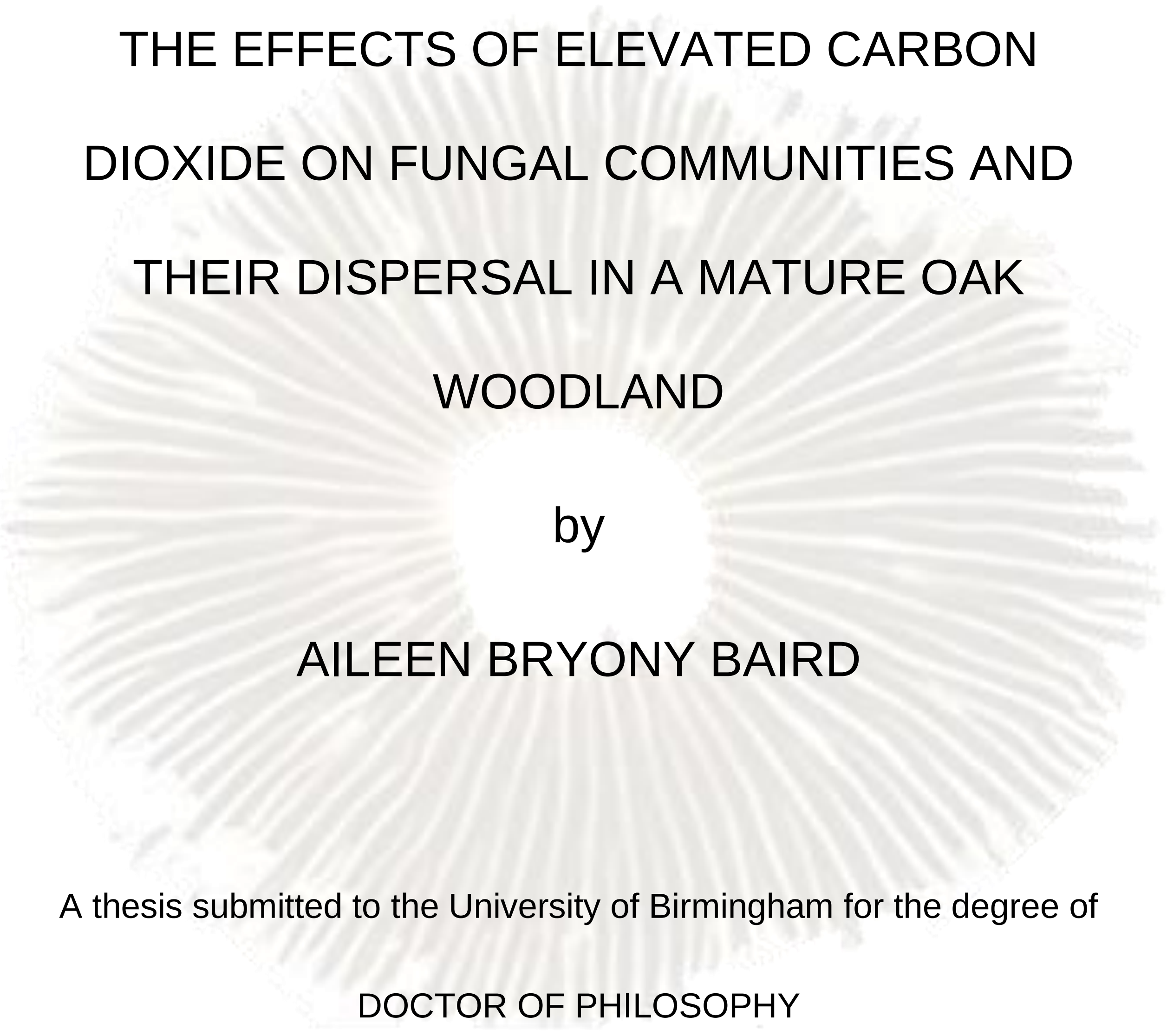

THE EFFECTS OF ELEVATED CARBON DIOXIDE ON FUNGAL COMMUNITIES AND THEIR DISPERSAL IN A MATURE OAK WOODLAND

by

AILEEN BRYONY BAIRD

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College of Life and Environmental Sciences

University of Birmingham

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ABSTRACT

Forests are a key habitat in our understanding of how the climate and biodiversity crises will affect our planet. Often overlooked in climate models, fungi play critical roles in carbon and nitrogen cycling in forests and are a vital part of woodland biodiversity. Although there is a considerable body of research using long time series to investigate the impacts of climate change on fungal fruiting in real time, and extensive laboratory study; there have been a limited number of climate manipulation facilities incorporating fungal studies into their research programmes.

To investigate the effects of elevated CO_2 on forest fungal communities, a variety of complementary studies were undertaken at the Birmingham Institute of Forest Research (BIFoR) Free Air Carbon dioxide Enrichment (FACE) facility from 2017 to 2021. The FACE facility is located within a mature deciduous oak (*Quercus robur*) woodland in Staffordshire, UK, where areas of the woodland are treated with +150ppm CO_2 during the oak growing season. A total of six studies were completed, studying the impacts of elevated CO_2 (eCO_2) on fungal communities at different stages of the fungal life cycle. Chapters 3 and 4 investigated airborne spore concentrations and their dispersal, Chapter 5 fungal fruiting patterns, and Chapters 6, 7, and 8 soil fungal communities.

Optical Particle Sensors (OPCs) were used to investigate the impacts of eCO_2 on bioaerosols in Chapters 3 and 4. There were stark differences in bioaerosol patterns between the 2018 and 2019 measurement campaigns, with differences in how eCO_2 appeared to affect bioaerosol concentrations. Relative humidity did not affect airborne bioaerosol concentrations, however other meteorological conditions such as wind speed and temperature did alter bioaerosol levels.

Using fungal fruiting surveys (Chapter 5), neither the number of fungal species fruiting or the weight of sporocarps were significantly affected by eCO_2 across the two-year survey campaign. Saprotrophic species were most commonly found fruiting, however some ectomycorrhizal species were also common, with significant overlap in the fungal communities fruiting in eCO_2 and aCO_2 arrays.

