturer ICT (see hyperlinks and Appendix A) is based on the Heat Field Deformation (HFD) principle (ref) and is a modern development of the HPC method using thermistor sensors. Its disadvantage is the need to ensure power is available locally to energise the thermistors continuously, but this is not a large overhead compared with heater energy requirements. A further study of energy usage and reliability of this method may be carried out if the current HPC method is found to be unsuitable.

Another, laboratory nursery based non intrusive heat pulse system trial (Helfter et al (2007)) uses infrared laser application and might seem to offer benefits, but is not commercially developed or trialled for large woody stem use in the field.

Finally, alternative non-intrusive methods not using heat have been considered (Table 4), but no suitable proven technique is yet available for use in the field.

Appendices

Ccalibration notes.

Wound effects and other problems concerning calibrations

In respect of calibration, Sun (2016) (in Amatya (2016) Ch 3 P 38 Sun et al) considers sapflow as a powerful tool for quantifying water use at whole stand levels so long as sample size and variability of individual tree samples is adequate.

In choosing a sapflow measurement method, consideration of calibration and wound effects can be critical if consistent comparative measurements are required. In respect of the wound, then minimising exposure of the wound to air can be a critical factor (verbal advice from Forestry Research). Also by restricting the contact area of the probe to a minimum, limiting the tangential width of the wound and by minimising depth of wound to only the essential xylem depth of interest, then effects can be limited to a practical minimum.

Consideration of the literature on this subject has focused on: wound effect during measurement (e.g. see Green, Clothier & Perie (2009)) and vulnerability of the tree following wounding (e.g. by increasing risk of disease incursion or animal attack), whatever the initial reason for the intrusion.

Volkman et al (2016) describes the effect on the tree physiology of the wound made for a measurement probe and also cites Moore *et al.* 2010 specifically in respect of sapflow sensors and considers that the localised xylem function post wounding may be increasingly affected as a result. Kramer & Boyer 1995 (cited Volkman et al 2016) has found that parts of the xylem affected may fill with air or exudates, or form calouses (Taiz & Zeiger 1991 cited Volkman et al (2016)).

van Mantgem & Stephenson (2004) considered paired trees of the *Abies* species one of which was subjected to incremental coring (which causes a larger wound than any of the sapflow methods) and found no difference in mortality over the experiment duration but also comment that other species may differ in response. A number of manual exist for incremental boring (e.g. Grissino-Mayer, H.D. (2003)) which highlight the need to allow the wound to heal naturally and not to apply any wound treatments.

In this respect North American industry studies in the 1980s, (e.g. Shigo and Shortie, W.C. (1983)) concerned trials of different treatment chemicals post wounding of white oaks and maples); these all concluded that no wound treatment is better than any intervention which tries to help the tree heal, even when the substances used were natural tree based chemicals such as aspirin & cinnamic acid.

in more recent reports, there is also emphasis on allowing the tree to heal naturally. The dendrochronologist [Dieter Eckstein](#) certainly did not report any adverse effects on trees, though his analysis may be subjective as he was a champion of the method. (Eckstein & Schweingruber (2009)).

Other authors (e.g. Wiedermann (2016) point out that the effects of wounding on xylem flow will vary with species. Ring porous species such as oak may react more to wounding in respect of the localised xylem flow, whereas Beech which is diffuse porous will not react so strongly. Whilst this means that measurement compensation will be more critical in oak species, the good news is that the ring porous species are protecting themselves more quickly to infection. Wiedermann(2016) also recommends a staged approach to installation as the trees are mostly affected during the first two weeks of the growing season, such that sequential installation of probes (e.g. 2 probes every 2-3 months) will help to pick up the effects and enable effective wound compensation to be applied to measurements.

Appendices

Measurement method	Description	Refs	Notes
Transpiration loss measurement	Method of sap movement measurement based on weight loss measurement as a result of transpiration	See Green, Clothier & Jardine (2003)	Only possible in small/ young trees
Wood moisture content	Measured in cubic metres/ cubic metre of wood (m^3m^{-3}) – this affects the speed of sap flow inversely	See Green, Clothier & Jardine (2003)	An inherent part of the HPC method.
nuclear magnetic resonance (NMR) & magnetic resonance imaging (MRI)	Nuclear magnetic resonance has been used to measure phloem and xylem sap flow velocities non-invasively. NMR requires high-tech equipment, this method is mainly confined to laboratory	(Xia et al. 1993, Peuke et al. 2001 as cited Helfter et al (2007) (to be researched). Lab studies (Windt et al. 2006, De Schepper et al. 2012). small-scale portable NMR, field conditions Windt and Blümler (2015) as cited Steppe,	this method 'can provide information about the spatial distribution of xylem and phloem flows. ' It is not practical for large trees.
Indicator-dilution techniques	This uses relationship between the mean concentration of a solute in a flowing solution and flow rate. Radioactive substance such as ^{32}P is used, monitored by a Geiger-Muller detector and	Fisher (1990), Cited Helfter (2007)	Phloem transport, lab only.

Table 4: Other methods used for measurement of flow

Additional Actions in respect of Sapflow measurement to mitigate Threats

Further work will be considered, including suggested actions listed, to ensure the threats of HPC method application are minimised.

1. Measurement of Heart wood/sapwood boundary to determine sapwood area – work has started on this and a less intrusive method has been identified using a combination of sonic and electrical tomography. In this method it is necessary to insert a series of 2mm nails into the bark and puncture the xylem boundary rather than drilling/ coring into the heartwood (see Bieker & Rust. (2010) as cited in Wang(2016)). This method can also be validated by applying the method to trees for which we have taken incremental cores of oakwood already and comparing results (University of Swansea partnership).

2. Another estimation method, similar to that used by Yan (2015) will be tried, based on trees already cored during the two year characterisation period (Loader, N (2017) unpublished data.). Yan estimated, relationships between the sapwood area (AS) and DBH using power curve equations for the estimation of all the trees each year and this method will be looked at for Mill Haft (Vertessy, 1995) as cited by Yan (2016).

Appendices

3. Measure canopy size along with LAI and also total evapo-transpiration from eddy flux tower to estimate WUE without calibrated sapflow measurement.

4. Whilst there is currently only limited availability to access the top canopy, there is a possibility of taking diurnal measurement of stomatal conductance to water in-situ, or from cut-twigs. Then, using environmental parameter data to calculate leaf transpiration and canopy spread or structure, the transpiration can be upscaled.

See also Appendix C for other instrumentation measures within this research stream (to be updated SQ during project progress).

Likely duration of project

The lead researcher aims to collect all data from the sapflow experiment within five years of commencement of the project (i.e. Sept 2016-2021). It is likely that each installation will need review each year to assess wounding effects and continued usefulness of probe, but reinstallation in the same tree is a possibility. Proposed timescales are therefore:

2016-2017 Yr 1 trialling of method in dominant oaks and installation of a minimum of 2 probes per oak tree, 19 trees in total (2x per array + trial tree). Phased approach to further installations.

2017-2018 Yr2 review existing results and installations. Leave or repeat installation. Additional installations in sub-species with similar or smaller probes.

2018-2019 Yr 3 review yr 2 results & installations. Leave or repeat installation on all species.

2019-2021 Yrs 4-5 assess and review all findings and decide future requirements.

Recommendations

This review and justification has summarised issues relating to sap flow measurement method and associated measurements to calculated sapflow density in a mature oak. It is recommended that:

1. Using strict biosecurity measures, the current available heat pulse method be trialled during 2017 in up to 6 oak trees (fitted during the first half of the year, but avoiding April the xylem growing season, to remain in place at least until they cease giving useful data). Subsequently a total of 19 oak trees could be fitted at any one time (limited by numbers of probesets).
2. That evidence of healing for those tree already subjected to incremental coring be used to justify the marginally intrusive sapflow approach.
3. That no measures to treat tree wounds will be used; probes will be left in situ unless needed elsewhere, or sterile metal dummy probes inserted once measurements are complete until full healing has taken place.

Conclusions

This report has been prepared in response to concern about tree wounds created by measurement techniques. It is concluded that the sapflow measurements have a very low risk of causing tree vulnerability over and above those already in place in the woodland due to historical stresses, especially if biosecurity measures are strictly applied..

References:

Amatya, D. (Ed.). (2016). *Forest hydrology : processes, management and assessment* / edited by Devendra M. Amatya [and three others]. GEN, Boston, MA : CAB International, [2016].

Appendices

Bauerle, W.L., T.H. Whitlow, C.R. Pollock and E.A. Frongillo. (2002) *A laser-diode-based system for measuring sap flow by the heat pulse method*. Agric. For. Meteorol. 110:275–284.

Bieker D, Rust, S. (2010). *Electric resistivity tomography shows radial variation of electrolytes in Quercus robur*. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 40:1189–1193. DOI: 10.1139/x10-076

Burgess, S. S. O., Adams, M. A., Turner, N. C., Beverly, C. R., Ong, C. K., Khan, A. A. H., & Bleby, T. M. (2001). *An improved heat pulse method to measure low and reverse rates of sap flow in woody plants*. Tree Physiology, 21(9), 589–598. JOUR. Retrieved from <http://dx.doi.org/10.1093/treephys/21.9.589>

Eller, C.B., Pereira, C. G. Oliveira, R S., Downey, A., Burgess, S . (no date) *Differences in Quality Between Old and New HRM Instruments*. Available at Differences_in_Quality_Between_Old_and_New_HRM_Instruments.pdf available at http://au.ictinternational.com/case-study-pdf/?product_id=1924 [accessed and downloaded SEQ 14-02-17]

Granier, Andre. (1985) *Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des arbres Annales des Sciences forestières*. Vol. 42. No. 2. EDP Sciences.

Green, S.R.& Clothier, B.E. (1988) *Water Use of Kiwifruit Vines and Apple Trees by the Heat-Pulse Technique*. J Exp Bot 1988; 39 (1): 115-123. doi: 10.1093/jxb/39.1.115 (& as cited Steppe 2015)

Green, Steve, Clothier, Brent and Jardine, Bryan. "Theory and practical application of heat pulse to measure sap flow." Agronomy Journal 95.6 (2003): 1371-1379.

Green S, Clothier B, Perie E (2009) *A re-analysis of heat pulse theory across a wide range of sap flows*. Acta Hort 846:95–103.

Grissino-Mayer, H.D. 2003. *A manual and tutorial for the proper use of an increment borer*. Tree-Ring Research, 59(2), 63-79.

Hannukainen, I., Hölttä, T., Sevanto, S., Nikinmaa, E., & Vesala, T. (2010) *Understanding tree water transport measurement techniques and their detailed information related to night time variations in stem diameter*. University of Helsinki, and Los Alamos National Laboratory, Los Alamos, New Mexico, USA

Available online from <http://www.atm.helsinki.fi/faar/reportseries/rs-109/abstracts/IrinaHannukainen.pdf>
[accessed SEQ 28-09-2016]

He, Bin et al (2016), *A new indicator of ecosystem water use efficiency based on surface soil moisture retrieved from remote sensing*, Ecological Indicators, Volume 75, April 2017, Pages 10-16, ISSN 1470-160X, <http://dx.doi.org/10.1016/j.ecolind.2016.12.017>.

Helfter, C., Windt, C., Van As, H., Mencuccini, M., & Hand, D. (2007). *Comparison of xylem flow velocities determined by MRI and a non-invasive heat pulse technique in Golden Alder and Silver Birch. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 146(4), S65–S66. <https://doi.org/10.1016/j.cbpa.2007.01.064>

Herbst, M., Roberts, J. M., Rosier, P. T. W., Taylor, M. E., & Gowing, D. J. (2007). *Edge effects and forest water use: A field study in a mixed deciduous woodland*. Forest Ecology and Management, 250(3), 176–186. <https://doi.org/10.1016/j.foreco.2007.05.013>

Appendices

- Hernandez-Santana, V., Fernández, J. E., Rodríguez-Dominguez, C. M., Romero, R., & Diaz-Espejo, A. (2016). *The dynamics of radial sap flux density reflects changes in stomatal conductance in response to soil and air water deficit*. *Agricultural and Forest Meteorology*, 218–219, 92–101. JOUR. <https://doi.org/http://dx.doi.org/10.1016/j.agrformet.2015.11.013>
- Hopmans, J. W., and M. T. van Genuchten (2005), *Vadose zone: Hydrological Process*, in *Encyclopedia of Soils in the Environment*, edited by D. Hillel, pp. 209–216, Elsevier, Amsterdam. As cited in Sprenger (2016) *N.B. Not yet fully researched*.
- Kučerová, A., Čermák, J., Nadezhdina, N., & Pokorný, J. (2010). *Transpiration of Pinus rotundata on a wooded peat bog in central Europe*. *Trees*, 24(5), 919–930. article. <https://doi.org/10.1007/s00468-010-0463-4>
- Loader, N (2015-7) – unpublished work for BIFoR re dendrometry and dendrochronology.
- Nadezhdina N, Čermák J, Nadezhdin V (1998) *Heat field deformation method for sap flow measurements*. In: *Proceedings of 4th international workshop on measuring sap flow in intact plants*. Židlochovice, Czech Republic, 3–5 Oct 1998. IUFRO Publications Publishing House of Mendel University, Brno, pp 72–92
(not accessed SQ, cited Kučerová et al (2010))
- Nadezhdina, N., Cermak, J., Meiresonne, L., & Ceulemans, R. (2007). *Transpiration of Scots pine in Flanders growing on soil with irregular substratum*. *Forest Ecology and Management*, 243(1), 1–9. JOUR. <https://doi.org/http://dx.doi.org/10.1016/j.foreco.2007.01.089>
- Eckstein, D., & Schweingruber, F. (2009). *Dendrochronologia—A mirror for 25 years of tree-ring research and a sensor for promising topics*. *Dendrochronologia*, 27(1), 7–13. JOUR. <https://doi.org/http://dx.doi.org/10.1016/j.dendro.2009.01.001>
- Shigo, A.L., and Shortle, W.C. 1983. *Wound dressings: Results of studies over 13 years*. *J. Arboric.* 9:317–329.
- Sprenger, M., H. Leistert, K. Gimbel, and M. Weiler (2016), *Illuminating hydrological processes at the soil-vegetation-atmosphere interface with water stable isotopes*, *Rev. Geophys.*, 54, 674–704, doi:10.1002/2015RG000515.
- Steppe, K., De Pauw, D. J. W., Doody, T. M., & Teskey, R. O. (2010). *A comparison of sap flux density using thermal dissipation, heat pulse velocity and heat field deformation methods*. *Agricultural and Forest Meteorology*, 150(7–8), 1046–1056. JOUR. <https://doi.org/http://dx.doi.org/10.1016/j.agrformet.2010.04.004>
- Steppe, K., Vandegehuchte, M. W., Tognetti, R., & Mencuccini, M. (2015). *Sap flow as a key trait in the understanding of plant hydraulic functioning*. *Tree Physiology*, 35(4), 341–345. JOUR. Retrieved from <http://dx.doi.org/10.1093/treephys/tpv033>
- Tatarinov, F. a, Kučera, J., & Cienciala, E. (2005). *The analysis of physical background of tree sap flow measurement based on thermal methods*. *Measurement Science and Technology*, 16(5), 1157–1169. <https://doi.org/10.1088/0957-0233/16/5/016>
- van Mantgem, P.J., & Stephenson, N. L. (2004). *Does coring contribute to tree mortality?* *Canadian Journal of Forest Research*, 34(11), 2394–2398. Retrieved from <https://search-proquest-com.ezproxyd.bham.ac.uk/docview/230520555?accountid=8630>
- Vandegehuchte MW, Steppe K (2013) *Sap-flux density measurement methods: working principles and applicability*. *Funct Plant Biol* 40:213–223.

Appendices

- Vandegehuchte MW, Burgess SSO, Downey A, Steppe K (2015) *Influence of stem temperature changes on heat pulse sap flux density measurements*. Tree Physiol 35:346–353.
- Verstraeten, W. W., Veroustraete, F., & Feyen, J. (2008). *Assessment of evapotranspiration and soil moisture content across different scales of observation*. Sensors, 8(1), 70-117.
doi:<http://dx.doi.org/10.3390/s8010070>
- Vertessy, R.; Benyon, R.; O'sullivan, S.; Gribben, P. *Relationships between stem diameter, sapwood area, leaf area and transpiration in a young mountain ash forest*. Tree Physiol. **1995**, 15, 559–567. as cited by Yan (2016)
- Volkman, T. H. M., Kühnhammer, K., Herbstritt, B., Gessler, A., & Weiler, M. (2016). *A method for in situ monitoring of the isotope composition of tree xylem water using laser spectroscopy*. Plant, Cell & Environment, (June), n/a-n/a. <https://doi.org/10.1111/pce.12725>
- Yan, Mei-Jie, Zhang, Jian-Guo, He, Qiu-Yue, Shi, Wei-Yu, Otsuki Kyoichi, Yamanaka, Norikazu and Du, Sheng,(2016) (authors from China/Japan) *Sapflow-Based Stand Transpiration in a Semiarid Natural Oak Forest on China's Loess Plateau* Published Forests 07-00227(licensee MDPI, Basel, Switzerland.): 9 October 2016 Open access online
- Wang, H., Guan, H., Guyot, A., Simmons, C. T., & Lockington, D. A. (2016). *Quantifying sapwood width for three Australian native species using electrical resistivity tomography*. Ecohydrology, 9(1), 83–92. <https://doi.org/10.1002/eco.1612>
- Zhang, L, Niu H, Wang S, Zhu X, Luo C, Li Y, Zhao X (2012) *Gene or environment? Species-specific control of stomatal density and length*. Ecology and Evolution, **2**, 1065-1070.
- Hyperlinks
Czechglobe: <http://www.czechglobe.cz/>
- ICT: <http://www.ictinternational.com/sfm1.html>
- Minolta: <http://www.konicaminolta.eu/en/measuring-instruments/products/colour-measurement/chlorophyll-meter/spad-502plus/introduction.html>)
- Tranzflo: <http://www.tranzflo.co.nz/>

Appendix 3: Chapter 3 Supplementary Material

The supplement to the Biogeosciences accepted version of Quick *et al.*, 2025 is available at the following link:

<https://doi.org/10.5194/bg-22-1557-2025-supplement>

The published version of this Appendix has been corrected on p.9 of the original.

Table S5 (corrected) follows:

Appendices

Previous (published) version.

Section 3.2

	Slope (litres d ⁻¹ mm ⁻¹)	SE	intercept	t	df	p
aCO2	3.86	1.25	-775	3.09	16	p<0.01
eCO2	3.55	0.31	-691	11.53	15	p<0.001
Ghost	1.20	0.47	75.4	2.54	21	p<0.05

Table S1: Linear regression model parameters for oak mean TWU ($\overline{TWU}$, litres d⁻¹) across a month (here July) versus bark radius (m) at insertion point. In this table slope, litres per day per millimetre bark radius, is calculated. Data from 2, 6, 12, 18 or 17 trees for years 2017, 2018, 2019, 2020 & 2021 which each have two probesets (E and W facing) fully operational across the month of interest are modelled by treatment for all years combined. July typically exhibits maximum mean TWU ($\overline{TWU}$) across summer months in control arrays. See also Notes to Table S3 re relative size ranges of trees in the three treatment types.

New corrected version (February 2026)

Section 3.2

	Slope (litres d ⁻¹ mm ⁻¹)	SE	intercept	t	df	p
aCO2	3.94	1.21	-812	3.25	16	p<0.01
eCO2	3.56	0.30	-691	11.94	15	p<0.001
Ghost	1.20	0.47	75.4	2.54	24	p<0.05

Table S2 (corrected): Linear regression model parameters for oak mean TWU ($\overline{TWU}$, litres d⁻¹) across a month (here July) versus bark radius (m) at insertion point. In this table slope, litres per day per millimetre bark radius, is calculated. Data from 2, 6, 12, 18 or 17 trees for years 2017, 2018, 2019, 2020 & 2021 (total 57 tree-year points) where each tree has two probesets (E and W facing) fully operational across the month of interest are modelled by treatment for all years combined. July typically exhibits maximum mean TWU ($\overline{TWU}$) across summer months in control arrays. See also Notes to Table S3 re relative size ranges of trees in the three treatment types.