In soil DNA barcoding studies (Chapters 6 – 8), soil horizon was shown to have a significant effect on fungal community composition, however there were no significant differences in communities between seasons. Elevated CO_2 consistently affected fungal community composition, however in the early years of treatment there were no effects of eCO_2 on community richness or relative abundances of fungal phyla or functional groups. After four years of eCO_2 treatment, there were some shifts in fungal relative abundance, for example, relative abundance of ectomycorrhizal fungi decreased in eCO_2 treatment arrays.

These results demonstrate that elevated CO_2 can have wide-ranging and diverse effects on fungal communities, and these effects are often not consistent between species, functional groups, seasons, and years. There has been a lack of fungal studies in FACE experiments worldwide, and several of the studies reported within this thesis are the first of their type in a forest FACE experiment. They provide a valuable contribution to our understanding of the effects of climate change on fungi in forest systems, and provide evidence for the importance of fungi in policy around woodland creation and the protection of existing woodlands.

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With thanks to my family - to Hester, my Dad, and in memory of my brilliant Mum. To James – for being eternally patient, caring, and encouraging (and not objecting when I wanted to store fungi and soil samples in our freezer…)

Finally, to all the members of the mycological community who have welcomed me with open arms - Thank you all!

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PUBLICATIONS

Chapter 1 of this thesis was published in January 2022 titled *" 'Can't see the wood from the trees' : the importance of fungi in the context of UK tree planting"* (Baird and Pope, 2022). The authorship of the paper was as follows: Aileen B. Baird and Francis D. Pope. ABB and FDP created the concept of the paper. ABB wrote the paper. Both authors discussed the structure and writing of the manuscript and contributed to edits.

Chapter 3 was published as a pre-print in Biogeosciences in July 2021 (Baird et al., 2021) titled *"Mass concentration measurements of autumn bioaerosol using low cost sensors in a mature temperate woodland Free Air Carbon Dioxide Enrichment (FACE) experiment: investigating the role of meterology and carbon dioxide levels"*. The paper underwent open peer review, but at the time of this submission of this thesis, no further decisions had been received. The final version of the manuscript was published in May 2022. As described in the manuscript, the authorship of the paper was as follows: Aileen B. Baird, Edward J. Bannister, A. Robert MacKenzie, Francis D. Pope. ABB and FDP designed the bioaerosol study as part of the FACE programme designed by ARMK. ABB collected and analysed the OPC data and prepared the manuscript. EJB wrote the model. All co-authors discussed the results and contributed to writing the manuscript.

CHAPTER1: THE IMPORTANCE OF FUNGI IN THE CONTEXT OF UK TREE PLANTING

1.1 Introduction

Climate change is the largest challenge facing the UK in the next 100 years. In the UK, the top ten warmest years have occurred since 1990, with 2008–2017 being on average 0.8°C warmer, and having 5% more rainfall, compared with the 1961–1990 baseline period (Met Office, 2019). The longest running temperature record in the UK indicates that current temperatures are on average 1°C warmer than pre-industrialisation (Met Office, 2019). A number of different climate projections are available for the UK depending on the extent of mitigation strategies achieved; the high emission scenario RCP8.5 predicts that summer temperatures will increase by 0.9°C–5.4°C by 2070 (Met Office, 2019).

In accordance with the 2015 Paris Agreement, and the target to keep global temperature increases below 2°C, the UK has a range of climate mitigation strategies, both to decrease carbon emissions by 68% by 2030 (compared with 1990 levels), but also to increase the UK carbon sink (Department for Business, Energy & Industrial Strategy, 2020). One of the measures set by the UK government to increase the carbon sink is to significantly increase forest cover from the current 13%, to 17–20% by 2050 (The Climate Change Committee, 2020). At present, the UK has a low percentage forest cover, at only 13%, compared with the average of 38% across Europe (*FAO Global Forest Resources Assessment*, 2015). Although the primary goal of this large tree planting scheme is to increase the national carbon sink, variables such as location and tree species amongst many others, will have significant impacts on how much carbon sequestration is achieved, in addition to the wider environmental and biodiversity impacts that a tree planting strategy on this scale could cause. It is therefore essential to consider, in-depth, the wider environmental contexts and consequences of this tree planting scheme.

The aim of climate mitigation tree planting initiatives, such as the UK strategy, is to increase the forest carbon sink by increasing the percentage of land under forest cover. The global net forest sink between 2001 and 2019 was estimated at -7.6 ± 49 $GtCO_2e$ per year, around 47% of which was in temperate woodlands, including those in the UK (Harris et al., 2021). Although a large proportion of this carbon sink is made up of tree biomass, an estimated 35% of this global forest carbon sink is made up of the deadwood, litter, soil and harvested wood (Pan et al., 2011).

Fungi are involved in almost all of the carbon stores in forests, but particularly in deadwood, litter, and soil, where saprotrophic fungi control decomposition of dead plant matter, and mycorrhizal fungal biomass store significant proportions of carbon in soil organic matter (Heilmann-Clausen et al., 2015). Fungi can also have effects on other sinks, for example pathogens affecting tree health and growth will affect carbon stored in tree biomass (trunk, leaves, roots) (Hicke et al., 2012).

Although fungi, and microbes more broadly, have significant impacts on forests, they are frequently poorly represented in forest carbon modelling, and rarely accounted for in tree planting initiatives (Ouimette et al., 2020). Several papers have demonstrated that including more in-depth specifications of soil and rhizosphere fungal processes could improve the current uncertainty in global climate modelling (Hararuk et al., 2015; Ouimette et al., 2020; Rinne-Garmston et al., 2019). Meanwhile, despite these complex roles that fungi play in forests, there is very limited policy and guidance to inform tree planting. There are currently no requirements to survey an area for rare fungi before tree planting (or completing other work), which contrasts with compulsory surveying for protected animal species in the UK such as bats or great crested newts. Only three fungi are listed on the global Red-List of species at high risk of extinction (which comprises a total of 45000 species), and although significant work has taken place to develop and inform it, the UK fungal Red List is not currently officially recognised (Dahlberg et al., 2010). A total of 61 fungal species are listed as priority species in the UK Biodiversity Action plan, and are recognised under Section 41 of the Natural Environment and Rural Communities Act; however, without requirements to survey, and with a lack of awareness of fungi, these species (and other fungi), are likely severely under-recorded (Department for Environment, Food & Rural Affairs and Natural England, 2022; JNCC, 2019). This lack of legal protection not only hinders the ability to protect rare fungal species and minimise biodiversity loss, but also discourages development of wider fungal ecology monitoring, and our overall understanding of the forest system. Without this vital data it is difficult to maximise the large carbon sequestration and biodiversity benefits of fungi in tree planting, whilst also mitigating appropriately against the plant and human disease risks. Considering this apparent disconnect between the importance of fungi, and the lack of policy surrounding them, in this review we highlight four key reasons why fungi should be included in all UK tree planting and restoration initiatives, identifying the current state of the research:

1. Fungi can cause severe tree disease
2. Fungi can cause significant human health burdens
3. Forest soil carbon and nutrient cycling is controlled by fungi
4. Climate change is already affecting fungi

Following these four reasons, we suggest a seven-step policy framework (Section 1.3) which aims to maximise carbon sequestration by fungi, mitigate against fungal threats, and protect rare fungi. This framework could be incorporated into existing tree planting guidance (e.g. Brancalion & Holl, 2020; Sacco et al., 2021).

1.2 Four reasons why fungi are important to consider when planting trees and restoring forested habitats

1.2.1 Fungi can cause severe tree disease

Fungi cause the most plant disease of any group of organisms and are responsible for several severe tree disease outbreaks in the UK in the past 50 years, most notably dutch elm disease (*Ophiostoma ulmi*) and ash dieback (*Hymenoscyphus fraxineus)* (Santini et al., 2013). Ash (*Fraxinus* spp.) dieback, caused by the fungus *H. fraxineus,* was first detected in the UK in 2012 and is currently the largest disease threat to British trees (Broome et al., 2019). *H. fraxineus* causes severe tree disease, and it is estimated that it will kill around 80% of UK ash trees and cost £14.8 billion over the next 100 years (£7.6 billion of which will occur over the next 10 years) (Hill et al., 2019; The Woodland Trust, 2021). In addition to the financial implications of losing trees, ash trees account for 12% of broadleaved trees in Great Britain, and form ecological associations with almost 1000 other species, including 68 free-living fungi and 548 lichens; demonstrating that their loss is likely to result in a both a significant loss of carbon storage and biodiversity (Forest Research, 2020; Mitchell et al., 2014). Other major tree disease outbreaks in the UK include dutch elm disease (*Ophiostoma ulmi*), which resulted in the loss of 30 million elm trees in the UK (Potter et al., 2011). In addition, the fungal-like oomycete *Phytopthora ramorum*, which is known to infect 109 host species (including oak trees), has been responsible for the loss of the majority of the UK's 154,000 acres of larch trees, and the fungus *Dothistroma septosporum*, which causes needle blight in conifers, predominantly affecting pine plantations in the UK (Fisher et al., 2012; Forest Research, 2021; Potter et al., 2011; The Woodland Trust, 2021). Tree disease is one of the largest threats to the health and survival of trees, with clear implications on the carbon sequestration outcomes of tree planting. It is therefore essential to consider the disease risk of the tree and wood products, the established diseases in a location, and the individual tree species at risk when establishing a plantation.

Climate change is also expected to impact the tree disease burden, and therefore affect any tree planting strategies currently being planned in the UK. La Porta et al. (2008) identify the key factors which could alter fungal disease risks as: a) abiotic stresses (e.g. drought), b) temperature and moisture changes altering sporulation and spore dispersal, c) migration of pathogens to a new geographical range, and finally d) new threats appearing because of a change in tree species composition. These four factors are all applicable to UK treescapes. Significant temperature and rainfall changes are already being measured in the UK, global trade and travel are increasing pathogen migration, and the introduction of a new national tree planting initiative, all of which could be expected to affect overall UK tree species composition. There is currently a lack of experimental studies testing these four effects of climate change in the UK on fungal pathogens, but non-UK studies suggest that changing conditions caused by climate change are likely to affect pathogens also present in the UK. For example, in their review, Woods et al. (2016) demonstrated that weather conditions strongly affect the life cycle of the fungal pathogen *D. septosporum*, suggesting that future climates are likely to promote disease growth. Model simulation and review have also demonstrated the likely expansion of *Phytophthora cinnamomi* under climate change (Bergot et al., 2004; Brasier and Scott, 1994).

1.2.2 Fungi cause significant human health burdens

Due to their high numbers of fruiting fungi, forests are a source of airborne fungal spores, which can spread over significant distances, causing high concentrations of fungal aerosols, not only close to forests, but also in nearby towns and cities (Sadyś et al., 2014). As forests are a large and significant source of fungal bioaerosols, the UK government initiative to increase the forest land area by 5–6% could also significantly increase the population-weighted aerosol exposure. Despite the numerous positive mental and physical health benefits of forests, a possible change in bioaerosol concentrations of this magnitude should also be taken seriously due to the negative human health implications of bioaerosols.

The most widely studied, and some of the most common UK aeroallergens are *Alternaria* and *Cladosporium* species, however many other fungal spores are prevalent in the atmosphere (e.g. basidiomycetes), and more research is needed to understand the impacts of other fungi (Caillaud et al., 2018; Gabriel et al., 2016; Grinn-Gofroń et al., 2019; Skjøth et al., 2016). Despite these gaps in our knowledge of airborne fungal spores, it is clear that they can cause a range of human diseases, with allergenic diseases such as childhood asthma being the most common (Caillaud et al., 2018; Harley et al., 2009; Rodrigues et al., 2016; Welsh et al., 2020). The global occurrence of allergic rhinitis (caused by pollen, in addition to other bioaerosols including fungal spores, pet dander etc) has been increasing for decades; however, the reasons for this remain unclear (Cox and Calderon, 2010). Symptoms reduce life quality and can be associated with an increased risk of asthma exacerbation, leading to hospitalisation (Compalati et al., 2010). Significant reductions in mental and physical health, and working and learning capabilities are commonplace in adults and children, which has implications for quality of life, as well as financial and healthcare system burdens (Wright, 2020). Several studies, both in the UK and in other countries, have demonstrated links between increased airborne fungal spore counts and hospital asthma admissions (Dales et al., 2003; D’Amato et al., 2020; Pulimood et al., 2007). As yet, there is limited research connecting forest bioaerosol concentrations or composition and human health outcomes.