Published supplement (final submitted Word version) follows (25 pages)

Supplementary information.
Section 2.2

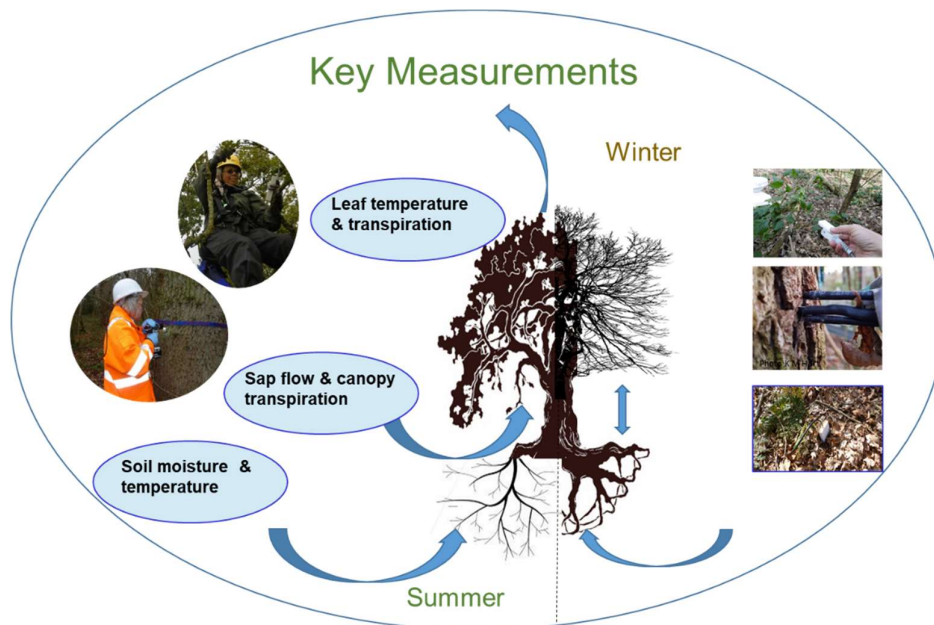


Figure S1: Key plant hydraulic related measurements.

Appendices

Tree ID	Array No.	Treatment	Install & Measure date	Initial Circumference (m)	Initial Average R _b at probeset (m)	2022 Circumference (m)	2022 Average R _b at probeset (m)	Average probeset height (m)
8467	1	eCO ₂	13/07/2017	1.640	<u>0.261</u>	1.656	<u>0.264</u>	1.13
8468	1	eCO ₂	13/07/2017	1.720	0.274	1.734	0.276	1.2
8673	2	aCO ₂	28/08/2018	2.360	0.376	2.372	0.378	1.26
8749	2	aCO ₂	28/08/2018	2.600	0.414	2.636	0.420	1.2
8351	3	aCO ₂	20/07/2017	1.710	0.272	1.726	0.275	1.19
9301	3	aCO ₂	20/07/2017	2.560	0.407	2.621	0.417	1.13
6621	4	eCO ₂	28/08/2018	2.350	0.374	2.372	0.378	1.3
6632	4	eCO ₂	05/09/2018	2.270	0.361	2.288	0.364	1.28
6382	5	aCO ₂	18/07/2018	2.210	0.352	2.220	0.353	1.3
6406	5	aCO ₂	18/07/2018	2.890	0.460	2.864	0.456	1.28
3755	6	eCO ₂	17/07/2018	2.260	0.360	2.278	0.363	1.23
5846	6	eCO ₂	17/07/2018	2.920	0.465*	2.968	0.472*	1.3
6027	7	none/ Ghost	21/07/2017	2.050	0.326	2.104	0.335	1.29
6021	7	none/ Ghost	21/07/2017	2.620	0.417	2.644	0.421	1.23
3642	8	none/ Ghost	24/07/2017	1.720	0.274	1.790	0.285	1.24
3634	8	none/ Ghost	24/07/2017	2.690	0.428	2.718	0.433	1.28
7476	9	none/ Ghost	05/01/2017	2.160	0.344	2.202	0.350	1.3
7331	9	none/ Ghost	05/07/2017	2.500	0.398	2.556	0.407	1.25

Table S3: Tree identification, array number, treatment type, species and installation/ measurement dates along with circumference and diameter at probeset insertion point height for target oak trees at start and in 2022. Underlined R_b values are smallest (initial & 2022). Largest R_b is marked with an asterix. See also Table S3 for summary data by treatment type.

Section 2.6

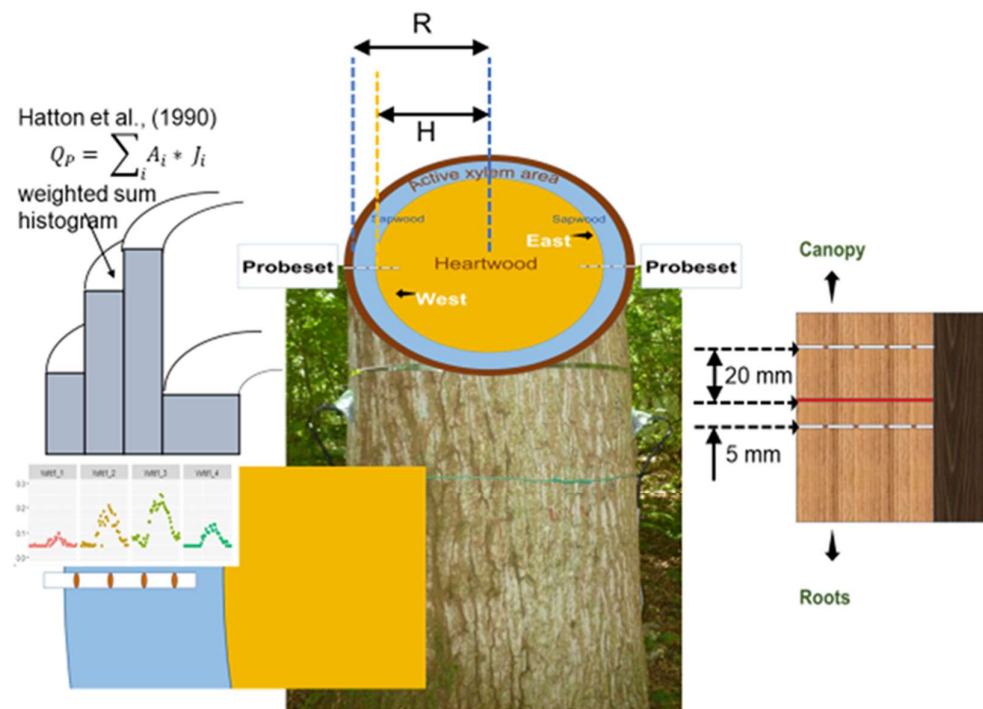


Figure S2: Showing sap probeset layout, spacing dimensions between probes and indicative illustration of Hatton et al., (1990) weighted sum histogram, where R (m) is the radius to the cambium and H (m) is the heartwood estimated radius, both at the probeset insertion height. All equations and variables also defined in Tables A1 and A2. Graphical insert is Fig. C1(b) in main text.

Appendices

Section 2.2 to 2.7

Description	Make	Model	Position	Parameter	Parameter symbol	Where	Units	Sampling rate	Logger rate
Shallow soil moisture. 12 cm water content reflectometry (WCR)	Campbell Scientific [Logan, USA]	CS655 ±3% v/v for typical soils	Inserted from ground level	Volumetric water content (VWC) + Surface soil temperature	<i>n/a</i>	Various all Arrays	% °C		At least every 30 mins
Tipping bucket gauges Half-hourly totals were compiled	Campbell Scientific	ARG100	Ground level	throughfall precipitation - accumulative	P_{fs}	all Arrays	mm		30 mins
FACE hemispherical solar radiation	Hukseflux pyranometer	LP02-03	Top canopy (c25 m)	Total Solar Radiation (A_v)	TG	A 1-6	Watt m ⁻²	10 secs	Every hour
FACE temperature sensor (thermistor)	Campbell Scientific	T107	Top canopy (c20 m)	Air Temp (A_v)	T_a	A 1-6	°C	10 secs	Every hour
Met tower precipitation	Texas Electronics (Dallas; Texas)	TR-525M	2 m above ground	Precipitation - accumulative	P	Met Towers	mm		15 minutes
Microcores	fCMC, Italy on behalf of UNIVERSITA' di PADOVA	Trephor (Rossi et al., n.d.)	At sap probe insertion height	15mm and 25 mm long, 2mm diameter cores for wood characteristics,	<i>n/a</i>				

Table S4: Soil, precipitation, FACE and Met tower instrumentation. Also microcorer equipment type.

Appendices

Section 2.7

2022	Tree	GH		Tree	aCO2		Tree	eCO2
	6027	2.104		8673	2.372		8467	1.656
	6021	2.644		8749	2.636		8468	1.734
	3642	1.79		8351	1.726		6621	2.372
	3634	2.718		9301	2.621		6632	2.288
	7476	2.202		6382	2.22		3755	2.278
	7331	2.556		6406	2.864		5846	2.968
mean circumference		2.34			2.41			2.22

sm	3642	1.79	sm	8351	1.726	sm	8467	1.656
med	7476	2.202	med	8673	2.372	med	6632	2.288
lg	3634	2.718	lg	6406	2.864	lg	5846	2.968

Table S5: To show tree stem circumference measures in 2022 for all trees. Also mean circumference (metres) and sizes of smallest (sm), largest (lg) and medium (med) sized trees in each treatment category.

Notes to Table S3.

All oak trees were of similar height (circa 25 m). Tree stem circumference at insertion height of probes was measured at installation (from 2017 onwards), using a standard tape measure, and checked manually in subsequent winters (Jan 2020-Feb 2022). The range and mean-per-treatment values of bark circumference (metres) for all target trees are tabulated and are summarised as follows: *Ghost* mean 2.34 m, range 1.79–2.72 m; aCO₂ mean 2.41 m (3% larger than the *Ghost* control mean), range 1.73–2.86 m; eCO₂ mean 2.22 m (5% smaller than the *Ghost* control mean), range 1.66–2.97 m.

Appendices

Section 2.8

Parameter	Author	Reported value	Conversion factor	Metric equivalent	Tree size
Sap flux (density) maximum	Tatarinov (2005)	0.1 kg cm ⁻² h ⁻¹	10 ⁴ / 3600	2.7778 x 10 ⁻¹ Kg m ⁻² s ⁻¹ [litres m ⁻² s ⁻¹]	Dbh>30cm, Height=29m
Sapflux max hourly (mean of 2x trees)	Sanchez-Perez (2008) HP	The hourly Maximum (total) fluxes were [30 litres h ⁻¹].		The hourly maximum fluxes were [30 litres h ⁻¹]. [July circa 380 (litres tree ⁻¹ day ⁻¹)] see below	Dbh=83cm Height=22
Total daily sapflux (summer max)	Čermák et al (1992) ref in Tatarinov (2005)*	200 kg tree ⁻¹ h ⁻¹		200 kg tree ⁻¹ h ⁻¹ [circa 1600 (kg tree ⁻¹ day ⁻¹)]	Dbh=30cm Height=29m
Total daily sapflux mean	Sanchez-Perez (2008) HP		N/A	[385 +/-41 l tree ⁻¹ day ⁻¹ July 1996]	Dbh=83cm Height=22
Total daily sapflux max *Q.suber	David (2013)	250 (kg tree ⁻¹ day ⁻¹)		250 (kg tree ⁻¹ day ⁻¹)	
Breast height Sap flux area (typical)	(Čermák et al (1992) & as quoted/Implied Tatarinov (2005)	2000 cm +2	10 ⁻⁴	2.0 x 10 ⁻¹ (m +2)	

Table S6: Comparison of sap parameters with other studies of oak.

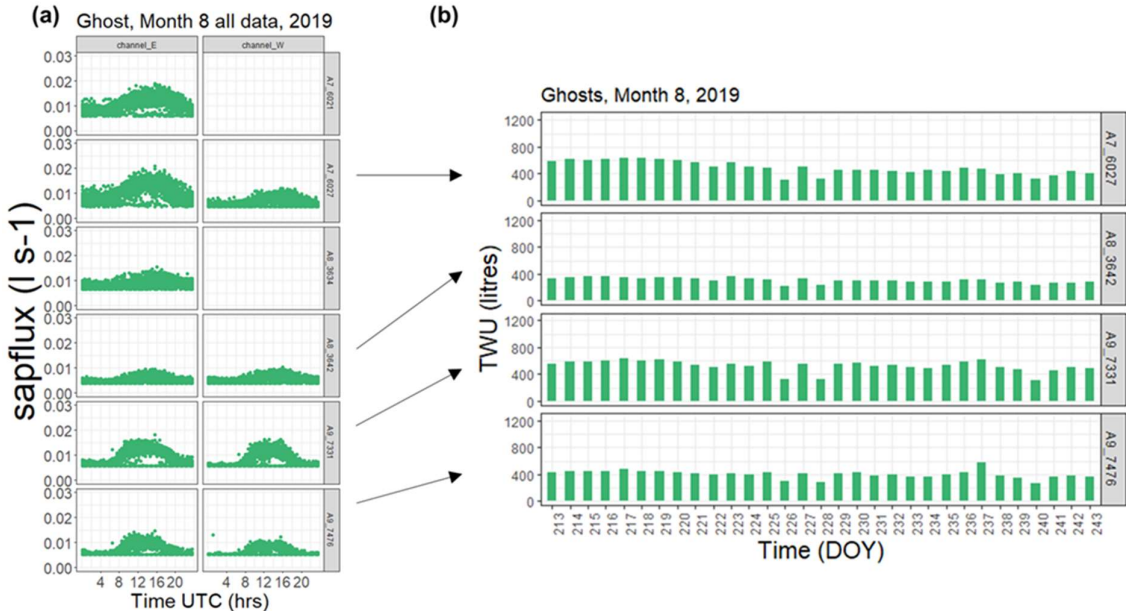


Figure S 3: Example *Ghost* Array xylem sap responses in August 2019. (a) diel (24hour) tree sap flux for all days in August 2019 are superimposed. E (left) and W (right) facing probesets for six *Ghost* trees show circumferential imbalance in xylem flux. All data for the individual month is superimposed across time-of-day sampling (hours, UTC). Frequency of sampling is every 0.5 hrs. Faulty probeset positions are shown blank. **(b)** Example of accumulative daily diurnal water usage (*TWU*) per tree totalled for E and W facing probesets across month 8 2019 for four *Ghost* trees having both E and W probesets functioning with the other two *Ghost* trees omitted due to faulty probesets. Time is day-of-year (DOY).

Appendices

Section 3.1

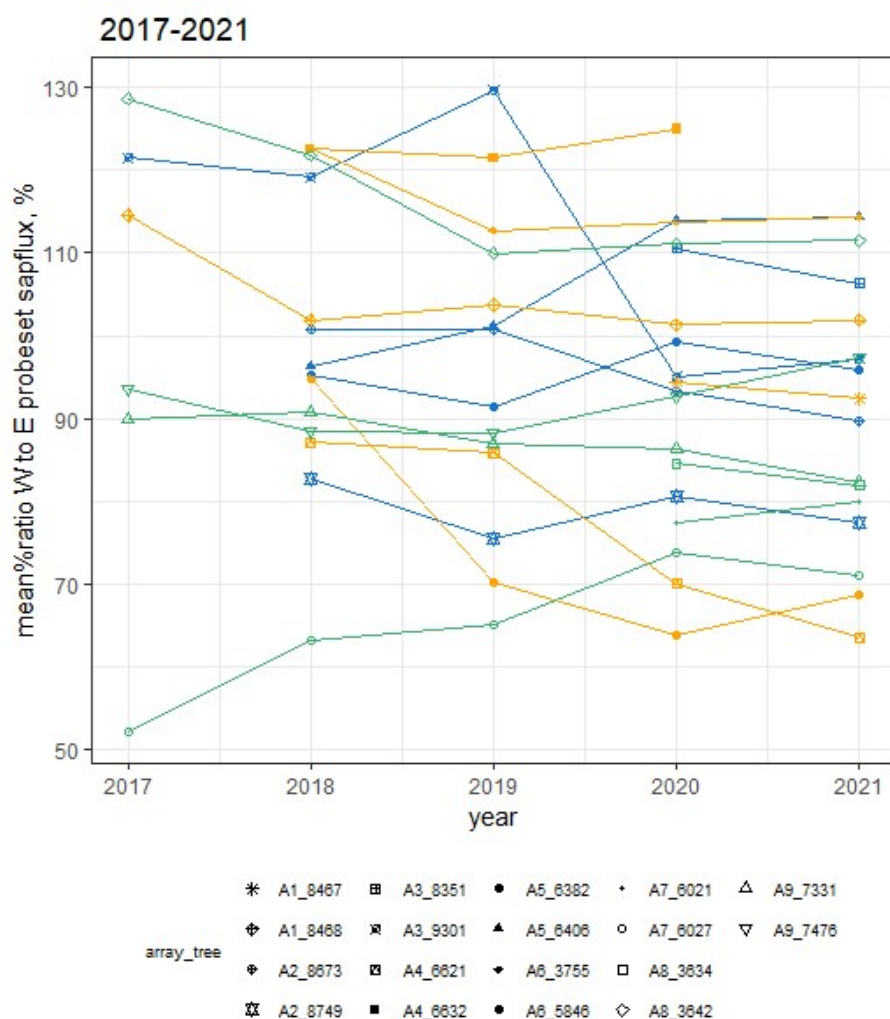


Figure S4: Imbalance of median sap flux values for each probeset by tree. Tree sapflux data for W-facing probeset as a percentage of E-facing sapflux data, averaged for each year of two probeset operation. Points are derived from means of monthly median probeset sapflux values for each tree. Note 2017 is only a partial year.

Appendices

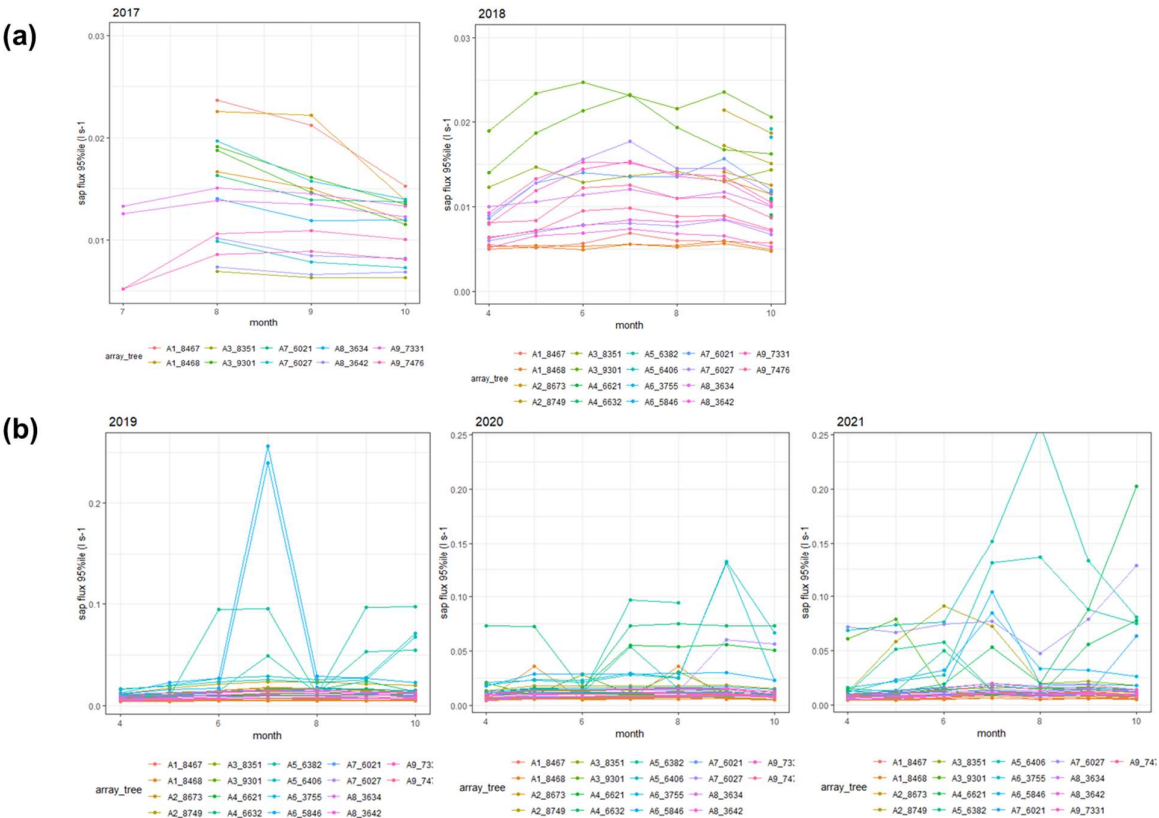


Figure S5: In years 2017 to 2021, summary monthly treatment season 95%ile sap flux (l s^{-1}) for each functional probeset all trees are shown. Colours represent individual trees and are also labelled with Array number (Ax). A1, A4, A6 are eCO_2 trees, A3, A2, A5 are aCO_2 trees, A7, A8, A9 are *Ghost* trees. (a) years 2017 and 2018, scaled to show a maximum of 0.03 l s^{-1} . (b) years 2019, 2020 and 2021, scaled to show a maximum of 0.25 l s^{-1} .

Section 3.2

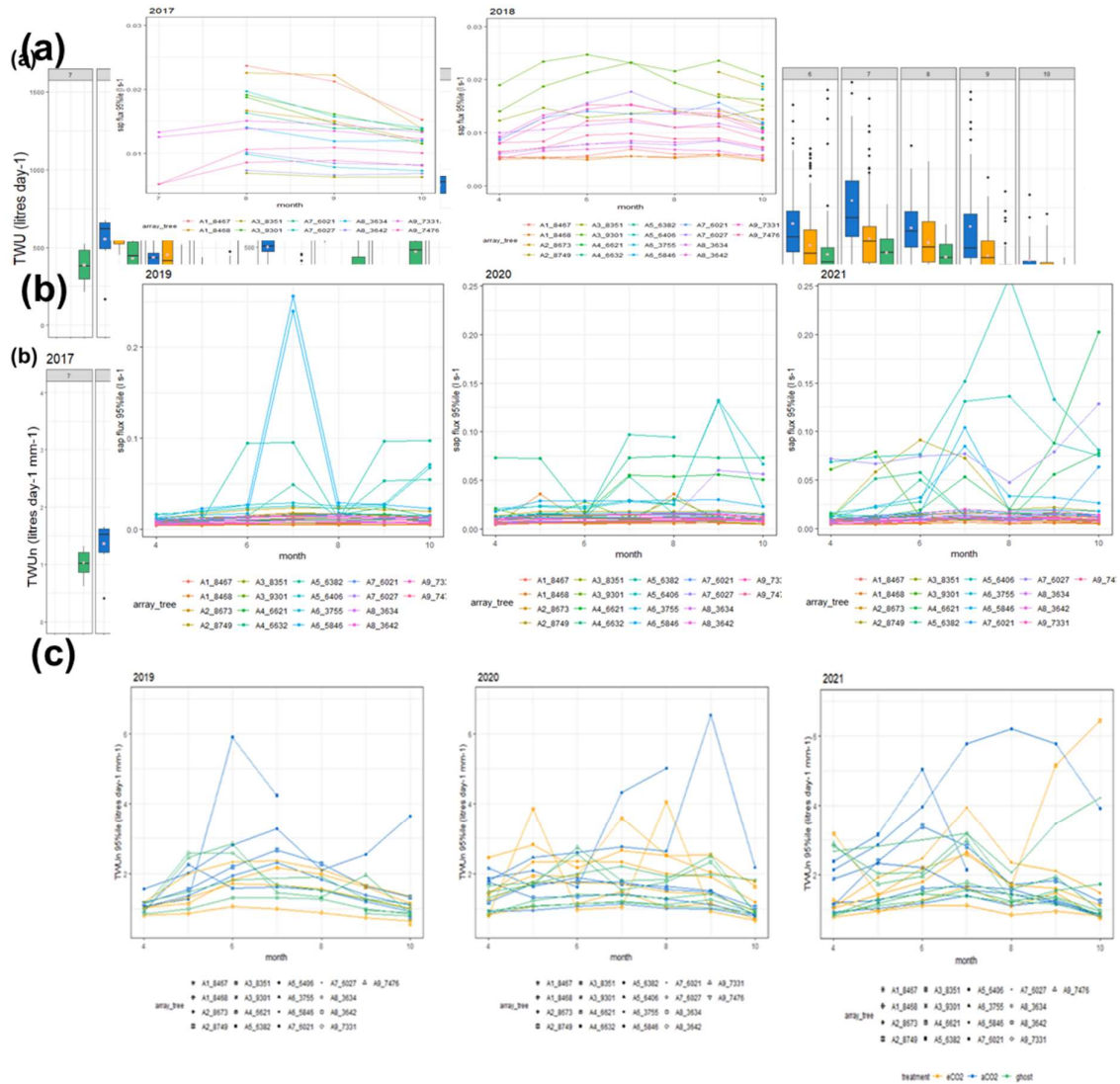
	Slope (litres $\text{d}^{-1} \text{ mm}^{-1}$)	SE	intercept	t	df	p
<i>aCO2</i>	3.86	1.25	-775	3.09	16	$p < 0.01$
<i>eCO2</i>	3.55	0.31	-691	11.53	15	$p < 0.001$
<i>Ghost</i>	1.20	0.47	75.4	2.54	21	$p < 0.05$

Table S7: Linear regression model parameters for oak mean TWU (TWU litres d^{-1}) across a month (here July) versus bark radius (m) at insertion point. In this table slope, litres per day per millimetre bark radius, is calculated. Data from 2, 6, 12, 18 or 17 trees for years 2017, 2018, 2019, 2020 & 2021 which each have two probesets (E and W facing) fully operational across the month of interest are modelled by treatment for all years combined. July typically exhibits maximum mean TWU (TWU) across summer months in control arrays. See also Notes to Table S3 re relative size ranges of trees in the three treatment types.

Appendices

Tree_ID	Array	year	SprdAvdia	TotSpread_dia	CanopyArea	treatment	barkradius	FirstLast
8467	1	2017	9.695	10.217	81.986	eCO2	0.26	First
8467	1	2022	8.377	8.904	62.264	eCO2	0.26	Last
8468	1	2017	9.545	10.092	79.999	eCO2	0.27	First
8468	1	2022	8.92	9.472	70.464	eCO2	0.28	Last
8673	2	2017	15.175	15.926	199.212	aCO2	0.38	First
8673	2	2022	11.448	12.203	116.963	aCO2	0.38	Last
8749	2	2017	12.48	13.308	139.088	aCO2	0.41	First
8749	2	2022	12.947	13.786	149.262	aCO2	0.42	Last
8351	3	2017	13.925	14.469	164.432	aCO2	0.27	First
8351	3	2022	12.398	12.948	131.667	aCO2	0.28	Last
9301	3	2017	16.375	17.19	232.079	aCO2	0.41	First
9301	3	2022	16.083	16.918	224.786	aCO2	0.42	Last
6621	4	2017	11.155	11.903	111.277	eCO2	0.37	First
6621	4	2022	14.598	15.353	185.139	eCO2	0.38	Last
6632	4	2018	11.49	12.213	117.14	eCO2	0.36	First
6632	4	2022	11.248	11.977	112.657	eCO2	0.36	Last
6382	5	2017	15.03	15.733	194.419	aCO2	0.35	First
6382	5	2022	12.573	13.28	138.511	aCO2	0.35	Last
6406	5	2017	15.795	16.715	219.431	aCO2	0.46	First
6406	5	2022	16.747	17.658	244.9	aCO2	0.46	Last
3755	6	2017	11.74	12.459	121.922	eCO2	0.36	First
3755	6	2022	11.232	11.957	112.284	eCO2	0.36	Last
5846	6	2017	15.315	16.244	207.253	eCO2	0.46	First
5846	6	2022	16.083	17.028	227.73	eCO2	0.47	Last
6021	7	2017	13.3	14.134	156.898	Ghost	0.42	First
6021	7	2022	14.635	15.477	188.123	Ghost	0.42	Last
6027	7	2017	14.42	15.073	178.428	Ghost	0.33	First
6027	7	2022	11.568	12.238	117.629	Ghost	0.33	Last
3634	8	2017	13.195	14.051	155.067	Ghost	0.43	First
3634	8	2022	11.39	12.255	117.949	Ghost	0.43	Last
3642	8	2017	10.675	11.222	98.916	Ghost	0.27	First
3642	8	2022	9.802	10.371	84.483	Ghost	0.28	Last
7331	9	2017	16.875	17.671	245.246	Ghost	0.40	First
7331	9	2022	16.785	17.599	243.246	Ghost	0.41	Last
7476	9	2017	9.685	10.373	84.501	Ghost	0.34	First
7476	9	2022	10.967	11.668	106.918	Ghost	0.35	Last

Table S8: Table of canopy spread v. bark radius and canopy spread diameter data for oak. Two separate repeat measures (First at installation year, Last early 2022) are listed. Bark radius is for the year of measurement.



Section 3.3

Figure S6: (a) Ghost and Infrastructure TWU (litres d⁻¹) boxplots years 2017–2019. Boxplots 2018, 2019, show mean daylight daily tree water usage (TWU , litres d⁻¹) combined by treatment for each treatment month April to October. (b) TWU_n (litres d⁻¹ mm⁻¹), i.e. TWU normalised by tree radius (mm) at stem probe insertion height. Numbers of trees with both sap probeset installations functioning differs by month and year from July 2017 (2 trees) until Oct 2019 (18 trees). Mean values, calculated from the entire range of data, are shown as spots (pink).

Figure S7: Comparison of monthly water usage data for all trees with individual tree ids., showing (a) sap flux (litres s⁻¹) 95%iles (2017–2018) (b) sap flux (litres s⁻¹) 95%iles (2019–2021), and (c) TWU_n (litres day⁻¹ mm⁻¹) 95%iles (2019–2021). Panels across seasons 3 to 5 of treatment) with Y axis commencing at 0.5 litres day⁻¹ mm⁻¹. (a) and (b) colours correspond to individual trees. (a) years 2017 and 2018, scaled to show a maximum of 0.03 l s⁻¹. (b) years 2019, 2020 and 2021, scaled to show a maximum of 0.25 l s⁻¹. (c) symbols are individual trees, colours are treatment type.

Note on Outliers

Outliers (Figures S7) influence tree water usage even when normalised for tree size. Peak variations of TWU (litres d⁻¹) and TWU_n (litres d⁻¹ mm⁻¹) (main document Fig. 5 and Fig. S5(c)), influenced by outliers, can be extreme for the infrastructure arrays. We cannot conclusively deduce inter-treatment comparisons without outlier temporal synchronicity or consistent removal prior to analysis. This check

Appendices

has now been done for TWU_n data across the season (2019–2021) as part of the analysis of variance. It is interesting to note that in 2021 for example, outliers appear to have been more prevalent in the aCO_2 trees in June, July and August, and more prevalent in the eCO_2 trees in June, July and September, with outliers in *Ghost* trees always being less frequent and smaller than for aCO_2 trees. This may imply a delayed effect of infrastructure present in eCO_2 compared to aCO_2 , so inter-monthly comparisons may be distorted when compared to each control treatment, or between controls.

Section 3.4

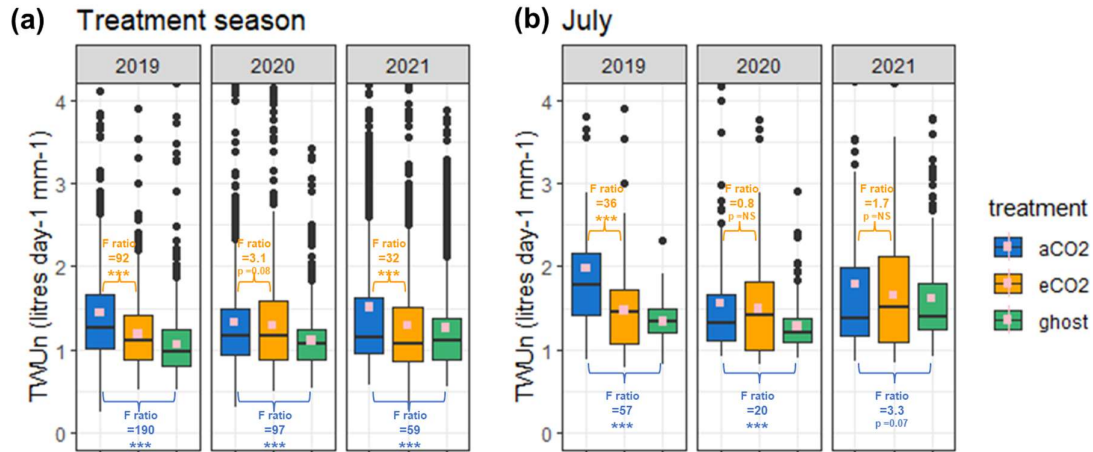


Figure S 8: Treatment comparison of TWU . For years 2019–2021 the TWU_n (litres d⁻¹ mm⁻¹) data is shown for the three treatment types. (a) The season data April–October is combined for each year. (b) July for each year is shown. The distributions are shown as box and whisker plots showing median and interquartile range (IQR, 25%ile to 75%ile) with whiskers calculated as 1.5 x IQR from the hinge and points for outliers. Mean values, calculated from the entire range of data i.e. season (a) or July (b) are shown as spots (pink). p-values and F ratios are indicated in orange (hypothesis 1, Table 4) for eCO_2 : aCO_2 one-way ANOVA model and blue (hypothesis 2, Table 5) for aCO_2 : *Ghost* one-way ANOVA model. Values for both treatment season (a) and July mean (b) TWU_n in each year are given. Tables also give % differences of both treatment season and July mean TWU_n .

	2019 p value	2019 F ratio	2019 %	2020 p value	2020 F ratio	2020 %	2021 p value	2021 F ratio	2021 %
Season	< 0.001	91.90	-19%	p>0.05, actual value 0.079	3.09	-3%	<0.001	32.27	-13.9%
July only	< 0.001	35.61	-26%	p>0.05, actual value 0.37	0.80	-4.5%	p>0.05, actual value 0.19	1.71	-7.3%

Table S 9: Hypothesis 1 CO_2 effects. One-way ANOVA p-value, F ratio, and % difference summary for mean eCO_2 TWU_n , compared with mean aCO_2 TWU_n , in years 2019–2021. Mean values are compared, calculated from the entire range of data for season data April–October (Fig. S8(a)) and July (Fig. S8b), for each year as shown. Bold typeface indicates p-value < 0.05.

	2019 p value	2019 F ratio	2019 %	2020 p value	2020 F ratio	2020 %	2021 p value	2021 F ratio	2021 %
Season	< 0.001	187.38	+37%	< 0.001	96.66	+20%	<0.001	58.81	+20%
July only	< 0.001	57.35	+48%	< 0.001	19.95	+22%	p>0.05, actual value 0.071	3.29	+9.9%

Table S 10: Hypothesis 2 infrastructure effects. One way ANOVA p-value, F ratio, and % difference summary for aCO_2 compared with *Ghost* TWU_n in years 2019–2021. Mean values are compared, calculated

Appendices

from the entire range of data for season data April–October (Fig. S8(a)) and July (Fig. S8(b)) for each year as shown. **Bold typeface indicates p-value <0.05.**

ANOVA model notes

Hypothesis 1 concerning the effect of CO₂ was tested by one-way ANOVA between $eCO_2 \overline{TWU_n}$ compared with $aCO_2 \overline{TWU_n}$ (Table S7, Fig. S8).

In 2019 and 2021 seasons, the ANOVA suggested a highly significant ($p < 0.001$), -19% to -13.9%, reduction in $eCO_2 \overline{TWU_n}$ compared with $aCO_2 \overline{TWU_n}$ (Fig. S8 blue-yellow comparisons, Table S7). In 2020, the 3% reduction was marginally significant ($p = 0.08$) for $eCO_2 \overline{TWU_n}$ vs. $aCO_2 \overline{TWU_n}$. For July-only results, the ANOVA suggested a highly significant ($p < 0.001$) -26% reduction in $eCO_2 \overline{TWU_n}$ compared with $aCO_2 \overline{TWU_n}$ in July 2019, whilst comparisons for July 2020 and July 2021 showed no significant differences (Fig. S8 blue-yellow comparisons, Table S7).

Hypothesis 2 concerning the effect of infrastructure is tested by one-way ANOVA between mean values of $aCO_2 \overline{TWU_n}$ compared with $Ghost \overline{TWU_n}$ (Table S8, Fig. S8 blue-green comparisons). For all 2019, 2020 and 2021 seasons, the ANOVA suggested a highly significant ($p < 0.001$) 37% to 20% increase in $aCO_2 \overline{TWU_n}$ compared with $Ghost \overline{TWU_n}$ (Fig. S8, Table S8). For July-only $\overline{TWU_n}$, the ANOVA suggested a highly significant ($p < 0.001$) 48% to 22% increase in aCO_2 compared with $Ghost \overline{TWU_n}$ for July 2019 and July 2020 (Fig. S8, Table S8). For July 2021, a marginal 10% effect for $aCO_2 \overline{TWU_n}$ vs. $Ghost \overline{TWU_n}$ was found ($p = 0.07$).

Section 3.3

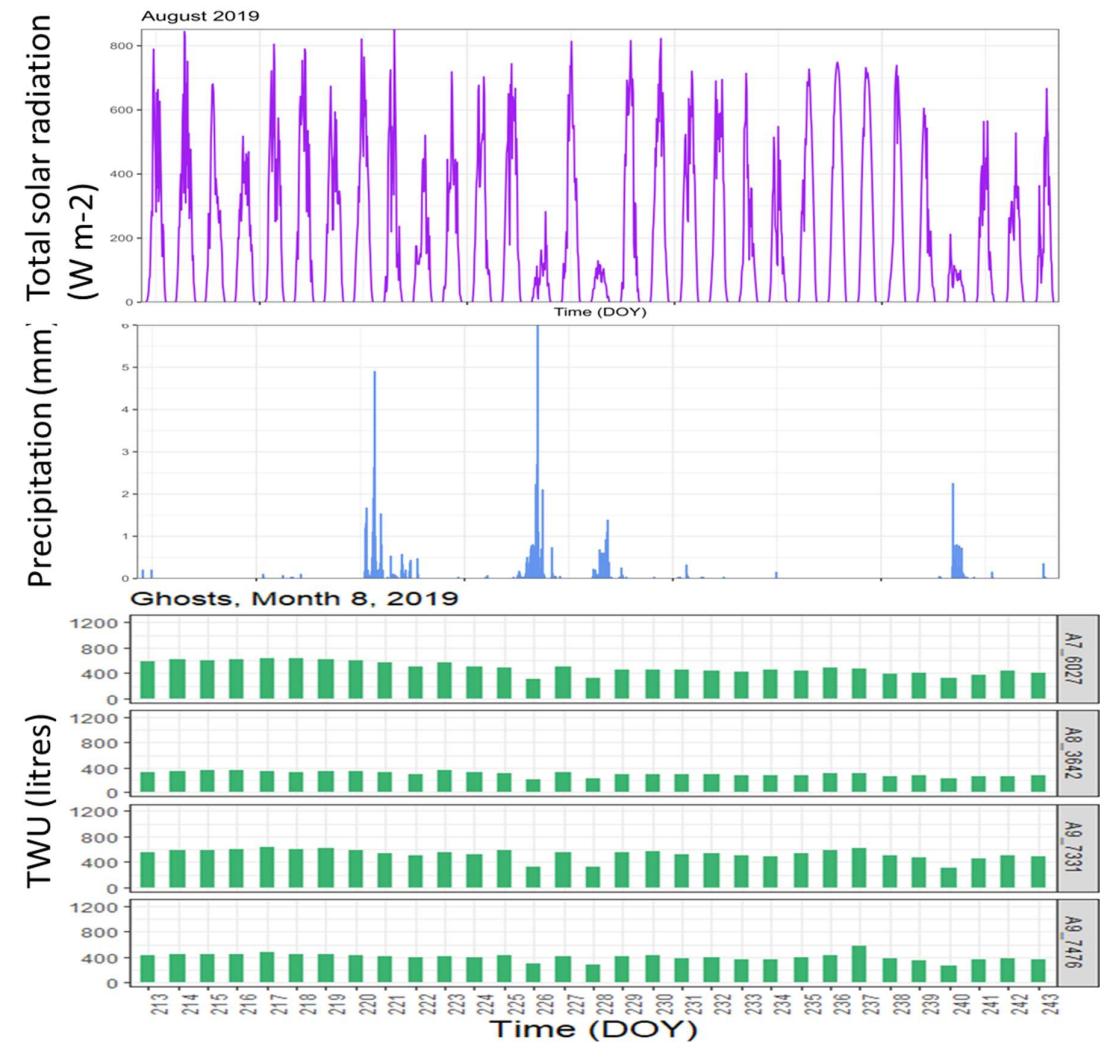


Figure S9: Example *TWU* (litres d⁻¹) per DOY v. solar radiation and precipitation for August 2019. Note if precipitation is nocturnal, then it does not influence *TWU* directly.

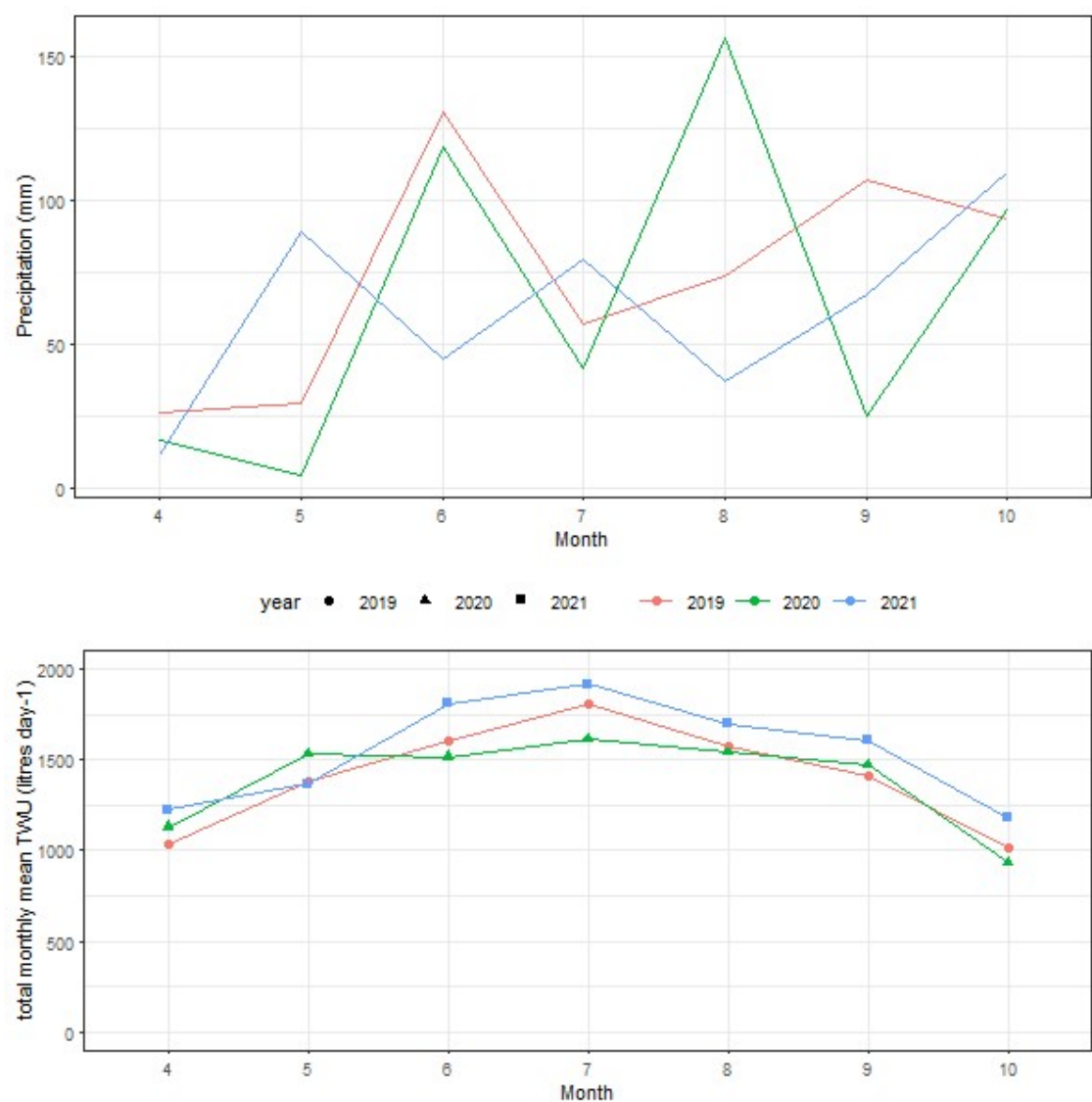


Figure S10: Monthly precipitation (mm) versus monthly mean TWU ($\overline{TWU}$, litres d⁻¹) for treatment season months in years 2019 -2021.