In addition to potential increases in population-weighted fungal spore concentrations due to increased tree numbers, climate change is also likely to influence fungal spore concentrations and seasonal patterns. There have been well-documented extensions to the length of the fungal fruiting season in the UK, both in the autumn and spring, which could in turn alter the seasonality of airborne fungal spore concentrations (Andrew et al., 2018b; Gange et al., 2007). Altered seasonality of airborne fungal spores could increase occurrences of combined allergenic reactions to pollen and fungal spores, as well as increasing the likelihood of seasonal cold and flu viruses being combined with the start or end of the airborne fungal spore season (D'Amato et al., 2015). Meteorological variables (e.g. rainfall, temperature, relative humidity, storms) have been demonstrated to affect airborne concentrations of fungal spores, all of which will be altered under a changing climate (Grinn-Gofroń et al., 2019; Sadyś et al., 2016a, 2016b). There is not an equivalently large body of research investigating the impact of elevated atmospheric CO_2 concentrations on fungal spore concentrations; however, the existing studies suggest that there are species-specific responses to CO_2. For example, Wolf et al. (2003) tested the response of 11 arbuscular mycorrhizal fungi to elevated CO_2 (eCO_2) at the BioCON FACE grassland experiment, but only a single *Glomus* species produced additional spores in the soil. Similarly, in a *Populus tremuloides* open-topped chamber CO_2 fumigation experiment, Klironomos et al. (1997) found that airborne fungal spore concentrations increased, which they suggested was due to corresponding increases in spore concentrations in the leaf litter.

1.2.3 Forest soil carbon and nutrient cycling is controlled by fungi

Over 50 years of research have demonstrated that fungi hugely influence carbon and nutrient cycling in forest soils (Gadgil and Gadgil, 1971). It is therefore essential to consider these fungi, and the implications (positive and negative) that they could have in a tree planting initiative which has a primary goal of increasing carbon sequestration (Gadgil and Gadgil, 1971; Rygiewicz and Andersen, 1994). A significant proportion of forest carbon is stored as fungal biomass, with some studies reporting up to 21% of net primary productivity (NPP) being allocated to ectomycorrhizal (ECM) fungi (Hobbie, 2006; López-Mondéjar et al., 2018). Cheeke et al. (2017) found that forests dominated by ECM fungi (as opposed to arbuscular mycorrhizal (AM) fungi) had three times more fungal biomass, representing a significant carbon sink. Trees predominantly associate with either AM or ECM fungi, so climate, disease, or planting-triggered shifts in tree species could have significant implications for mycorrhizal type and therefore belowground carbon storage.

In addition to carbon stored as fungal biomass, fungal saprotrophs also release significant quantities of carbon during decomposition of deadwood and leaf litter (Tláskal et al., 2021). Deadwood and other dead plant material have up to 50% of their dry mass made up of carbon, some of which is released by heterotrophic respiration during decomposition by fungi (and other microbes and soil fauna). The potentially “competing” interactions between saprotrophic fungi (decomposing and releasing CO_2) and mycorrhizal fungi (harvesting nutrients and storing carbon as fungal biomass) have long been debated with numerous papers investigating “Gadgil” and “priming” effects, but there still is not a consensus within the scientific community on a mechanism by which saprotrophs may interact with mycorrhizal fungi, and the combined effects that this may have on the total carbon balance of forests (Fernandez and Kennedy, 2016; Frey, 2019). It seems likely that soil nutrient availability, primarily nitrogen, is at least partly responsible for the current variability (and therefore uncertainty) in the mycorrhizal-saprotroph interactions, and also potentially causes shifts in the type of mycorrhizal fungi seen- which may explain results such as those seen by Cheeke et al. (2017) (Averill et al., 2018; Hobbie, 2006; Kicklighter et al., 2019; Parihar et al., 2020; Schulte-Uebbing and de Vries, 2018; Treseder and Allen, 2000).

As well as the current uncertainties regarding the balance between mycorrhizal carbon storage and saprotrophic carbon release, we also have a limited understanding of how climate change will affect these processes. Several studies have reported that ECM fungi are more susceptible to climatic changes than other fungal groups, and could consequently decrease in richness, potentially causing decreases in soil fungal biomass (Bennett and Classen, 2020; Miyamoto et al., 2018; Steidinger et al., 2020; Větrovský et al., 2019). Sapsford et al. (2017) discussed a “chicken and egg” theory about tree decline and mycorrhizal fungi, whereby it is unclear whether global stressors are causing tree loss due to lack of mycorrhizas or *vice versa*. Major changes to the abundance of mycorrhizal fungi in the soil could have significant implications on how much carbon is stored by woodlands.

1.2.4 Climate change is already affecting fungi

There is strong evidence from the UK, and wider studies in similar temperate climates, that fungal fruiting seasonal patterns and community structures are already significantly affected by climate change. On average, fungal fruiting seasons have been lengthening, both starting earlier, and ending later, with many species also having an additional spring fruiting season in addition to the extended autumn fruiting season (Ágreda et al., 2016; Andrew et al., 2018a, 2018b, 2016; Boddy et al., 2014; Gange et al., 2007; Kauserud et al., 2012). At the BangorFACE experiment, researchers found that ECM sporocarp (fungal fruit body) biomass increased under elevated CO_2 treatments, with similar increases in ECM fruit body production shown at Aspen FACE experiment (Andrew and Lilleskov, 2009; Godbold et al., 2015). Whilst in investigations of temperate forest data, Ágreda et al. (2016) found that fungal fruit body yields were strongly positively correlated with temperature, and that although effects were species-specific, more fungal species decreased fruiting under climate change than fungi which increased fruiting.