Appendices

Section 3.4

Levene's test and model_CO2 for eCO₂ compared with aCO₂: Table S9.

All Levene tests (mean and median) for seasonal data 2019 and 2021 are significant; also for July data in 2019. This implies heterogeneity of variances of the data. For 2020 season and July 2020 and 2021 the test is not significant meaning ANOVA model can be validated for parametric assumptions.

	2019	2019	2020	2020	2021	2021
Season	F(1, 2065) =	Pr(>F)	F(1, 2491) =	Pr(>F)	F(1,2167) =	Pr(>F)
mean	58.697	***	0.474	0.4912	40.141	***
median	39.179	***	0.0332	0.8555	13.69	***
July	F(1, 300) =	Pr(>F)	F(1, 367) =	Pr(>F)	F(1,304) =	Pr(>F)
mean	11.07	***	0.7235	0.3956	5.3071	0.02191 *
median	7.7959	**	0.003	0.9563	0.8713	0.3513

Table S11: Levene's test mean and median CO₂ effect eCO₂ compared with aCO₂. Bold results (2020 and 2021) are non-significant exhibiting homogeneity of variances.

Levene's test and model_inf for aCO₂ compared with Ghost: Table S10

All tests both for seasonal and for July data 2019, 2020 2021 are significant demonstrating the heterogeneity of variances of the data.

	2019	2019	2020	2020	2021	2021
Season	F(1, 1962) =	Pr(>F)	F(1, 2491) =	Pr(>F)	F(1, 2438) =	Pr(>F)
mean	96.972	***	112.05	***	130.93	***
median	70.25	***	78.943	***	53.131	***
July	F(1, 277) =	Pr(>F)	F(1, 367) =	Pr(>F)	F(1, 338) =	Pr(>F)
mean	35.15	***	35.026	***	19.54	***
median	26.984	***	19.905	***	7.989	**

Table S12: Levene's test mean and median infrastructure effect aCO₂ compared with Ghost. No results are non-significant so homogeneity of variances is not proven.

Notes on Levene tests.

Despite limitations to the comparison of mean daily values, given the non-linear nature of the *TWU* data exhibiting variable outliers, the one-way ANOVA models using *TWU_n* provide a good initial indication of treatment comparisons when sufficient periods of time (here whole season or whole of July) of continuous data are compared between treatments, in line with the Central Limit Theorem.

Appendices

Section 2.5 and 3.4

Annual mean solar radiation and air temp from FACE monitoring (top of 6 infrastructure arrays)
2017 – 2018 2019-2020 2021-

Extracted from 30min FACE
data files.
Jan 2022 & Jun 2022

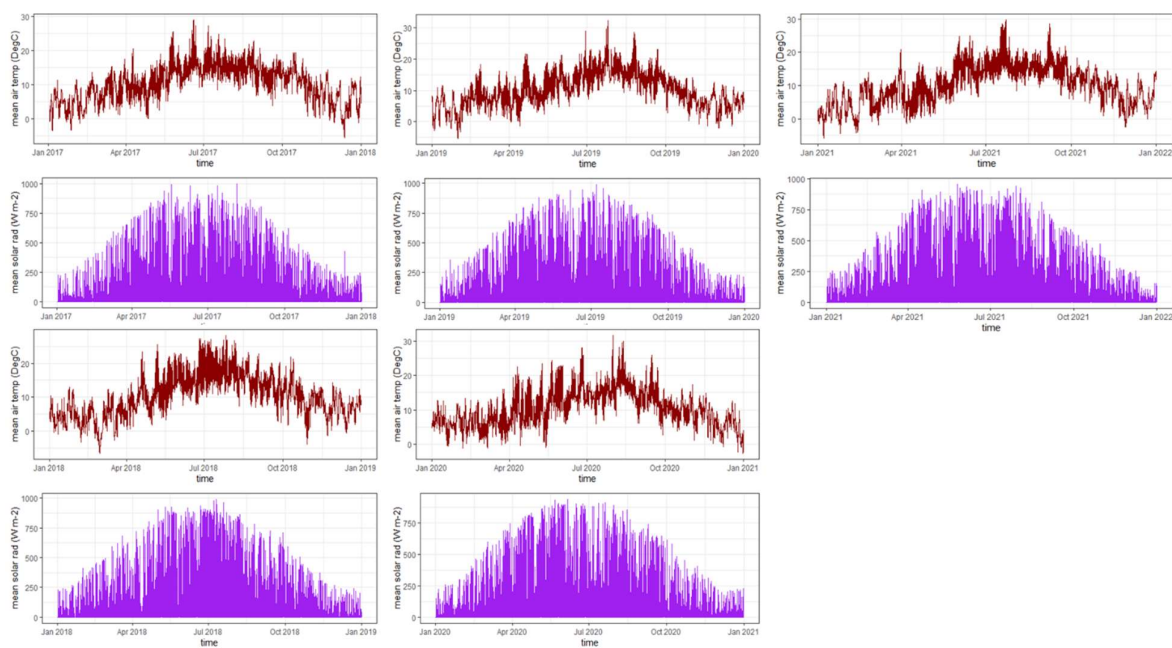


Figure S11: Annual mean solar radiation (W m^{-2}) and air temp (degrees C) for 2017 to 2021 from FACE monitoring (top of six infrastructure arrays).

Appendices

Section 3.4

Season	Tree numbers removed	No. of tree data used	Max number of days in season	Total treedays modelled
CO₂ model (eCO₂: aCO₂)				
2019	A1_8467 and A3_8351 low data	10	214	2047
2020	none	12	214	2493
2021	A4_6632 (failed) A5_6382 low data (98 days)	10	214	2071
Infrastructure model (aCO₂: <i>Ghost</i>)				
2019	A3_8351, A7_6021 & A8_3634 low data	9	214	1841
2020	none	12	214	2493
2021	A5_6382 low data	11	214	2342

Table S 13: Data used in glmm models for hypothesis 1 and hypothesis 2

	2019	2020	2021
σ^2	0.07	0.08	0.11
$\tau_{00 \text{ DOY}}$	0.03	0.03	0.04
$\tau_{00 \text{ treelabel}}$	0.01	0.01	0.02
ICC	0.37	0.35	0.34
Marginal R^2 / Conditional R^2	0.041 / 0.399	0.003 / 0.348	0.046 / 0.371

Table S 14: Detailed statistics from the glmm model to test hypothesis 1

	2019	2020	2021
σ^2	0.08	0.06	0.08
$\tau_{00 \text{ DOY}}$	0.03	0.03	0.03
$\tau_{00 \text{ treelabel}}$	0.02	0.01	0.01
ICC	0.37	0.35	0.34
Marginal R^2 / Conditional R^2	0.097 / 0.433	0.061 / 0.390	0.029 / 0.355

Table S 15: Detailed statistics from the glmm model to test hypothesis 2

Appendices

Section 4

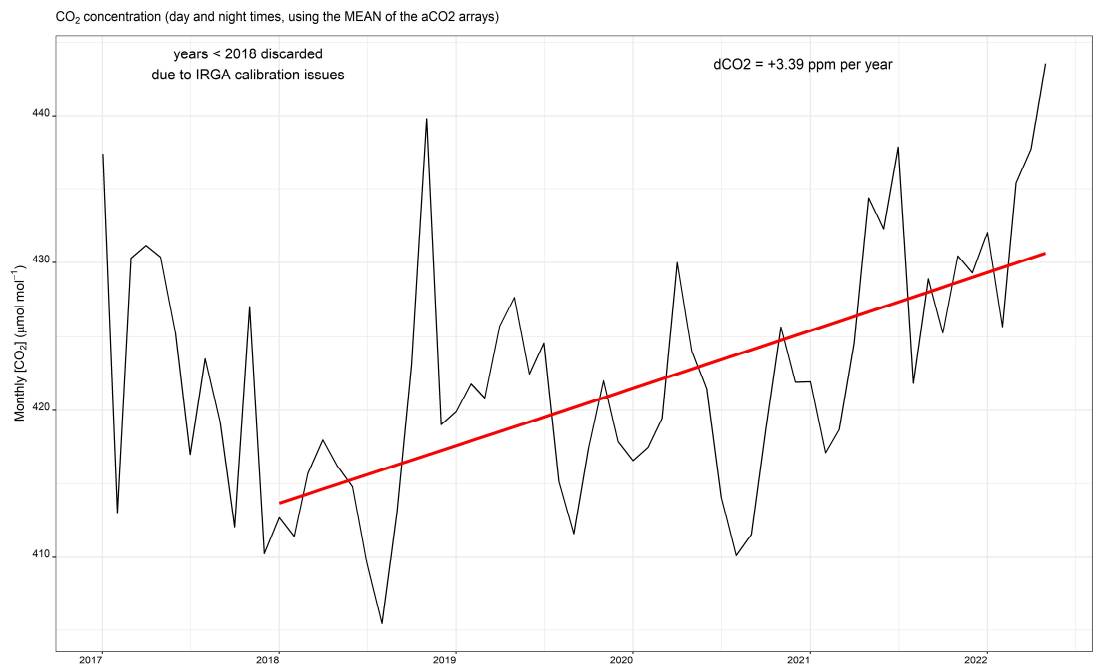


Figure S12: Ambient CO₂ concentration at BIFoR FACE experiment during years 2018 to 2021 (day and night times, using the mean of the aCO₂ arrays). The red line represents the slope of the increase over that period. Note pre-2018 data are calibrated differently.

Example visualisation (referenced Section 2.9).

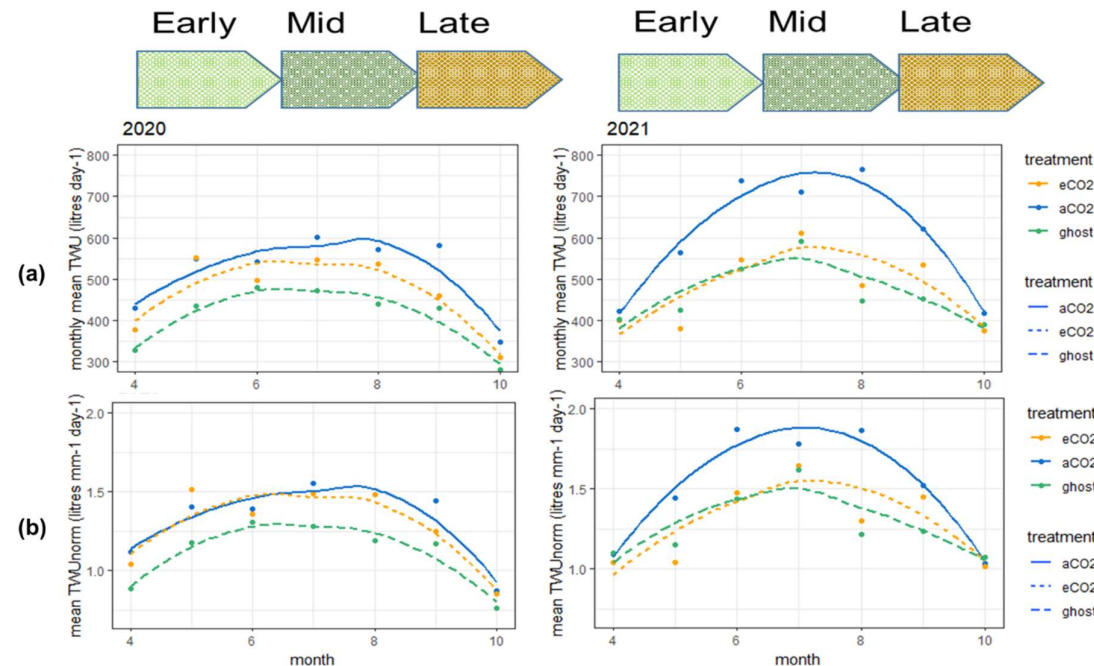


Figure S13: Seasonal changes in water usage (a) $\overline{TWU}$, (litres d⁻¹ month⁻¹), grouped by treatment (up to 6 trees), for each treatment month in 2020 & 2021. (b) $\overline{TWU}_{norm}$, (litres d⁻¹ mm⁻¹ month⁻¹), grouped by treatment (up to 6 trees), for each treatment month in 2020 & 2021. The lines use method = 'loess' and formula 'y ~ x'.

Note on monthly mean TWU and TWU_n ($\overline{TWU}$ and $\overline{TWU_n}$).

Figure S13 indicates $\overline{TWU}$ (litres d⁻¹ month⁻¹) and $\overline{TWU_n}$ (litres d⁻¹ mm⁻¹ month⁻¹) for each treatment season month in 2020 and 2021 to more clearly visualise those data. The seasonality of $\overline{TWU}$ and $\overline{TWU_n}$ at senescence is different in the two years.

Supplement Appendix S-A.

Environmental and soil parameters.

For plants, incident precipitation affects their function in several ways during the leaf-on season. Firstly, water droplets fall on the leaves which combined with lack of sun can prevent full photosynthesis. The canopy water mostly evaporates or may drip to ground. Secondly throughfall (P_{fs} , mm) reaching ground level may either: runoff the surface being lost to the soil, infiltrate increasing soil moisture content (providing some necessary support for root rehydration and plant water intake) or evaporate. Lastly, the soil water percolates through the saturated soil layers to replenish the water table.

Tor-ngen et al. (2014) found no significant interaction effects between sap flux derived canopy conductance eCO_2 changes and soil moisture (using relative extractable water).

Seasonal weather

Local reference precipitation (P_r , mm) for the period of interest (November 2015 to December 2021) in both the meteorological year and the tree hydraulic year averages to approximately 748 mm yr⁻¹ (Table S14). All years are reported, for completeness and to provide a broader context for the years for which we report sapflux data. We focus on hydraulic Years 3–5 (2019–2021) to interpret the rank ordering of our results for eCO_2 treatment effect on TWU (Hypothesis 1) in the light of interannual climate variability. From Table S7, above, the magnitudes of both the seasonal and July-only eCO_2 treatment effects are ranked (lowest to highest) in the order 2020, 2021, 2019.

Effects of precipitation on deciduous tree water usage

Table S14 reports Early leaf-on (May to June), Mid leaf-on (July to August) and Late leaf-on (September to October) variability in P_r ; the pattern is shown qualitatively in Figure S14. Dormant (November to February) and pre-budburst (March to April) P_r are also shown, with the preceding dormant season typically providing about 50% of the precipitation in the plant hydraulic year. Replenishment of perched water table and ground water reservoir levels, influences utilisation by old growth oak (Süßel and Brüggemann, 2021) during summer drought. The wettest and driest years and leaf-on seasons in respect of the tree hydraulic year are also marked (Table S14). Year 2 of the study (2017–2018) was the driest hydraulic year (HY) overall despite a very wet pre-budburst. During the growing season P_r averages 499 mm (Table S14). HY 5 (2020–2021) was the wettest of these three years overall but exhibited less than average growing season precipitation. HY 3 (2018–2019) had the wettest growing season.

The rank order of growing season TWU_n follows that of growing-season precipitation: i.e. 2021, 2020, 2019 (lowest to highest), which indicates the control of TWU_n by water supply, but the association is

Appendices

indicative at best. The rank order of eCO₂ treatment effects follows that of sub-seasonal precipitation, both May-June and Sep-Oct: i.e., 2020, 2021, 2019.

For the treatment season, monthly throughfall (P_f , mm), i.e. average precipitation received at soil level, and monthly interception (P_i , mm), are shown in Fig. S15, across the five treatment years. Reference precipitation April–October (100%) minus treatment season throughfall (P_{fs} , %), by month and treatment season, gives interception percentage (P_{is} , %). Treatment season throughfall averages 59% per month. Annual throughfall averages 64% across the five years presented.

Throughfall percentages are influenced by changes to canopy cover and affected by herbivory defoliation (recorded in May 2018 and 2019). Another driver of high throughfall is heavy rain, which reduces interception. The rank order of interception minima (excluding early leaf-on and defoliation periods) is 6% July 2019, 7% in August 2020 and 11% in October 2019. Throughfall has not yet been quantitatively associated with TWU or $\overline{TWU}$ due to the complexity of mixed effects in respect of other environmental influences (e.g. solar radiation, Figs. S9 & S11, and monthly precipitation versus $\overline{TWU}$, Fig. S10). To fully assess these interception factors LAI data is required which was not available for all years of this study. We present interception and throughfall (Fig. S15) here to inform future analyses through modelling.

FACE Treatment season label		Reference Precipitation P_r (mm)						
Year		2016	2017	2018	2019	2020	2021	Mean
Annual (Jan-Dec)		871.3	713.2	625.9	758.9	741.3	780.7	748.6
Tree hydraulic year		2015-6	2016-7	2017-8	2018-9	2019-20	2020-21	
Annual (Nov-Oct)		<u>913.5</u>	720.9	<u>649.2</u>	718.2	721.4	759.3	747.1
% anomaly to mean 2015-2021		22.3	-3.5	-13.1	-3.9	-3.4	1.6	0.0
November – Feb	Dormant	<u>296.6</u>	273.4	225.7	<u>151.9</u>	244.3	296.3	248.0
March – April	Pre-budburst	153.6	68.3	<u>178.2</u>	74.4	<u>34.2</u>	35.7	90.7
May – June	Early leaf-on	<u>202.3</u>	106.2	<u>61.5</u>	160.3	123.0	134.0	131.2
July – August	Mid leaf-on	153.9	157.4	<u>64.5</u>	130.8	<u>198.4</u>	116.5	136.9

Appendices

September – October	Late leaf-on	<u>107.1</u>	115.6	119.4	<u>200.8</u>	121.6	176.8	140.2
March – Oct	Growing	<u>616.9</u>	447.4	<u>423.5</u>	566.4	477.1	463.0	499.1
eCO ₂ effect (%)		<u>n/a</u>	n/a	<u>n/a</u>	-19	-3	-14	

Table S 16: Precipitation totals and percentage deviations from mean across the seasons and years of interest. 2016 (2015/2016) included as a pre-treatment year. Calendar years are shown in rows 1-2; hydraulic year is used in the remainder of the table. Underline is maximum and underline is minimum of the years. Where data is not available it is marked “n/a”.

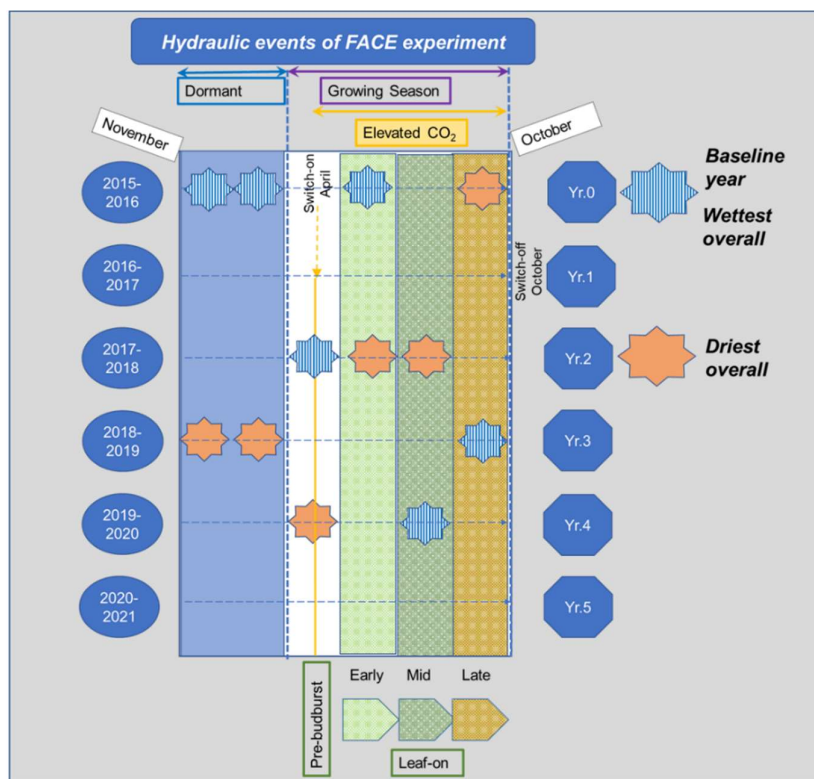


Figure S 14: Hydraulic events during period November 2015 to October 2021, baseline Year 0 and Years 1 to 5 of the FACE experiment. Year 2 (2017–2018) was the driest hydraulic year overall and year 3 (2018–2019) was the wettest treatment growing season (Table S14) including a very wet late leaf-on season.



Figure S 15: Local monthly precipitation, shown as stacked throughfall (P_r mm) and interception (P_i mm), at BIFoR FACE for treatment seasons 2017 to 2021. Percentage throughfall is indicated above each combined bar.

Appendices

Shallow soil moisture response to treatment season precipitation

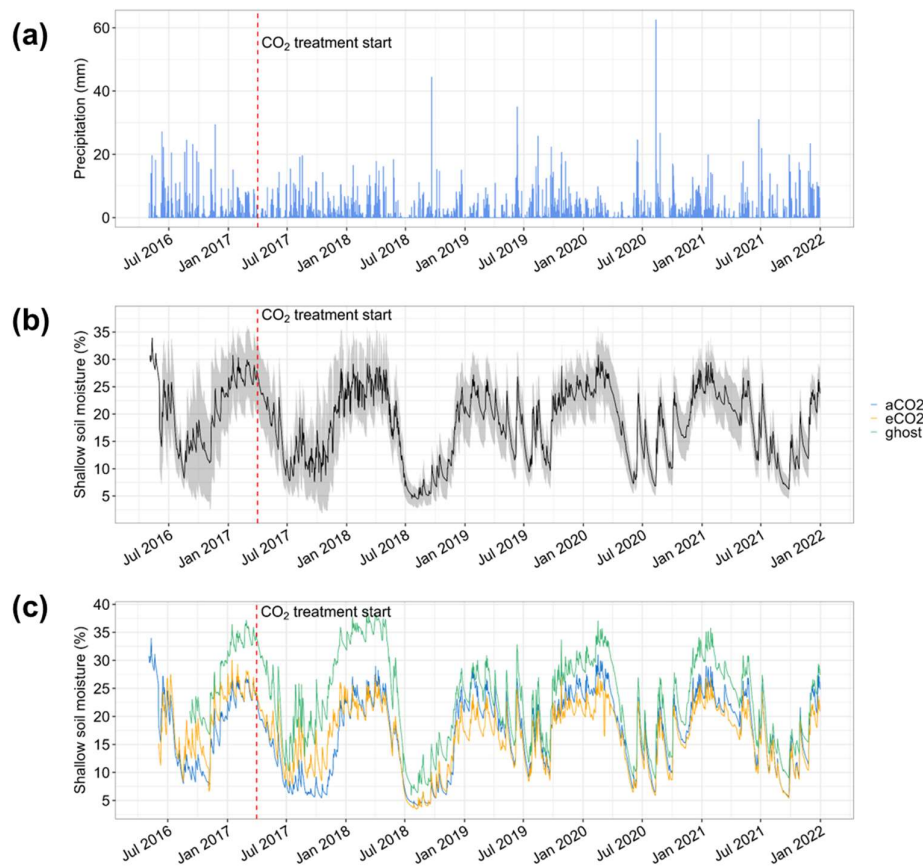


Figure S 16: Years 2016–2021 (a) daily precipitation (b) daily shallow soil moisture + sd averaged across all treatment arrays (c) daily mean shallow soil moisture by treatment. Extended from MacKenzie et al., 2021.

Throughfall during the treatment season (Fig. S15) directly affects Volumetric Water Content (VWC) and may therefore influence *TWU* from budburst to senescence. The extent of shallow (0 to 20cm depth) soil moisture depletion during drought and its effects on water usage by BIFoR FACE control (*aCO₂* and *Ghost*) oaks has been reported by Rabbai et al. (2023). Shallow soil moisture availability decreases progressively across the leaf-on season even in wet summers (MacKenzie et al., 2021 and Fig. S16).

Comparing Figs. 5 (main text) and S16, the opposite seasonalities of *TWU* and VWC are evident with autumn–winter dormant season providing VWC recharge. Tree water usage drives the leaf-on seasonal reduction in VWC, although the leaf season VWC cycle is strongly modulated by precipitation (e.g., Fig. S16, summer 2020). On shorter, sub-seasonal and daily timescales, the relationship between *TWU* and VWC is not expected to be simple. We defer a full account of sub-seasonal *TWU* v. VWC relations to future work, but note here in passing that, for example during the most pronounced continuous dry period of the observation period (June to July 2018, Fig. S16), there appears to be no inter-year difference in median *Ghost* tree diurnal sap flux (Fig. 2(a)), 95%ile sap flux (Fig. S5), or median *TWU* (Fig. S6) in all *Ghost* trees.

Appendices

References

- Čermak, J., KUČERA, J., and ŠTĚPÁNKOVÁ, M.: Water consumption of full-grown oak (*Quereus robur* L.) in a floodplain forest after the cessation of flooding, <https://doi.org/10.1016/b978-0-444-98756-3.50034-4>, 1991.
- David, T. S., Pinto, C. A., Nadezhdina, N., Kurz-Besson, C., Henriques, M. O., Quilhó, T., Cermak, J., Chaves, M. M., Pereira, J. S., and David, J. S.: Root functioning, tree water use and hydraulic redistribution in *Quercus suber* trees: A modeling approach based on root sap flow, *For. Ecol. Manage.*, 307, 136–146, <https://doi.org/10.1016/j.foreco.2013.07.012>, 2013.
- Hemery, G. E., Savill, P. S., and Pryor, S. N.: Applications of the crown diameter–stem diameter relationship for different species of broadleaved trees, *For. Ecol. Manage.*, 215, 285–294, <https://doi.org/https://doi.org/10.1016/j.foreco.2005.05.016>, 2005.
- Rossi, S., Anfodillo, T., and Menardi, R.: Trephor: A New Tool for Sampling Microcores from tree stems, *IAWA J.*, 27, 89–97, <https://doi.org/https://doi.org/10.1163/22941932-90000139>, n.d.
- Sánchez-Pérez, J. M., Lucot, E., Bariac, T., and Trémolières, M.: Water uptake by trees in a riparian hardwood forest (Rhine floodplain, France), *Hydrol. Process.*, <https://doi.org/10.1002/hyp.6604>, 2008.
- Tatarinov, F. a, Kučera, J., and Cienciala, E.: The analysis of physical background of tree sap flow measurement based on thermal methods, *Meas. Sci. Technol.*, 16, 1157–1169, <https://doi.org/10.1088/0957-0233/16/5/016>, 2005

Appendix 4: Chapter 4 Supplementary Material

Supplement to:

Linking *Quercus robur* tree-water usage to soil-water dynamics within a forest FACE experiment.

Susan Quick^{1,2}, Giulio Curioni^{1,2}, A. Robert MacKenzie^{1,2}, Stefan Krause^{1,2,3,4}

¹School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, B15 2TT, UK

²Birmingham Institute of Forest Research, University of Birmingham, Birmingham, B15 2TT, UK

³Laboratoire d'écologie des hydrosystèmes naturels et anthropisés, University Claude Bernard, Lyon1, Lyon, France (LEHNA)

⁴Institute for Global Innovation, University of Birmingham, Birmingham, B15 2TT, UK

Correspondence to: Susan Quick (sue_quick@yahoo.co.uk), Stefan Krause (s.krause@bham.ac.uk)
official correspondence author

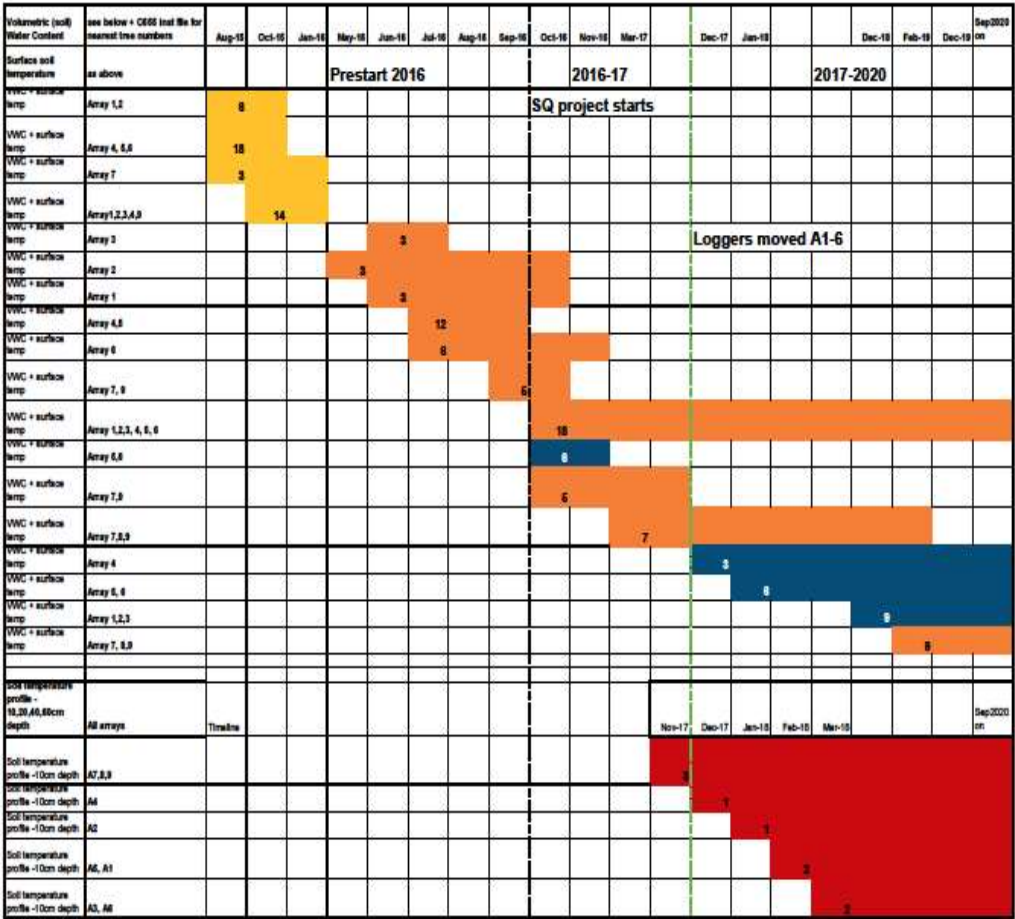


Figure S 17: Timeline of shallow soil moisture and soil temperature probe additions years 2015 to 2020.

Quality checks on soil transducers

During quality checks we removed small numbers of soil-water data outliers in each study year prior to further averaging by time or treatment. The averaging method used for groups of shallow CS655 probes differed to that for other manufacturers' probes.

Data quality of all shallow soil-moisture and soil temperature data (i.e. the CS655 both VWC and surface temperature, 107 Temperature data, Enviroscan profile soil-moisture data and Delta-T profile soil-moisture and temperature data) were checked and processed as follows. An algorithm was pre-applied to these data to remove clear outliers using the following method. For each year, and for each individual sensor raw

Appendices

time series (i.e. the individual CS655 data before averaging by group) we used high and low thresholds (e.g. for VWC (>0.8 and <0.001) to eliminate extreme values and double-checked the remaining data for outliers, sudden breaks and missing data problems. A running median was calculated with a short window length (usually 3 or corresponding to 1 or 2 hours). Next the absolute difference between the smoothed signal and the original one was calculated. Then the percentile of this difference was calculated using all the specific year's data from the selected sensor and depth (using $p = 99.9\%$ for temperature and $p = 99.99\%$ for the other parameters). Finally, all values from the original time series greater than the corresponding percentile threshold were removed (assign NA). This method removed only a small number of evident outliers. Soil temperature (here surface temperature) usually has a stable signal so the lower percentile threshold of 99.9%-ile was used. Soil-moisture exhibits real spikes during heavy rainfall events, hence a higher threshold of 99.99%-ile was used. Some specific smoothing and thresholds were also set following visualisation for the Enviroscan probes during preparation of the 'day' files. Table S1 summarises the quality thresholds applied.

Soil transducer types	Parameter type	Percentile calculated	Minimum threshold	Maximum threshold
CS655, 107, Delta-T	soil temperature (various depths)	99.9	temp_low <- (-15) # if soil temperature is below -15 degrees C it is considered an outlier.	temp_up <- (50) # if soil temperature is greater than 50 degrees C it is an outlier.
CS655, Enviroscan, Delta-T	vlc (various depths)	99.99	vlc_low <- 1% # vlc less than 1% considered unrealistic	vlc_up <- 80 # vlc more than 80% unrealistic

Table S 17: Pre-filtering applied to raw data from soil transducers. See data processing scripts for more detailed information.

Appendices

	Treat- ment	2017		2018		2019		2020		2021		2022	
		Fld. Cap.	Wilt pt.	Fld. Cap.	Wilt pt.	Fld. Cap.	Wilt pt.	Fld. Cap.	Wilt pt.	Fld. Cap.	Wilt pt.	Fld. Cap.	Wilt pt.
A1	eCO ₂	23.65	5.84	19.8	3.3	18.43	7.53	21.65	4.99	22.78	4.35	19.61	3.5
A2	aCO ₂	31.3	6.76	29.33	4.8	29.26	10.84	NA	NA	NA	NA	24.99	4.68
A3	aCO ₂	28.56	4.52	27.52	4.26	24.96	8.72	24.45	4.46	26.59	4.63	26.52	4.47
A4	eCO ₂	26.91	4.85	22.4	3.27	24.03	5.29	26.79	6.14	26.64	5.44	23.06	4.01
A5	aCO ₂	20.16	4.47	21.35	3	21.64	6.62	28.33	5.4	26.6	5.62	24.94	4.9
A6	eCO ₂	34.13	8.83	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A7	Ghost	39.62	11.81	41.4	5.07	16.9	5.8	32.77	9.99	32.76	10.26	NA	NA
A8	Ghost	NA	NA	35.8	8.82	29.39	13.01	NA	NA	NA	NA	31.13	6.81
A9	Ghost	34.02	8	36.19	5.19	29.51	11.37	36.59	8.11	36.5	8.05	32.8	7.1
mean		29.8	6.9	29.2	4.7	24.3	8.6	28.4	6.5	28.6	6.4	26.2	5.1
sd		6.3	2.6	7.9	1.9	5.0	2.8	5.5	2.1	5.0	2.3	4.6	1.4

Table S 18: Field capacity (measured as θ_{max}) and wilt point (measured as θ_{min}) measured per hydraulic year for Campbell Scientific 655 WVC probesets adjacent to Enviroscan profile probesets in treatment arrays. NA indicates no Enviroscan data for that year (*add back in the values for CS655 and plot them even if there are no Enviroscan probes working?*). Pink shading indicates lowest value and blue shading the highest measuring point values for the θ_{max} or θ_{min} values in that hydraulic year.

Appendices

hydyear	treatment	array	tree_ID	sensor	fld_cap (%)	%delta fldcap	wilt_pt (%)	%delta wiltpt	max_min (%)	%delta maxmin
2017	eCO2	A1	8437	CS655	23.65	59.8	5.84	45.7	17.81	64.4
2017	eCO2	A1	8437	ENV	9.51		3.17		6.34	
2017	aCO2	A2	8749	CS655	31.3	18.0	6.76	-73.2	24.54	43.1
2017	aCO2	A2	8749	ENV	25.68		11.71		13.97	
2017	aCO2	A3	9301	CS655	28.56	55.7	4.52	42.9	24.04	58.2
2017	aCO2	A3	9301	ENV	12.64		2.58		10.06	
2017	eCO2	A4	6632	CS655	26.91	81.6	4.85	63.9	22.06	85.4
2017	eCO2	A4	6632	ENV	4.96		1.75		3.21	
2017	aCO2	A5	6387	CS655	20.16	80.3	4.47	53.5	15.69	87.9
2017	aCO2	A5	6387	ENV	3.98		2.08		1.9	
2017	eCO2	A6	3752	CS655	34.13	62.0	8.83	-4.5	25.3	85.3
2017	eCO2	A6	5846	ENV	12.96		9.23		3.73	
2017	Ghost	A7	6021	CS655	39.62	96.8	11.81	97.3	27.81	96.6
2017	Ghost	A7	6027	ENV	1.27		0.32		0.95	
2017	Ghost	A9	7331	CS655	34.02	72.9	8	23.1	26.02	88.2
2017	Ghost	A9	7331	ENV	9.22		6.15		3.07	
2018	eCO2	A1	8437	CS655	19.8	41.5	3.3	15.2	16.5	46.7
2018	eCO2	A1	8437	ENV	11.59		2.8		8.79	
2018	aCO2	A2	8749	CS655	29.33	8.3	4.8	-10.0	24.53	11.8
2018	aCO2	A2	8749	ENV	26.91		5.28		21.63	
2018	aCO2	A3	9301	CS655	27.52	50.1	4.26	46.2	23.26	50.8
2018	aCO2	A3	9301	ENV	13.73		2.29		11.44	
2018	eCO2	A4	6632	CS655	22.4	81.8	3.27	44.6	19.13	88.2
2018	eCO2	A4	6632	ENV	4.07		1.81		2.26	
2018	aCO2	A5	6387	CS655	21.35	82.9	3	40.0	18.35	89.9
2018	aCO2	A5	6387	ENV	3.65		1.8		1.85	
2018	Ghost	A7	6021	CS655	41.4	73.9	5.07	98.2	36.33	-151.7
2018	Ghost	A7	6027	ENV	10.81		0.09		10.72	
2018	Ghost	A8	3642	CS655	35.8	4.8	8.82	-1.7	26.98	-23.5
2018	Ghost	A8	3642	ENV	34.07		8.97		25.1	
2018	Ghost	A9	7331	CS655	36.19	12.5	5.19	100.0	31	65.6
2018	Ghost	A9	7331	ENV	31.66		0		31.66	
2019	eCO2	A1	8437	CS655	18.43	42.5	7.53	21.0	10.9	-296.1
2019	eCO2	A1	8437	ENV	10.6		5.95		4.65	
2019	eCO2	A1	8467	CS655	29.26		10.84		18.42	41.6
2019	aCO2	A2	8749	CS655	24.96	3.7	8.72	-67.0	16.24	
2019	aCO2	A2	8749	ENV	24.04		14.56		9.48	

Appendices

hydyear	treatment	array	tree_ID	sensor	fld_cap (%)	%delta fldcap	wilt_pt (%)	%delta wiltpt	max_min (%)	%delta maxmin
2019	aCO2	A3	9301	CS655	24.03	46.7	5.29	32.3	18.74	-62.9
2019	aCO2	A3	9301	ENV	12.8		3.58		9.22	
2019	eCO2	A4	6632	CS655	21.64	79.2	6.62	56.0	15.02	-598.1
2019	eCO2	A4	6632	ENV	4.5		2.91		1.59	
2019	aCO2	A5	6387	CS655	16.9	74.7	5.8	56.4	11.1	-841.4
2019	aCO2	A5	6387	ENV	4.27		2.53		1.74	
2019	Ghost	A7	6021	CS655	29.39	63.3	13.01	88.0	16.38	-96.3
2019	Ghost	A7	6027	ENV	10.8		1.56		9.24	
2019	Ghost	A8	3642	CS655	29.51	-9.9	11.37	100.0	18.14	28.8
2019	Ghost	A8	3642	ENV	32.42		0		32.42	
2019	Ghost	A9	7331	CS655	30.59	2.5	7.51	100.0	23.08	44.2
2019	Ghost	A9	7331	ENV	29.83		0		29.83	
2020	eCO2	A1	8437	CS655	21.65	35.1	4.99	12.8	16.66	-105.9
2020	eCO2	A1	8437	ENV	14.06		4.35		9.71	
2020	aCO2	A3	9301	CS655	24.45	7.9	4.46	-20.2	19.99	-20.4
2020	aCO2	A3	9301	ENV	22.51		5.36		17.15	
2020	eCO2	A4	6632	CS655	26.79	71.5	6.14	40.1	20.65	-479.0
2020	eCO2	A4	6632	ENV	7.64		3.68		3.96	
2020	aCO2	A5	6387	CS655	28.33	74.8	5.4	40.2	22.93	-484.1
2020	aCO2	A5	6387	ENV	7.13		3.23		3.9	
2020	Ghost	A7	6021	CS655	32.77	51.2	9.99	93.1	22.78	-86.1
2020	Ghost	A7	6027	ENV	15.99		0.69		15.3	
2020	Ghost	A9	7331	CS655	36.59	13.6	8.11	-7.2	28.48	19.6
2020	Ghost	A9	7331	ENV	31.6		8.69		22.91	
2021	eCO2	A1	8437	CS655	22.78	39.5	4.35	-22.3	18.43	-159.6
2021	eCO2	A1	8437	ENV	13.78		5.32		8.46	
2021	aCO2	A3	9301	CS655	26.59	11.0	4.63	-1.3	21.96	-11.8
2021	aCO2	A3	9301	ENV	23.66		4.69		18.97	
2021	eCO2	A4	6632	CS655	26.64	42.2	5.44	-9.2	21.2	-122.0
2021	eCO2	A4	6632	ENV	15.39		5.94		9.45	
2021	aCO2	A5	6387	CS655	26.6	46.3	5.62	9.1	20.98	-145.4
2021	aCO2	A5	6387	ENV	14.28		5.11		9.17	
2021	Ghost	A7	6021	CS655	32.76	47.6	10.26	32.2	22.5	-179.2
2021	Ghost	A7	6027	ENV	17.15		6.96		10.19	
2021	Ghost	A9	7331	CS655	36.5	4.4	8.05	0.4	28.45	40.0
2021	Ghost	A9	7331	ENV	34.89		8.02		26.87	
2022	eCO2	A1	8437	CS655	19.61	26.3	3.5	4.3	16.11	-82.8

Appendices

hydyear	treatment	array	tree_ID	sensor	fld_cap (%)	%delta fldcap	wilt_pt (%)	%delta wiltpt	max_min (%)	%delta maxmin
2022	eCO2	A1	8437	ENV	14.46		3.35		11.11	
2022	aCO2	A2	8749	CS655	24.99	-7.0	4.68	-7.9	20.31	-1.6
2022	aCO2	A2	8749	ENV	26.75		5.05		21.7	
2022	aCO2	A3	9301	CS655	26.52	10.7	4.47	4.5	22.05	1.9
2022	aCO2	A3	9301	ENV	23.68		4.27		19.41	
2022	eCO2	A4	6632	CS655	23.06	27.5	4.01	-6.0	19.05	-60.6
2022	eCO2	A4	6632	ENV	16.73		4.25		12.48	
2022	aCO2	A5	6387	CS655	24.94	31.1	4.9	13.9	20.04	-87.5
2022	aCO2	A5	6387	ENV	17.19		4.22		12.97	
2022	Ghost	A8	3642	CS655	31.13	-2.9	6.81	17.5	24.32	2.7
2022	Ghost	A8	3642	ENV	32.03		5.62		26.41	
2022	Ghost	A9	7331	CS655	32.8	-1.6	7.1	10.4	25.7	100.0
2022	Ghost	A9	7331	ENV	33.31		6.36		26.95	
max						96.8		100.0		100
min						-9.9		-73.2		-841.4

Table S 19: Comparison of field capacity (θ_{max}), wilt point (θ_{min}) and their difference ASW, calculated from data of two probeset types CS and Enviroscan during hydraulic years 2017 to 2022 (further failures after that latter year). Adjacent columns show %age differences between the data values for CS and those for Enviroscan.