In addition to changes in fungal fruiting phenology, the spatial and host distributions of fungi are likely to change in response to climate change. For example, in 2017, the first

Périgord black truffle (*Tuber melanosporum*) was harvested in the UK, which is the northernmost record of this fungus (Thomas and Büntgen, 2017). Gange et al. (2018) showed that in the north of the UK, ECM fungal fruiting has increased and saprotrophic fruiting decreased, with the opposite trends seen in the south, which they linked with increasing autumnal mean daily temperatures and rainfall, as well as concurrent phenological changes in the fungal host trees due to elevated CO_2 and other climatic changes. The Wood Ear fungus, *Auricularia auricula*, was originally only found growing on a single host tree (*Sambucus nigra*, elder), but over the last 50 years has extended its host range to 16 tree species (Gange et al., 2011).

These changes in fruiting, spatial distributions, and tree hosts demonstrate the effects that climate is already having on fungal populations, which has clear implications for forests and tree planting. To the best of our knowledge, there have been no studies investigating the impact of fungal community composition on tree establishment or tree community composition. We therefore have a limited understanding of the early stages of tree establishment in-situ, and how seeds and saplings interact with mycorrhizal, saprotrophic, and pathogenic fungi during this time. It is unclear how, for example, planting on intensively managed lowland agricultural soils (and the fungi present in these soils) would affect the success of tree planting long-term, and whether this would differ from land managed in a different way.

However there have been multiple studies investigating the impact of trees (and plantations in particular) on fungal communities. There is evidence to suggest that tree species composition and biodiversity-promoting forest management strategies do affect the species composition of fungal communities in forests (Asplund et al., 2019; Brazee et al., 2014; Gunina et al., 2017; Jönsson et al., 2017; Kutszegi et al., 2020, 2015; Müller et al., 2007; O'Hanlon and Harrington, 2012a; Purahong et al., 2018; Rodriguez-Ramos et al., 2021; Tomao et al., 2020; Varenius et al., 2016). However, the overall fungal richness is often not affected by tree species composition, and several studies have demonstrated the potential of plantation forests (including non-native tree species) to support and maintain fungal populations (Humphrey, 2005; Humphrey et al., 2000; Komonen et al., 2016; Leski et al., 2019; Newton et al., 2002; O'Hanlon and Harrington, 2012b; Quine and Humphrey, 2010). In a comparison of ectomycorrhizal fungal richness and community composition in ancient (>1000 years) vs over-mature planted forest (~180 years), fungal richness and community composition were strongly correlated with tree diversity, and were similar across both ancient and mature planted forests (Spake et al., 2016) . This shows that plantation forests can have good fungal biodiversity outcomes, with the authors also suggesting that older plantation stands could act as "ecological corridors", allowing fungi (particularly dispersal-limited and rare fungi) to travel between the sparsely situated ancient woodlands in the UK (Spake et al., 2016). These data demonstrate that good biodiversity outcomes can be achieved with plantations, particularly older plantations. However, it is important to remember that changes in community composition are still likely, particularly in young plantations, and this may result in the loss of more rare fungi, which are also more susceptible to climate change (Lonsdale et al., 2008; Zhou et al., 2020).

1.3 Maximising the benefits of forest fungi whilst mitigating against negative outcomes: Seven policy guidelines

Tree planting is an important component of the UK's climate mitigation strategy, and has the potential to result in significant carbon sequestration, as well as numerous other benefits. It is also clear that fungi have significant positive and negative effects on tree planting outcomes, and additionally that woodland creation on this scale could have significant impacts on fungi. It is therefore essential to consider fungi in any well-planned tree planting strategy.

Given the research gaps that remain, we promote a “precautionary principle” approach, as a strategy to protect the fungal biodiversity and highest carbon sequestration outcomes as far as possible despite our knowledge gaps of the system (Kriebel et al., 2001). Based on this principle and the current state of the knowledge, we have outlined seven policy recommendations (Figure 1-i) that can either be immediately started or implemented on a short (five year) timescale, that would mitigate some of the potential negative consequences of forest fungi, protect existing biodiversity, and promote increased carbon sequestration; and would complement ongoing and future forest research.

A summary of these policy recommendations is listed below:

A. Monitor tree fungal disease emergence and spread, including in source material trade (e.g. seeds and saplings)

B. Choose tree species combinations appropriate to the specific habitat, and appropriate for biodiversity and carbon storage goals

C. Develop and implement a widely accessible fungal spore forecast to complement existing pollen forecasts

D. Protect existing ancient and mature woodlands

E. Promote planting on suitable land types, avoiding grasslands and wetlands

F. Assess proposed and existing forest sites, ideally using a combination of fungal fruit body surveys and eDNA techniques

G. Develop and implement the UK Fungi Red List into UK law

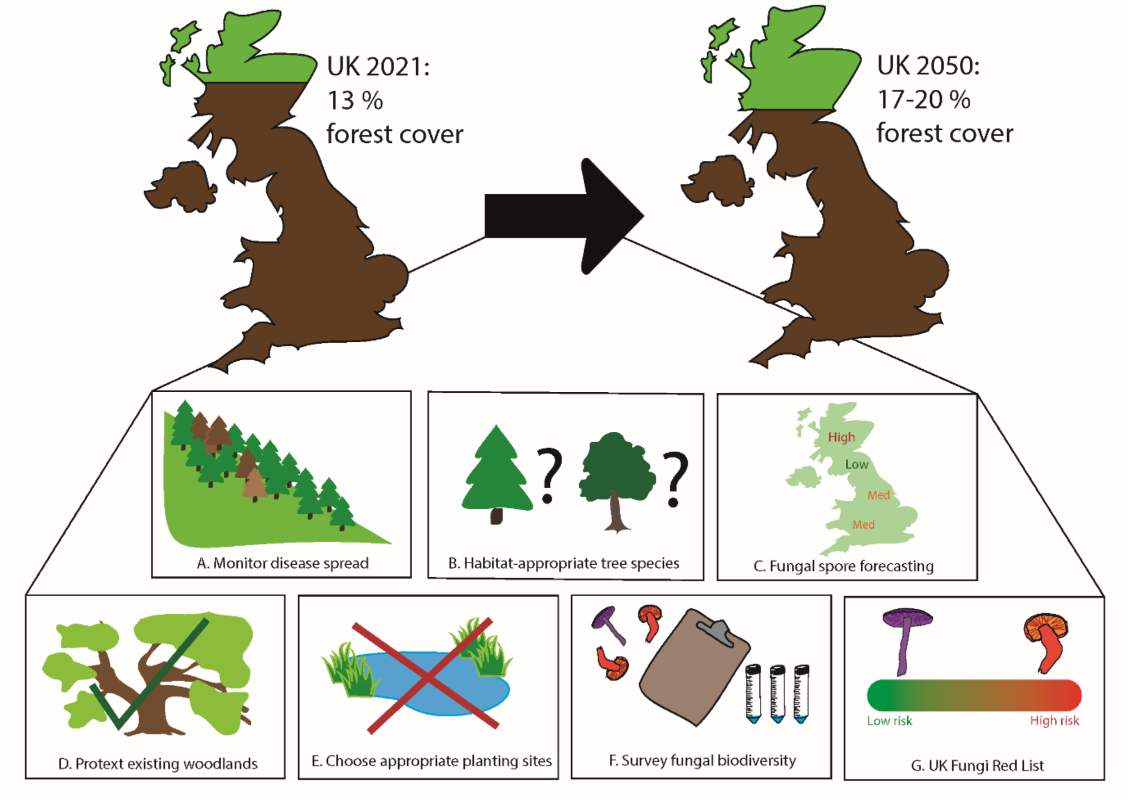


Figure 1-i Conceptual diagram showing the seven guidelines to consider in the large UK tree planting initiative. The seven guidelines have different, but overlapping goals, which refer to the four main areas in which fungi are important in forests. Guideline A and B aim to minimise harm from fungal tree pathogens. Guideline C aims to minimise harm from human lung allergic reactions and disease from airborne fungal spores. Guidelines A, C, D and E are designed to maximise carbon sequestration. Finally, guidelines F and G aim to protect fungal biodiversity, both in existing and new forests.

1.3.1 Monitor tree fungal disease emergence and spread, including in source material trade (e.g. seeds and saplings)

Monitoring the progression, and effects, of fungal disease on UK forest health requires early detection systems so that disease spread can be managed. The UK Plant Health Risk Register is a tool designed improve the information on plant diseases available to government, industry, and stakeholders. The Register currently lists 1215 pests, 173 of which are fungi (Department for Environment, Food & Rural Affairs, 2021). Other initiatives include the community science project “Observatree”, which trains volunteers to identify and report tree pests and diseases (Observatree, Forest Research, 2018).

Continuing to gather field data on fungal pathogens as climate change progresses is important, as risks may be altered significantly depending on temperature and meteorological conditions.

Finally, genetic approaches to studying disease susceptibility are developing further; for example, a significant number of projects are currently investigating why some ash tree individuals are resistant to ash dieback. There is the potential of developing genetically modified trees which are more disease or climate resistant, but in these cases it is important to consider public and forest manager responses, as there is often reluctance towards any type of genetic modification initiative (Jepson and Arakelyan, 2017; Marzano et al., 2019).

1.3.2 Choose tree species combinations appropriate to the specific habitat, and appropriate for biodiversity and carbon storage goals

In addition to managing the spread of emerging diseases, it is also important to deal with the consequences of diseases: tree death. Where severe tree diseases cause death, replacement trees will be needed in existing forests, as well as carefully choosing alternative trees for new plantations. In a series of papers, Mitchell et al. (2016a) studied the suitability of replacement trees for ash (in the context of replicating biodiversity), advocating an approach that considers the wider functioning of the ecosystem in addition to the species-specific biodiversity implications (e.g. plant, fungus, animal species that form associations with ash that may be lost if ash is lost). They suggest that oak (*Quercus* spp.) and beech (*Fagus sylvatica*) are the most likely species to replace ash by natural regeneration, with sycamore (*Acer pseudoplanatus*) identified as a good non-native plantation candidate (Broome et al., 2019; Mitchell et al., 2016a, 2016b). Most importantly, these works show that there is not a single tree species that is an appropriate replacement for ash in all habitats, with different replacement trees suiting different habitats, as well as considering combinations of trees.

In addition to highlighting that replacement tree species choices are often habitat-specific, the approach of Mitchell et al. as not been completed for all the major UK tree species that are currently under threat from fungal pathogens, and so the information is not universally available. The best recommendations for choosing replacement trees can be to look to the best available research at the time, as well as drawing on expert and local knowledge of the habitat and choosing site-specific trees, remembering that natural regeneration is often an excellent option, particularly for already existing mixed forests (Mitchell et al., 2019, 2016b; Sacco et al., 2021).

Finally, although we are lacking in data from large scale mixed species plantation experiments, it seems highly likely from current research that monoculture forests should be avoided entirely, as they are poor for biodiversity, are likely more susceptible to disease, as well the risk of the death of an entire forest in the case of a new invasive disease (Verheyen et al., 2016).

1.3.3 Develop and implement a widely accessible fungal spore forecast to complement existing pollen forecasts

Despite the uncertainties in the effects of tree planting and climate change on airborne fungal spores, the consequences of fungal-induced asthma attacks or other allergenic lung diseases can be severe, particularly in children, and so the precautionary principle should be applied. Pollen forecasting is already widespread and well used by the British public, appearing on most UK weather forecasts. Annual fungal spore data and forecasting is becoming available online, but is not currently broadcast to the public at the same level as pollen forecasting (Midlands Asthma and Allergy Research Association, 2021; University of Worcester, 2021). Integrating fungal spore forecasting into the current pollen forecasting approach would make information more widely accessible to the public, would allow at-risk persons to manage the personal risk of high concentration fungal spore events, and allow health systems to plan for high demand periods. These systems would easily integrate into existing systems, and minimise individual risk, whilst continuing with large-scale forest establishment and the numerous other benefits it offers.

1.3.4 Protect existing ancient and mature woodlands

There are many benefits of establishing new forests, and the evidence does show that plantations can support diverse fungal communities. However, it is still unclear how quickly fungal communities and carbon storage establishes in a new plantation, how climate change will affect forest fungi, and what effect the differing fungal communities between plantations and mature woodlands has on forest functioning. It is clear that a plantation cannot exactly replicate an existing forest. It is therefore essential to protect our existing forests, as well as establishing new wooded areas (Abrego et al., 2016b; Pasanen et al., 2014; Sacco et al., 2021). These existing forests already have significant carbon stocks, and are essential habitats for fungi, particularly rare fungi which exist in smaller ecological niches.