Independent soil moisture calibration methods (extract from Huang et al , 2016)

To check the accuracy of the TDR, the monitoring data could be calibrated by a comparison with gravimetric water content measurements between the saturated soil water content and the permanent wilting point. Gravimetric measurements obtained from conventional oven dry weights can be multiplied by bulk density data. The regression equation is as follows:

$$Y = 0.751X + 3.01 \quad R^2 = 0.953 \quad p > 0.05 \quad n = 9$$

where X is the soil moisture measured by TDR and Y is the soil moisture calculated using the oven-dry method multiplied by the soil bulk density (Zhu et al., 2014).

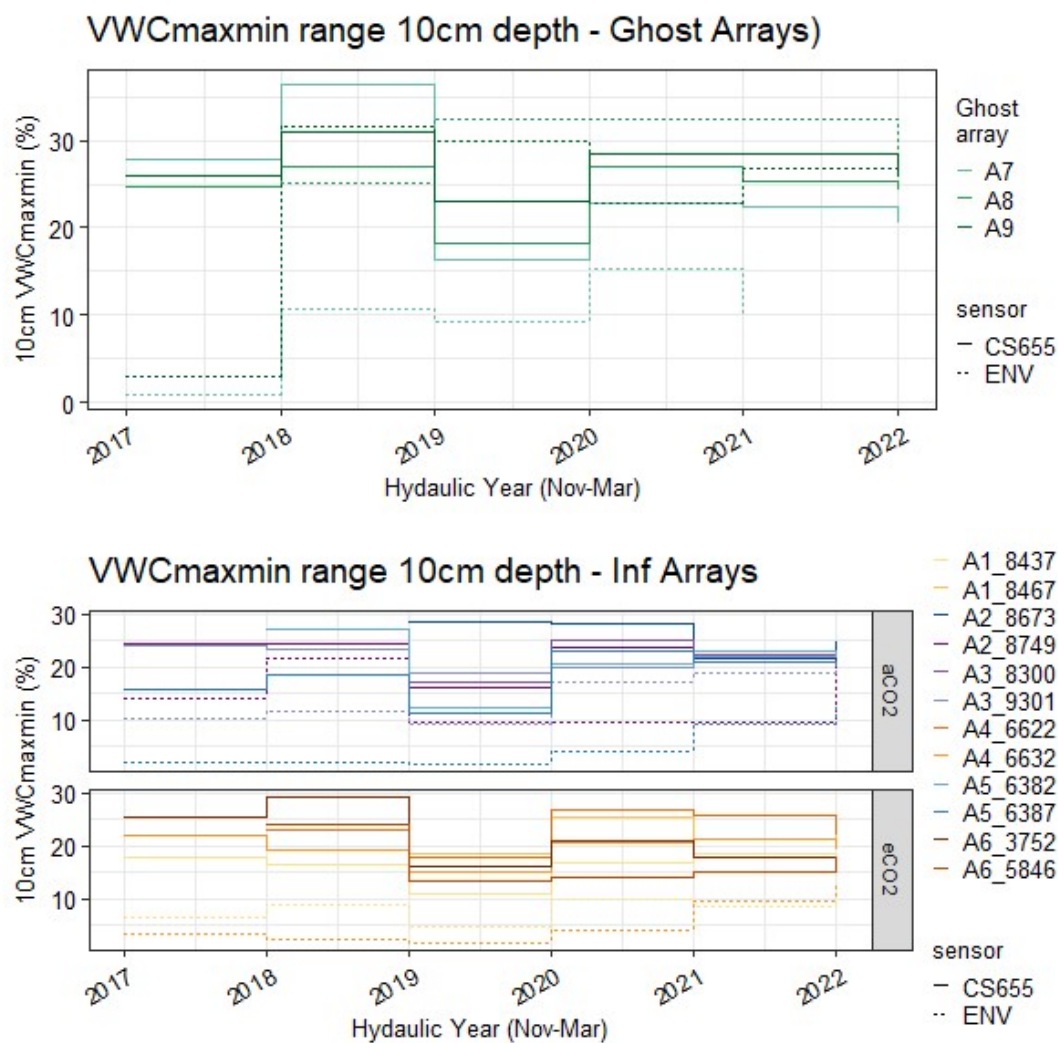


Figure S 18: Showing the maximum- minimum range of VWC for shallow soil moisture transducers CS655 (12 cm depth) and Enviroscan (10 cm depth -single Enviroscan transducer per array). a) shows the Ghost array data, b) shows the infrastructure array data (i.e. eCO₂ and aCO₂). The maximum value reflects the previous winter period and the minimum is the within-season value.

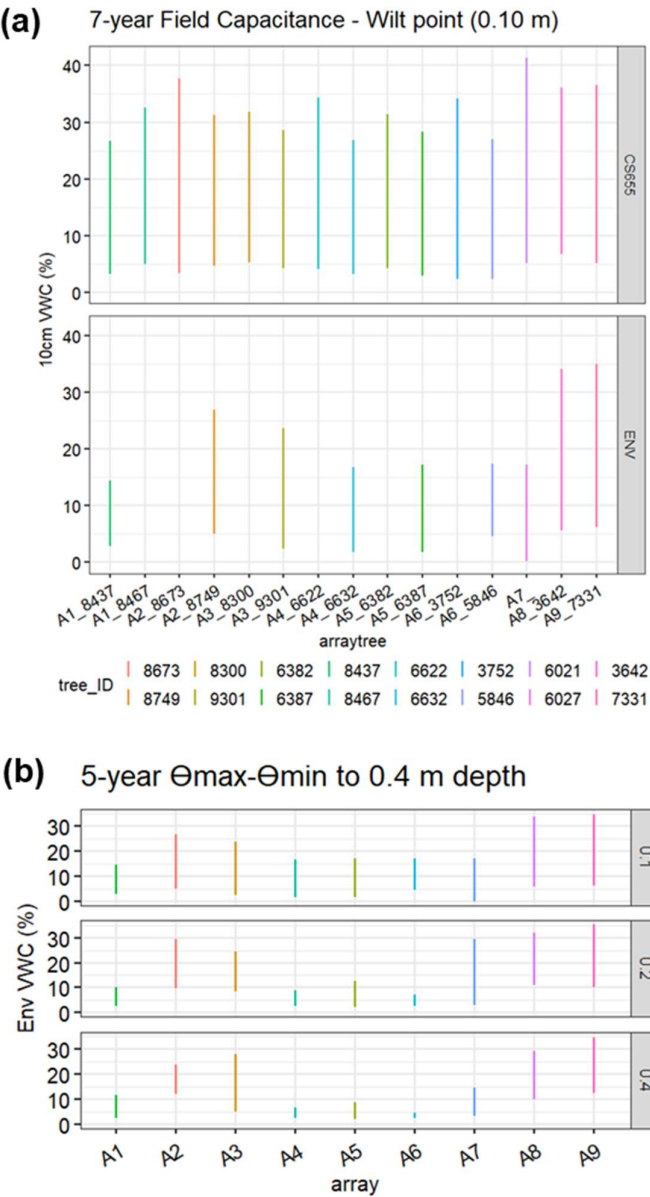


Figure S 19: Range of available soil water (ASW, %) from field capacitance (Θ_{max}) to permanent wilt point (Θ_{min}) (a) 7-year period max-min, comparing CS and Enviroscan from VWC data (b) 5-year period from Enviroscan VWC data.

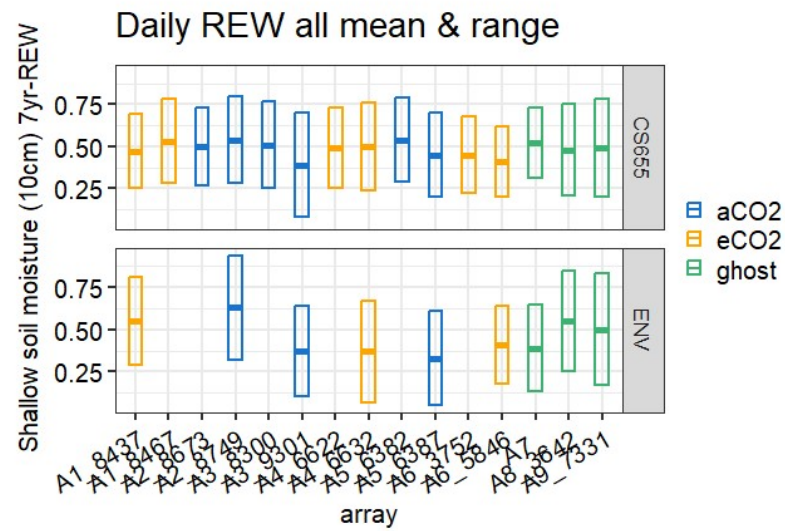


Figure S 20: To show range of REW_{7yr} (mean and 95%-iles) across the 6 years of the study 2018-2023 for each ~ 10 cm shallow soil-moisture position of CS sensor cluster (top) or Enviroscan probeset (bottom). Values of REW> 1 have been omitted. The gaps in the lower Enviroscan plot indicate positions where there were no probesets installed.

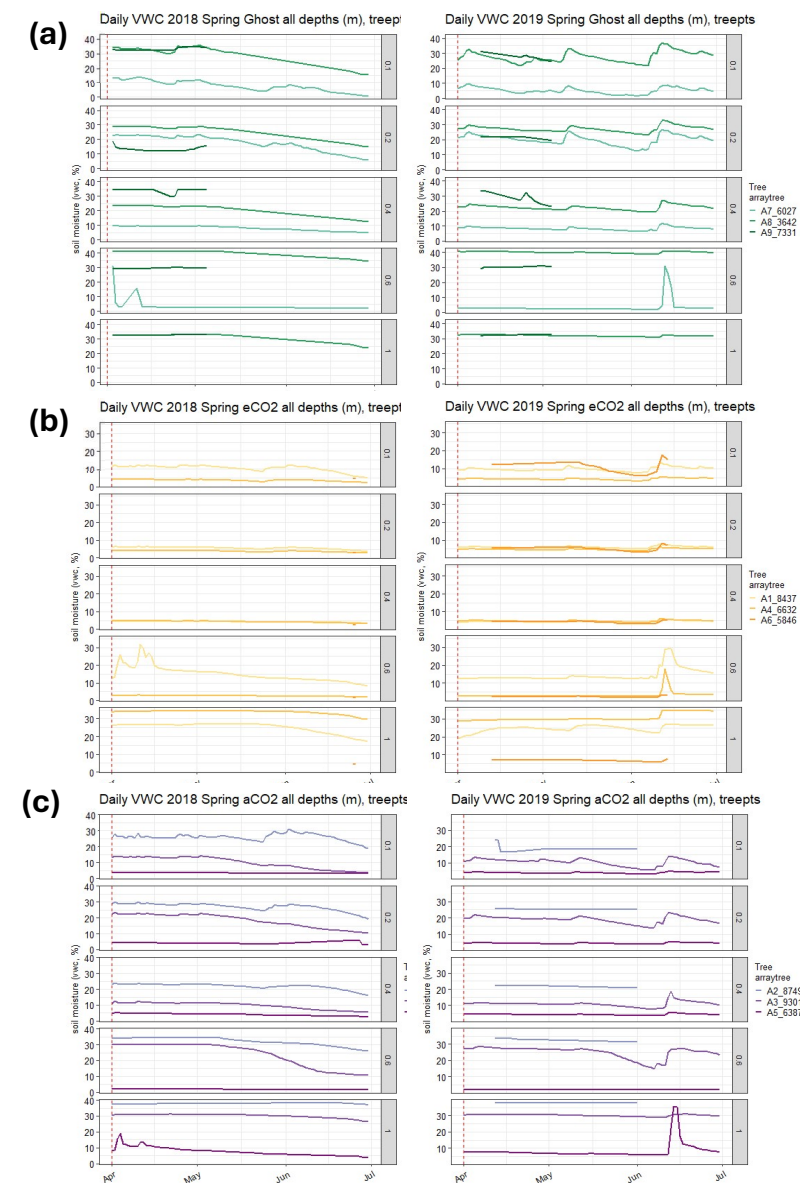


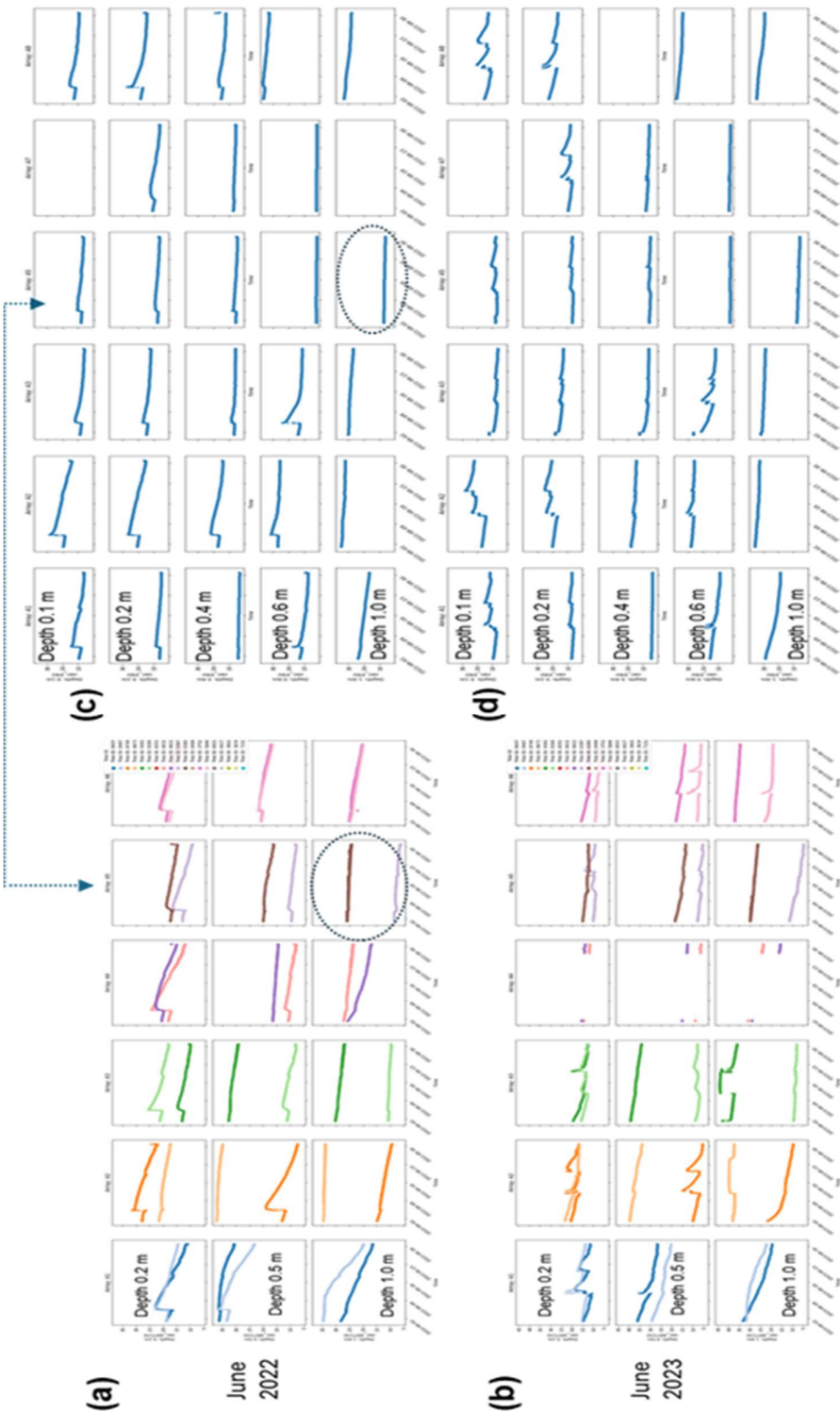
Figure S 21: Individual tree position Enviroscan profile VWC at all depths (i.e. 10cm, 20 cm, 40 cm, 60 cm, 100 cm). (a) *Ghost* Array data, (b) *eCO₂* Array data, (c) *aCO₂* Array data. This shows intermittent failures of some Enviroscan sensors during early 2018 and early 2019 seasons (i.e. April to June). Similar visualisation was carried out for other study years (2020-2023). See also Table 2 (main article) for reliable infrastructure sensors & Table S4 for sensor failures across each treatment season.

Appendices

	Treatment	2017	2018	2019	2020	2021	2022	2023
A1	eCO ₂	NF	OK	OK	OK	OK	OK	OK
A2	aCO ₂	OK	OK	OK	F	F	OK	OK
A3	aCO ₂	OK	OK	OK	OK	OK	OK	OK
A4	eCO ₂	NF	OK	OK	OK	OK	OK	F
A5	aCO ₂	OK	OK	OK	OK	OK	OK	OK
A6	eCO ₂	OK	F	F	F	F	F	F
A7	<i>Ghost</i>	OK	OK	OK	OK	OK	F	F
A8	<i>Ghost</i>	NF	OK	OK	F	F	OK	OK
A9	<i>Ghost</i>	OK	F	OK	OK	OK	OK	F

Table S 20: Enviroscan probe failures during the related seasons. OK indicates working probe, NF indicates probe not installed in that year, F indicates a failure of one or more depth positions (N.B. A7 probe had no data from 1 m depth due to installation problems).

Figure S 22: Comparison of infrastructure VWC profile probeset sub-daily data for June 2022 and June



2023 from a) Delta-T probes and b) Enviroscan probes. Colours do not indicate treatment on this figure. There are two sets of Delta-T data for each of the infrastructure arrays 1 to 6. (Note: *Ghost* data is only monitored by Enviroscan working probes so not shown.)