Some experiments have investigated the possibility of translocating soil or individual rare fungi in order to replicate existing ancient woodlands and protect rare species (Abrego et al., 2016b). There is a lack of evidence for the impact of soil translocations on fungal communities in the donor or recipient woodland as the limited number of studies have focussed on plant communities, and the practice of individual fungal translocations is also very new. Existing studies have demonstrated that translocations do not replicate the donor site entirely, and the phenological timing and gentle soil handling is important to maintain as much biodiversity as possible (Craig et al., 2015). Rare and infrequently occurring plant species were also shown not to survive translocation (Buckley et al., 2017). Translocation should not replace the protection and conservation of ancient habitats, and may also not provide a solution if a species is being excluded by climatic changes (Nordén et al., 2020; Pérez et al., 2012).

1.3.5 Choose tree-planting sites carefully, avoiding grasslands and wetlands.

In addition to the protection of existing forest sites, sites for new forest plantations should be chosen carefully to promote carbon storage and avoid new carbon loss. Suitable target sites include previously forested areas, rather than grasslands, moorlands and peatlands which have all been shown to result in no net carbon sequestration or even significant carbon losses when trees are planted (Sacco et al., 2021; Veldman et al., 2015). For example, a recent study by Friggens et al. (2020) in Scotland showed that net carbon sequestration was not achieved when planting native tree species into heather moorland. A number of studies also suggest that natural regeneration of previously forested areas may have the greatest benefits for carbon sequestration and biodiversity, with significantly less requirements for expertise, time, and money than large tree planting strategies (Lewis et al., 2019; Sacco et al., 2021).

1.3.6 Develop and implement the UK Fungi Red List into UK law.

Tree planting is likely to have large impacts on the fungal ecology of the site, particularly for rare species, however without a good understanding of the species at risk, or laws that protect them, it is difficult to make lasting conservation changes.

The primary target for conserving fungal diversity must be to continue the development and implementation of the UK fungal Red List (Clubbe et al., 2020; Dahlberg et al., 2010). Fungal conservation is still limited in the UK by the lack of policy. Without the legal requirements to protect fungi, it is difficult to enforce biodiversity promoting initiatives when the primary goals of tree planting projects are not biodiversity focussed (e.g. carbon sequestration, timber production). However, as numerous studies have shown (e.g. Section 1.2.3), changes in the fungal community composition and abundance can also affect the carbon storage in a system, and the lack of understanding of the fungal kingdom also hinders progression for the other tree planting goals.

The development of the Fungi Red List is dependent on data, which can be challenging given the lack of financial and infrastructure support for mycology. Recent initiatives to improve the data on UK threatened fungal species include the successful “Lost and Found Fungi Project” (LAFF Project) which called on community mycologists to submit field records of 100 potentially threatened UK fungi (Royal Botanic Gardens, Kew, 2019; The British Mycological Society, 2015). The LAFF Project also ran a series of DNA sequencing workshops using Bento Lab devices to improve community access, and improve fungal identification for people without access to laboratories (Bento Lab, 2021; Dr FunGi, 2019). In addition, the new “Darwin Tree of Life” fungal launch is an ambitious project, aimed at collecting and barcoding all (approximately 17000) known fungal species in the UK, involving close collaboration between community mycologists and researchers (Darwin Tree of Life, 2020). Finally, the UK is home to the largest fungarium in the world, host to 1.25 million fungal specimens, and is a wealth of data. Exploiting these already existing collections is an important source of data, as well as being a useful historical dataset to investigate the effects of climate change on fungal communities (Andrew et al., 2019; Royal Botanic Gardens, Kew, 2021).

1.3.7 Assess proposed and existing forest sites, ideally using a combination of fungal fruit body surveys and eDNA techniques

Climate change is already affecting fungal fruiting patterns, as well as altering fungal host ranges, and changing the geographical range where fungi can exist. However, it remains unclear exactly how these changes to fungal phenology affect the reproductive success of fungi, their functioning in forest systems, and carbon sequestration.

Without understanding of the effects of fungal biodiversity loss or change on forest ecosystems, the approach must be to preserve fungal communities; both to preserve the functioning of forest ecosystems, as well as for the inherent value of the fungi (Heilmann-Clausen et al., 2015). It seems likely that plantations can support diverse fungal communities, despite these communities probably being different in their composition from existing mature and ancient woodlands.

To more fully understand the effects that tree planting has on fungal communities, a site surveying approach combining both fungal fruiting body surveys and eDNA techniques both allows the identification of rare fungi that may be extirpated by tree planting, but also to assess the changes in fungal communities over time (Runnel et al., 2015). Potential planting sites should be surveyed for rare fungi before planting (as would be required for rare species in other kingdoms). In addition to surveys pre-planting, surveys at regular intervals after planting would assess the fungal populations over time, and provide further information of how young plantation forests can support or alter fungal communities (Abrego et al., 2016a). These surveys should also be completed on established forests of a variety of ages and management styles, to allow comparison between new plantations and other forest types. These measurements could then be integrated into a wider network of forest monitoring measurements, improving our understanding of the wider system as well as the fungal communities.

Site surveys can be challenging, particularly due to the time and expertise required. Field mycology and taxonomy skills are becoming increasingly rare, with most field mycology experts being amateurs, and often remaining separate from academic researchers (British Mycological Society, 2008; Buyck, 1999; Wilson, 2017). Even with the sudden rise in popularity (and concurrent decrease in price) of molecular tools such as high throughput DNA sequencing, these technologies are still unavailable to the vast majority of people due to lack of expertise and funding. One solution to the lack of mycology expertise is to identify suitable indicator species of fungi, which are both relatively easily identified, in addition to being good indicators for the state of the rest of the habitat. These type of indicator species have been used before, but are often chosen without much consideration, therefore the development of a suitable list of species by UK forest mycology experts could be a useful and important tool (Halme et al., 2017).