Appendices

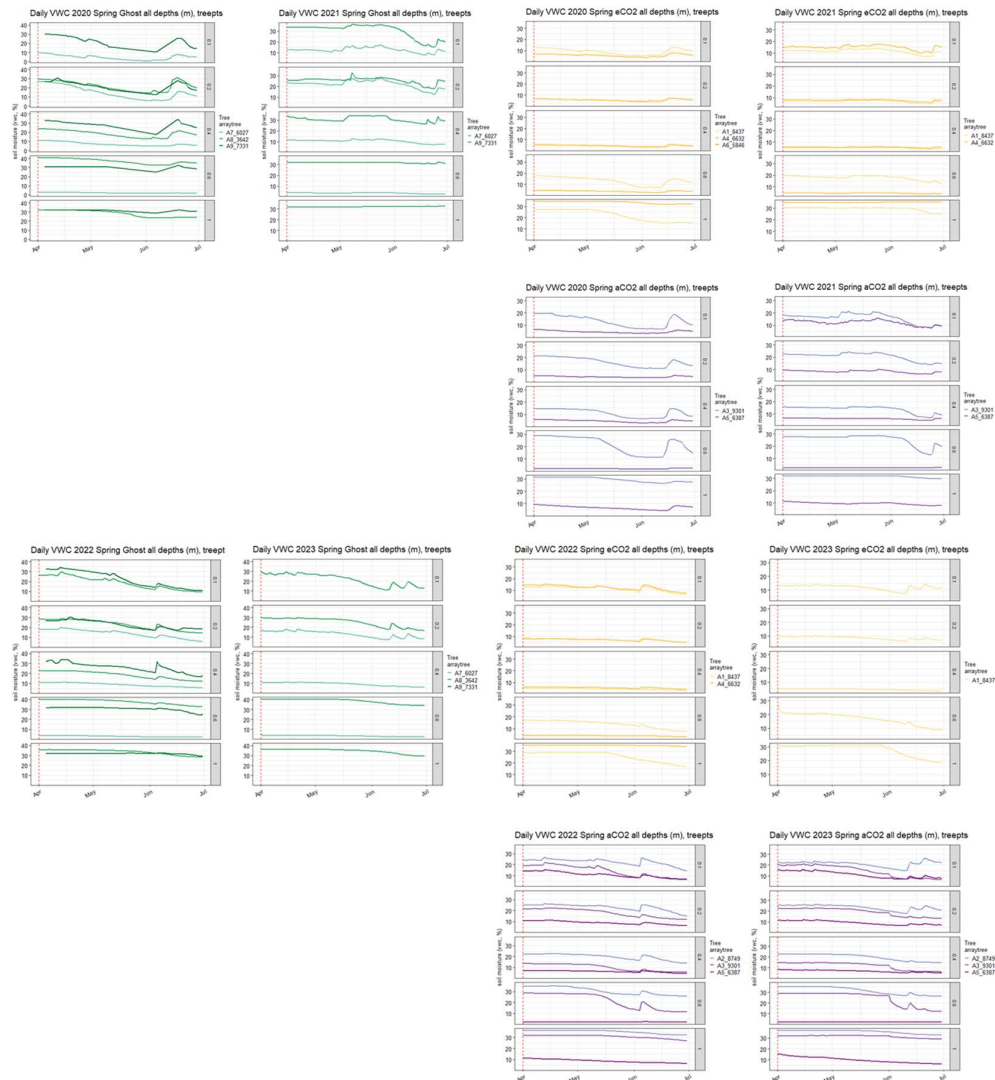


Figure S 23 : Showing detailed Enviroscan probe data for season 2020 to 2023 by array probeset. See Table S2 for failure details in each year.

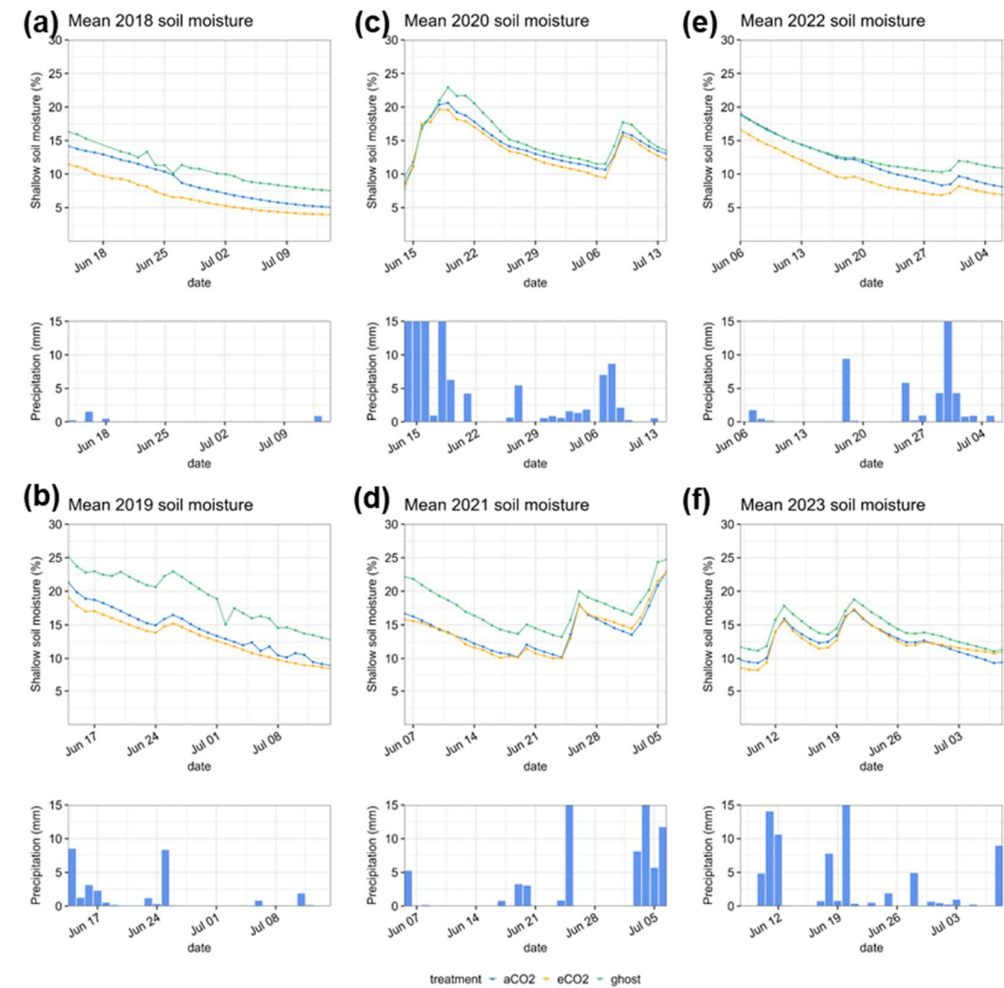


Figure S 24: Showing shallow (10-12cm) soil-moisture VWC (all treatments) and precipitation for drought periods (7+ consecutive days with < 2mm precipitation) for June to July in seasons 2 to 7 in season order ((a) season 2, 2018 to (f) season 7, 2023. Table S5 shows related data.

Appendices

Year	treatment	number days	start_doy	vwcm ^{max} %	end_doy	vwcm ^{min} %	midvw ^c %	pcpn rate mm	-ve slope % d-1
2018	aCO ₂	31	165	14.18	195	5.07	9.63	0.10	0.304
2018	eCO ₂	31	165	11.46	195	3.96	7.71	0.10	0.250
2018	ghost	31	165	16.28	195	7.53	11.91	0.10	0.292
2019	aCO ₂	19	177	16.47	195	8.95	12.71	0.16	0.418
2019	eCO ₂	19	177	15.17	195	8.47	11.82	0.16	0.372
2019	ghost	19	177	22.94	195	12.80	17.87	0.16	0.563
2020	aCO ₂	9	180	13.49	188	10.86	12.18	0.76	0.329
2020	eCO ₂	9	180	12.79	188	9.69	11.24	0.76	0.387
2020	ghost	9	180	14.32	188	11.51	12.92	0.76	0.351
2021	aCO ₂	12	158	16.26	169	10.60	13.43	0.08	0.515
2021	eCO ₂	12	158	15.56	169	10.34	12.95	0.08	0.475
2021	ghost	12	158	21.86	169	13.94	17.90	0.08	0.720
2022	aCO ₂	12	157	19.03	168	12.44	15.74	0.21	0.599
2022	eCO ₂	12	157	16.62	168	9.63	13.13	0.21	0.635
2022	ghost	12	157	18.74	168	12.70	15.72	0.21	0.549
2023	aCO ₂	7	172	17.19	178	12.40	14.80	0.39	0.798
2023	eCO ₂	7	172	17.35	178	11.92	14.64	0.39	0.905
2023	ghost	7	172	18.79	178	13.77	16.28	0.39	0.837

Table S 21: VWC ranges, slopes and maximum number of days of drought during June to July in seasons 2 to 7 (2018 to 2023). Day of year (doy) is indicated.

Horizon / depth	aCO₂ (% of total)	eCO₂ (% of total)	eCO₂/aCO₂ %
O	41 (33%)	59 (34%)	+44.7
A	25.5 (20%)	41 (23%)	+58.4
B	24 (19%)	33 (19%)	+38.1
30-50cm	20 (16%)	29 (16%)	+44.8
50-70cm	9 (7%)	9 (5%)	+1.1
>70cm	5.6 (5%)	6 (3%)	-7.8
Total (g m⁻²)	125.5	177	40.5 %

Table S 22: To show proportions of fine root (<1mm dia.) biomass (g m⁻²) from 1m soil cores. (Handy et al. in prep).

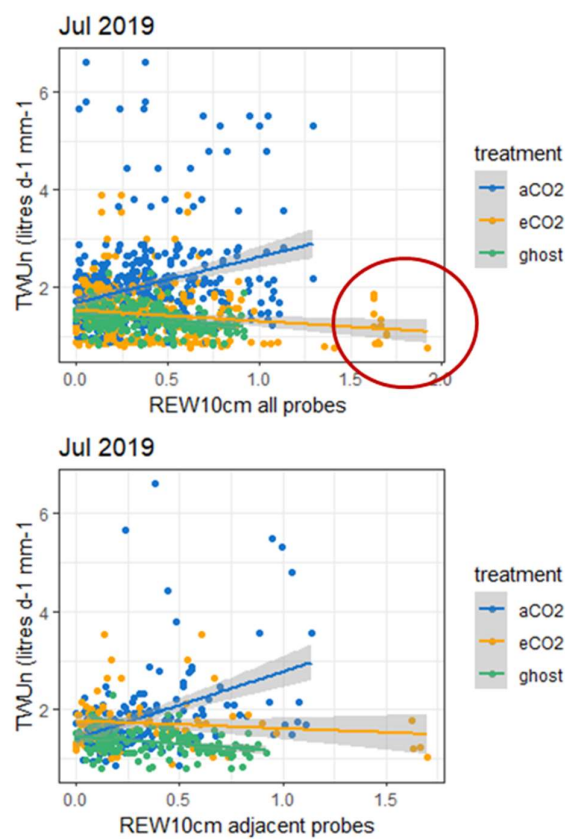


Figure S 25: Correlative scatterplots REW10cm against TWUn – first dataset for 2019. Top figures are all shallow probes (CS and Enviroscan). bottom are restricted to those closest to the relevant sap flow monitoring tree. (suppressed y-axis circa 0.7 l d⁻¹ mm⁻¹)

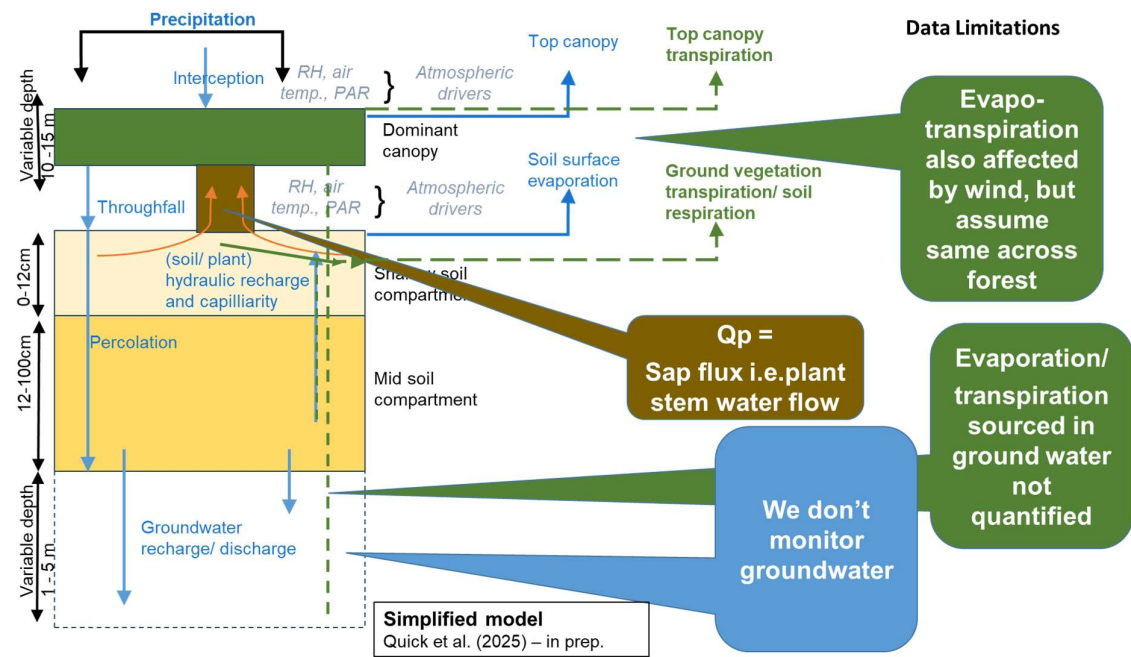


Figure S 26: Process model example

Appendix 5: Chapter 6 Supplementary Material

Chapter 6 supplement.

Leaf and stem: transpiration dynamics of dominant oak and sub-dominant trees at BIFoR FACE experiment.

Methods and Materials

Table S 23: List of tree numbers showing array number, treatment, species and parameters measured in each. Stomatal conductance and leaf temperature were measured for all CAS, in-situ and green-leaf cut samples.

Tree-label	array	Treatment	Species	sap flux	CAS top	CAS years	In-situ bottom	In-situ bottom yrs.	Green-leaf cut	Green-leaf yrs.	Notes
7331	9	Ghost	oak	Y	N	NA			Y	2019-2021	-
7476	9	Ghost	oak	Y	N	NA			N	NA	
602-1	7	Ghost	oak	Y	N	NA			Y	2019-2021	-
6027	7	Ghost	oak	Y	N	NA			N	NA	
3634	8	Ghost	oak	Y	N	NA			N	NA	
3642	8	Ghost	oak	Y	N	NA			Y	2019-2021	-
8467	1	eCO ₂	oak	Y	N	NA			N	NA	
8468	1	eCO ₂	oak	Y	Y	2019-2021			N	NA	
8351	3	eCO ₂	oak	Y	N	NA			N	NA	
9301	3	eCO ₂	oak	Y	Y	2019-2021			Y	2020-2021	-
5846	6	eCO ₂	oak	Y	Y	2020-2021			Y	NA	
3755	6	eCO ₂	oak	Y	N	NA			N	NA	
6382	5	aCO ₂	oak	Y	N	NA			Y	2020-2021	-
6406	5	aCO ₂	oak	Y	Y	2019-2021			N	NA	
8673	2	aCO ₂	oak	Y	Y	2019-2021			Y	2020-2021	-
8749	2	aCO ₂	oak	Y	N	NA			N	NA	
6621	4	aCO ₂	oak	Y	Y	2019-2021			Y note 1	2020-2021	-
6632	4	aCO ₂	oak	Y	N	NA			Y note 1	2020-2021	-
8437	1	eCO ₂	oak	N	N	NA			Y	2020-2021	-
8446	1	eCO ₂	oak	N	N	NA			Y	2020-2021	-
3686	6	eCO ₂	oak	N	Y	2019-2021			N	NA	
5848	6	eCO ₂	sycamore	Y	Y	2020-2021	Y	2020-2021			
6026	7	Ghost	sycamore	Y	N	NA	Y	2020-2021			
998-2	4	eCO ₂	hawthorn	Y	N	NA	Y	2020-2021			
7458	9	Ghost	sycamore	F	N	NA	Y	2020			F failed

Appendices

Tree-label	array	Treatment	Species	sap flux	CAS top	CAS years	In-situ bottom	In-situ bottom yrs.	Green-leaf cut	Green-leaf yrs.	Notes
											install-ation
7482	9	Ghost	sycamore	Y	N	NA	Y	2020-2021			
6381	5	aCO ₂	sycamore	Y			Y	2021			
6638	4	eCO ₂	sycamore	Y			Y	2021			
9378	1	eCO ₂	sycamore	Y (F)			Y	2021			F intermittent fault
5847	6	eCO ₂	sycamore	Y			Y	2021			
6385	5	aCO ₂	hawthorn	Y			Y	2021			
6386	5	aCO ₂	hawthorn	Y			Y	2021			
8435A	1	eCO ₂	hawthorn	Y			Y	2021			
8435B	1	eCO ₂	hawthorn	Y			Y	2021			
8352	3	aCO ₂	hawthorn	Y			Y	2021			
8350	3	aCO ₂	hawthorn	Y			Y	2021			

Appendices

Table S 24: Structural traits of subdominant stems selected for sap flow and leaf stomatal conductance monitoring. Stem radius (R_b , m) at installation and in 2022 are shown. Projected canopy area (A_c , m²) and tree height (H , m) are shown measured by clinometer at various dates 2020-2022.

Tree ID	Array No.	Treatment	Species	Installation date	Probe type	Initial Circumference (m)	Initial Av. R_b at probeset (m)	2022 Circumference (m)	2022 Average R_b at probeset (m)	Projected canopy Area A_c (m ²)	Tree height TH (m)
8435A	1	eCO ₂	hawthorn	21/10/202	short	0.372	0.059	0.364	0.058	12.1	10.7
8435B	1	eCO ₂	hawthorn	22/10/202	short	0.368	0.059	0.369	0.059	31.8	13.6
9378	1	eCO ₂	sycamore	12/05/202	short	0.325	0.052	0.358	0.057	22.3	11.3
8350	3	aCO ₂	hawthorn	14/04/202	short	0.345	0.055	0.342	0.054	22.4	9.0
8352	3	aCO ₂	hawthorn	22/04/202	short	0.56	0.089	0.57	0.091	39.7	12.0
9982	4	eCO ₂	hawthorn	18/03/202	long	0.54	0.086	0.538	0.086	20.5	9.0
6638	4	eCO ₂	sycamore	03/11/202	short	0.65	0.103	0.678	0.108	24.6	13.9
6385	5	aCO ₂	hawthorn	24/09/202	short	0.528	0.084	0.548	0.087	40.6	10.4
6386	5	aCO ₂	hawthorn	24/09/202	short	0.5	0.080	0.504	0.080	35.7	9.6
6381	5	aCO ₂	sycamore	27/10/202	short	0.43	0.068	0.46	0.073	19.5	8.4
5848	6	eCO ₂	sycamore	31/05/201	long	1.17	0.186	1.19	0.189	120.9	16.7
5847	6	eCO ₂	sycamore	12/05/202	short	0.685	0.109	0.68	0.108	38.4	13.6
6026	7	none/ Ghost	sycamore	18/03/202	long	0.442	0.070	0.463	0.074	47.4	12.4

Appendices

Tree ID	Array No.	Treatment	Species	Installation date	Probe type	Initial Circumference (m)	Initial Av. R_b at probes et (m)	2022 Circumference (m)	2022 Average R_b at probes et (m)	Projected canopy Area A_c (m ²)	Tree height TH (m)
7482	9	none/ <i>Ghost</i>	sycamore	09/07/2019	long	0.57	0.091	0.578	0.092	74.6	12.8

Table S 25: Instrumentation types and sampling details for environmental variables.

Description	Make	Model	Position	Parameter	Symbol	Where	Units	Sampling rate	Logger rate
FACE tower	Hukseflux pyrano-meter	LP02-03	Top canopy (c25 m)	Total Solar Radiation (Av)	$TG(q_s)$	A 1-6 (mean)	Watt m ⁻²	10 secs	Every hour
	Campbell Scientific	T107	Top canopy FACE (c20 m)	Air Temp (Av)	T_c	A 1-6 (mean)	°C	10 secs	Every hour
Met Tower	Kipp & Zonen (Delft; The Netherlands)	NR Lite 2	~23 m	Net Radiation	R_n	4 x Met Towers (mean)	Watt m ⁻²		30 minutes
	LI-COR Biosciences (Lincoln; USA)	LI-190R Quantum Sensor	~23 m	Photo-synthetically Active Radiation	PAR	4 x Met Towers (mean)	μmol of photons m ⁻² s ⁻¹		30 minutes
	Vaisala	HMP155RH + DTR503	~25 m (top) & 1.4 m (bottom) above ground	Relative Humidity/ Temperature	RH (RH_{top} & RH_{bot}) and T_{air} (T_{atop} & T_{abot})	4 x Met Towers (mean)	% and °C		30 minutes
	Vaisala (Helsinki; Finland)	PTB210 by	~25 m (top)	Barometric/ (ambient, top) pressure	p	4 x Met Towers (mean)	hPa		30 minutes

Appendices

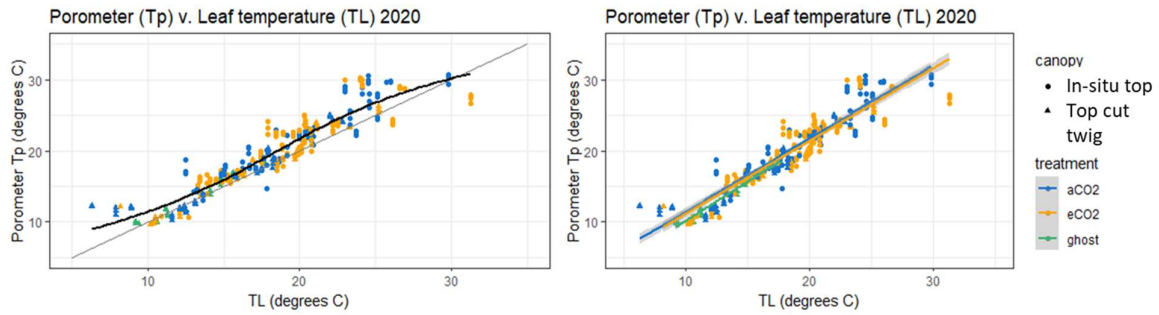


Figure S 27: Comparison of oak porometer leaf temperature T_p ($^{\circ}\text{C}$) and IR thermometer readings during 2020). Straight grey line is 1:1 ratio. (a) regression curve for all IR thermometer readings (first of each set on each leaf measured) showing non-linear relationships across the leaf temperature range and (b) linear regression lines coloured by treatment. Pale grey line is 1:1 relationship. Y-axis zero is suppressed.

Figure S1 shows IR leaf temperature (T_L) data from 2020 and 2021. The calculated IR leaf temperature for 2019 used a model based on the expected IR leaf temperature (porometer leaf temperature T_p plus a constant) for temperatures between 10 and 25 degrees C. Comparison of the two sources of leaf temperature, showing non-linearity at low and high temperatures, is illustrated in Fig. S1(a) for 2020, with a consistent effect across all treatments (Fig. S1(b)).

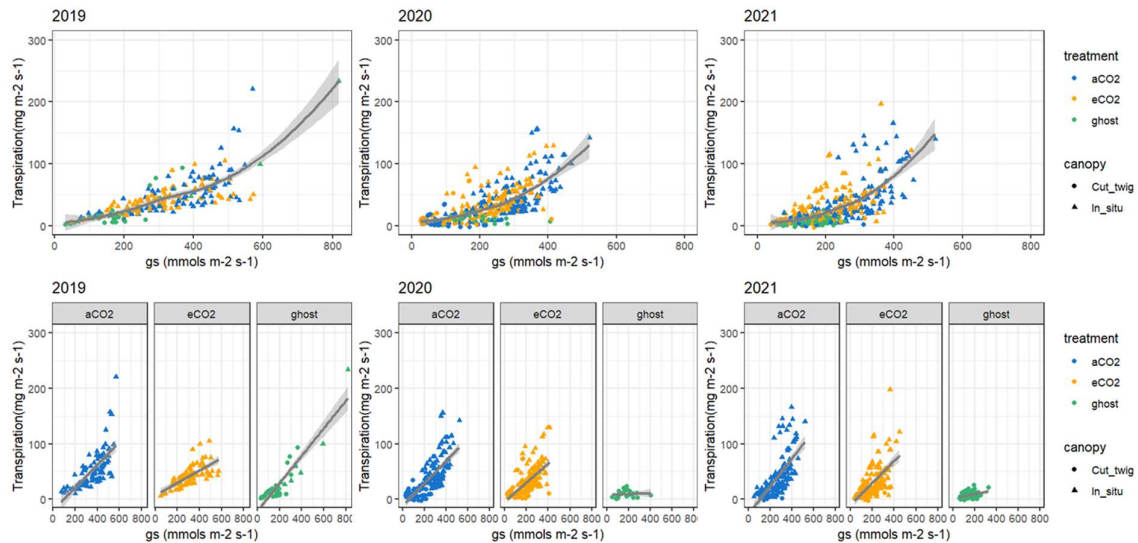


Figure S 28: Visualisations of oak leaf transpiration versus stomatal conductance following calculations. All data are show by year (top row) and then separated into treatment facets (bottom row).

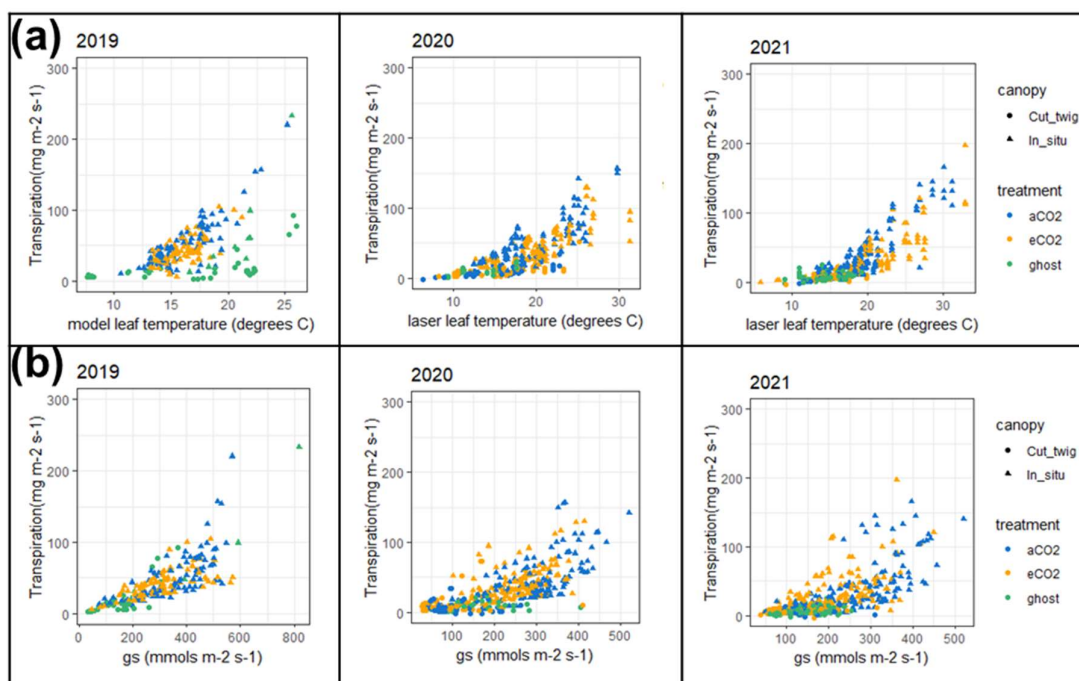


Figure S 29: Oak correlative scatter plots. (a) transpiration versus IR laser leaf temperature 2019, 2020 and 2021. Note 2019 model leaf temperature (Section 2.6.1) derived from porometer readings and adjusted to match IR readings in other years (see methods text). (b) transpiration versus stomatal conductance 2019, 2020, 2021.

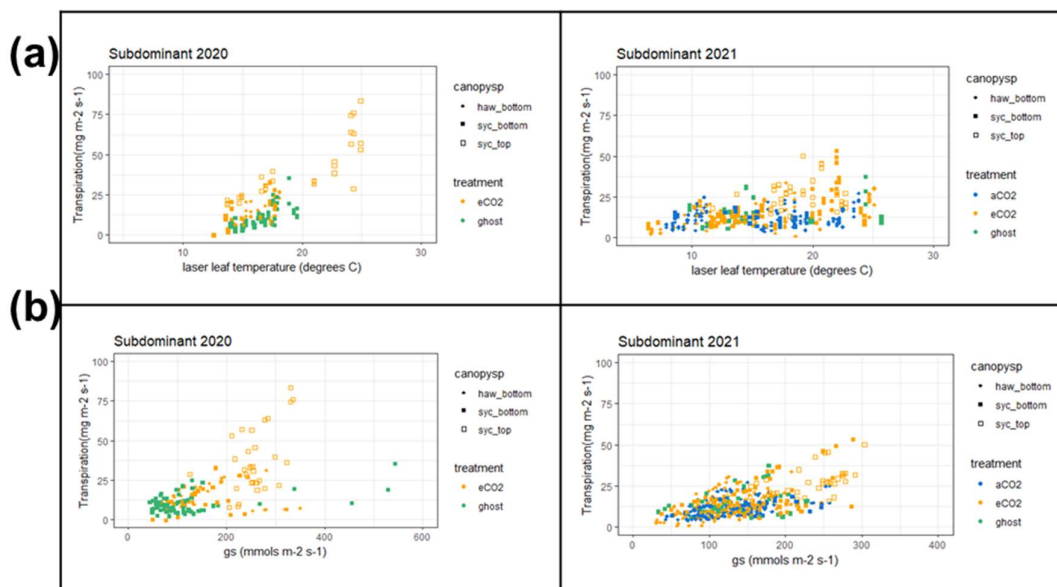


Figure S 30: Subdominant correlative scatter plots of (a), leaf transpiration versus laser leaf temperature 2020 and 2021. (b), transpiration versus stomatal conductance 2020, 2021 respectively.

Summary of tests

1. Levene tests

model_CO2 for eCO2 compared with aCO2 leaf transpiration results

Tests both for seasonal 2019 & 2020 are significant. For July-Aug data 2019 and 2021 are significant. For 2021 season and July-Aug 2020 the test is not significant meaning ANOVA model could be validated.

Table S 26: Leaf transpiration Levene's test median CO2 effect (eCO2 compared with aCO2). Bold results (mid-season 2020 and whole-season 2021) are non-significant exhibiting homogeneity of variances.

	2019	2019	2020	2020	2021	2021
Season	F(1, 170)	Pr(>F)	F(1, 352) =	Pr(>F)	F(1,299) =	Pr(>F)
median	11.5	***	5.37	*	2.1253	0.1459
July-Aug	F(1, 40) =	Pr(>F)	F(1, 165) =	Pr(>F)	F(1,151) =	Pr(>F)
median	9.407	**	0.397	0.5296	5.5851	*

Comparing with whole tree transpiration results (Table S5) have similar tendencies except for whole season 2021. This may be explained by the restriction of in-situ leaf results to the middle of the day when homogeneity is better.

model_CO2 for eCO2 compared with aCO2- sap flux results

All tests both for seasonal 2019 and 2021 are significant; also for July data in 2019. For 2020 season and July 2020, 2021 the test is not significant meaning ANOVA model can be validated.

Table S 27: Sap flux based Levene's test mean and median CO2 effect eCO2 compared with aCO2. Bold results (2020 and 2021) are non-significant exhibiting homogeneity of variances. (extract from Quick et al. (2025) supplementary material).

	2019	2019	2020	2020	2021	2021
Season	F(1, 2065) =	Pr(>F)	F(1, 2491) =	Pr(>F)	F(1,2167) =	Pr(>F)
mean	58.697	***	0.474	0.4912	40.141	***
median	39.179	***	0.0332	0.8555	13.69	***
July	F(1, 300) =	Pr(>F)	F(1, 367) =	Pr(>F)	F(1,304) =	Pr(>F)
mean	11.07	***	0.7235	0.3956	5.3071	0.02191 *
median	7.7959	**	0.003	0.9563	0.8713	0.3513

2. Shapiro-Wilk normality test summary

Table S 28: Shapiro-Wilk normality test results for whole season and July-August only for all oak leaf transpiration data.

	2019	2019	2020	2020	2021	2021
Season	W =	P =	W =	P =	W =	P =
transpiration	0.837	***	0.873	***	0.792	***
stomatal conductance	0.985	0.066 .	0.979	***	0.981	***
July-Aug	W =	P =	W =	P =	W =	P =
transpiration	0.789	***	0.854	***	0.851	***
stomatal conductance	0.971	0.366	0.991	0.3728	0.982	0.0452 *

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

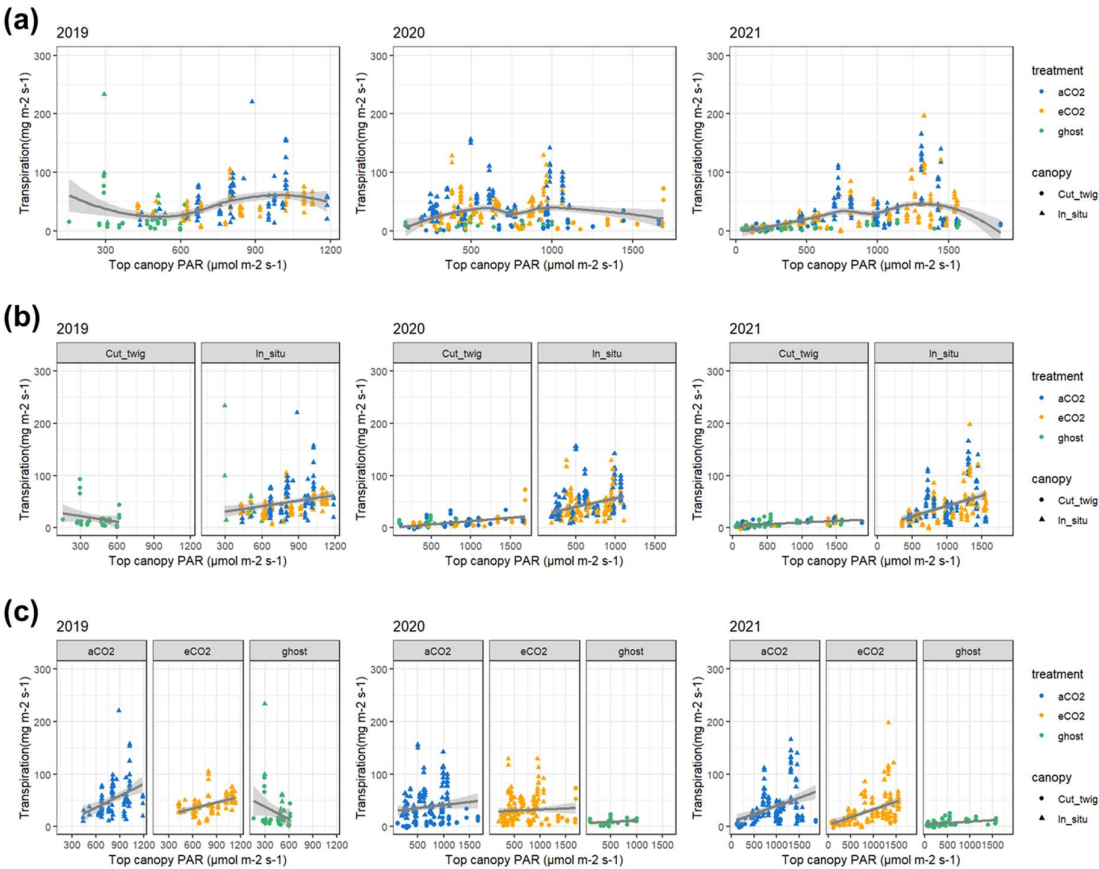


Figure S 31: Correlative graphics of all oak leaf transpiration against top-canopy PAR. Top row (a) faceted by season 2019, 2020, 2021 with modelled loess curves. Middle row (b) each season's data faceted by canopy measurement type in-situ and cut-twig. Bottom row (c) each season's data faceted by treatment type aCO₂ , eCO₂ , ghost.

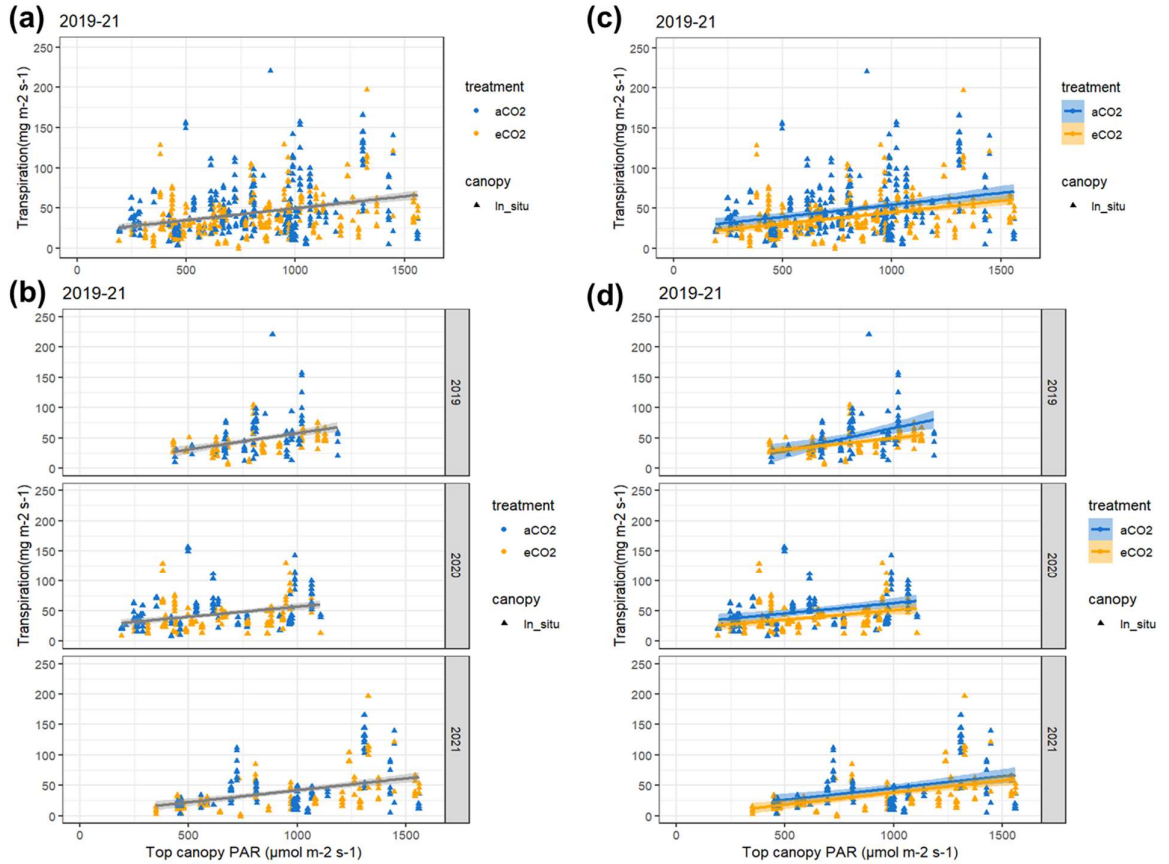


Figure S 32: Correlative graphics of infrastructure in-situ measured oak leaf transpiration against top-canopy PAR. (a) seasons 2019 to 2021 with combined linear modelling (black line). (b) faceted season's data each with with combined linear modelling (black line). (c) seasons 2019 to 2021 with separated linear modelling by treatment colours aCO₂ , eCO₂. (d) faceted season's data each with linear modelling by treatment colours aCO₂ , eCO₂.

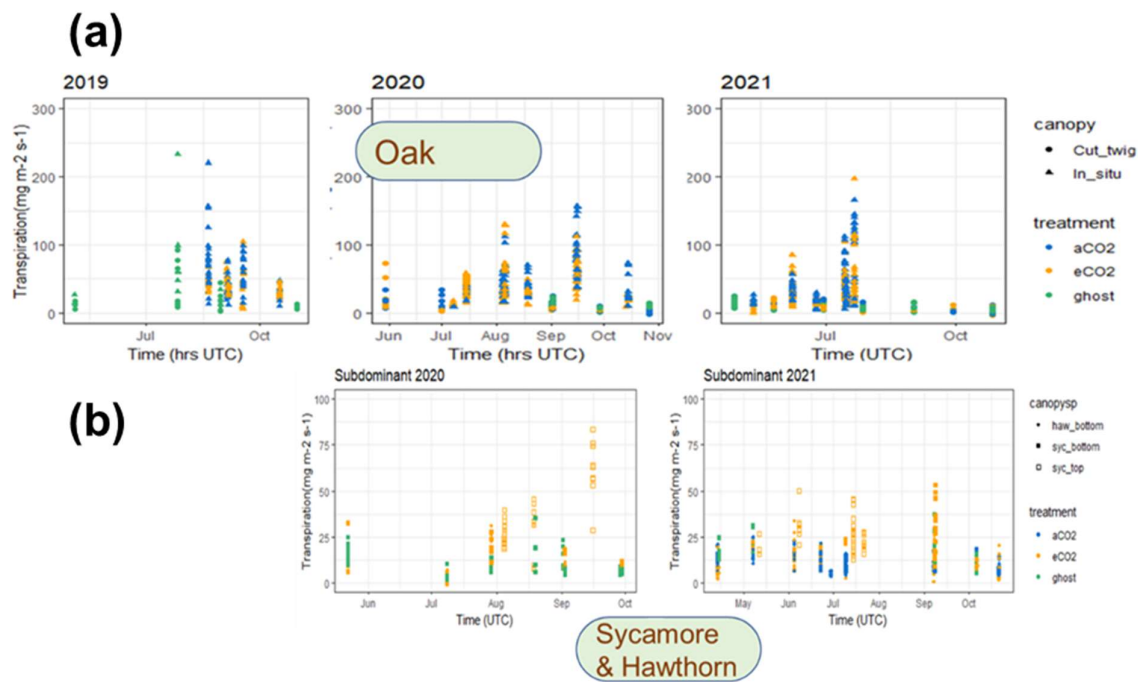


Figure S 33: Leaf transpiration for days on which leaf data were collected in 2019 – 2021 a) for oaks and b) for subdominant trees. Note differing y axis scales for subdominants compared with oak graphs.