1.4 Conclusion

In summary, the new UK tree planting strategy to increase forest cover from 13% to 17 – 20% has the potential to result in climate mitigation benefits; however, it is likely to significantly affect UK ecology, and requires careful planning to result in success.

We have highlighted four key reasons (sections 1.2.1 – 1.2.4) why fungi are essential to consider in tree planting initiatives, identifying both the benefits of fungi for carbon sequestration and biodiversity, and the disadvantages of fungi for plant and human health. We have identified the current state of UK forest mycology research, and identified seven policy recommendations (Figure 1-i and sections 1.3.1 – 1.3.7) that should be implemented during the planning stage of this national tree planting strategy. These recommendations aim to maximise the benefits of fungi for carbon sequestration, minimise the harm to plants and humans from fungal risks, and to protect fungal biodiversity from the potential large ecological changes that tree planting on this level will result in.

CHAPTER 2: AN INTRODUCTION TO PREVIOUS AND CURRENT MYCOLOGICAL RESEARCH AT FOREST FACE EXPERIMENTS

2.1 History of FACE Experiments

The ability to mimic and manipulate climatic and meteorological factors is an increasingly important methodology in large ecological experiments, as we attempt to understand what the effects of anthropogenic climate change will be on the planet. Free Air Carbon dioxide Enrichment (FACE) experiments are a type of experiment designed to mimic the effects of elevated atmospheric CO_2 in-situ. Aiming to minimise other disruption and manipulation of the experimental sites, FACE facilities are entirely open to the air, allowing eCO_2 to freely disperse around the experimental plots. This FACE methodology has been applied to a wide variety of ecosystems, including grasslands, savanna, staple crop plants, wetlands, desert, and forests (FACE Data Management System, 2021).

FACE technology is a particularly useful methodology for studying forest ecosystems, which by their nature are difficult to replicate (even on a single species scale) in laboratories, glasshouses, and open-top chamber field experiments, due to the large size of trees as well as the long time scale required for trees to grow to maturity. Table 2-i summarises the nine forest FACE experiments globally, including the seven experiments in the “first generation” which have now ceased operation, and the two “second generation” experiments currently running. The first generation of forest FACE experiments predominantly studied young plantation forests in temperate climates, with trees aged between 0 and 30 years at the start of the eCO_2 fumigation. The second generation of FACE experiments are located in mature forests and cover a wider ecological and geographic range. The two established second generation FACE experiments are EucFACE, a subtropical eucalyptus woodland in Australia, and BIFoR FACE, a temperate oak woodland in England. A third experiment, Amazon FACE, is located in a tropical rainforest in Brazil and is currently nearing the end of baseline data collection before FACE infrastructure construction. A fourth and final experiment, SwedFACE, is proposed for a hemiboreal conifer forest in Sweden (AmazonFACE, 2023; Norby et al., 2016). This second generation of FACE experiments aims to address the research gaps not covered by the first generation; in particular, how mature trees respond to eCO_2, but additionally, how differing nutrient limitations interact with eCO_2, how more highly biodiverse forests respond to eCO_2, and how a wider range of water and temperature ranges interact with eCO_2 (Norby et al., 2016). This thesis focussing on fungal communities at BIFoR FACE contributes to the questions on how ecosystem biodiversity in highly biodiverse forests responds to eCO_2, as well as strongly linking with the nutrient and eCO_2 interaction questions.

Table 2-i. Details of all previous and existing forest FACE experiments, including information on their location, start and end dates, forest type, CO_2 enrichment level, experimental design and tree age, published fungal research from each experiment and the response of the fungi to eCO_2 (Boddy, 2021; Crous et al., 2015; Dawes et al., 2011; Gielen et al., 2005; Gimeno et al., 2016; Hart et al., 2019; Karnosky et al., 1999; Miglietta et al., 2001; Norby et al., 2016, 2006; Norby and Zak, 2011; Pepin and Körner, 2002; Schlesinger et al., 2006; Smith et al., 2013; Teklehaimanot et al., 2002).

Name	Location	Start date	End date	Forest type	Mycorrhizal type	CO_2 enrichment	FACE experimental design	Tree age at fumigation start	Published fungal research for each FACE experiment	Fungal response to eCO_2 per paper, and substrate type tested
Duke FACE / FACTS I	Orange County, North Carolina, United States	1994 (1996)	2011	Loblolly pine (*Pinus taeda*)	Obligate ectomycorrhizal	+200 ppm	8x 30 m diameter plots, 4x CO_2 treatment, 4x control	10 years	McCormack et al., 2017 Chen et al., 2016 Hobbie et al., 2014 Pritchard et al., 2014 Weber et al., 2013 Weber et al., 2012 Feng et al., 2010 Garcia et al., 2008 Parrent and Vilgalys, 2007 Parrent et al., 2006 McElrone et al., 2005	Positive (mycorrhiza) N/A (isotopic analysis) N/A (isotopic analysis) Mixed (mycorrhiza) Mixed (unmodified soil) Neutral (unmodified soil) Neutral (unmodified soil) Mixed (mycorrhiza) Mixed (modified soil) Mixed (unmodified soil) Positive (pathogen)

Oak Ridge / ORNL FACE	Tennessee, United States	1997	2010	Sweetgum (*Liquidambar styraciflua*)	Arbuscular mycorrhizal	+150 ppm	5x 25 m diameter plots, 2x CO_2 treatment, 3x control	10 years		
ASPEN FACE/ FACTS II/ Rhinelander FACE	Rhinelander, Wisconsin, United States	1998	2010	Trembling aspen (*Populus tremuloides*), Paper birch (*Betula papyrifera*), Sugar maple (*Acer saccharum*)	Ectomycorrhizal and arbuscular mycorrhizal	+200 ppm	12x 30 m diameter plots, 3x CO_2 treatment, 3x CO_2 and O_3, 3x O_3, 3x control	Saplings planted at start of experiment	Dunbar et al., 2014 Andrew and Lilleskov, 2009 Chung et al., 2006	Neutral (unmodified soil) Positive (sporocarps) Neutral (unmodified soil)
POPFACE/ EuroFACE	Viterbo, Italy	1999	2005	Poplar spp. (*Populus alba, Populus nigra, Populus euramericana*)	Ectomycorrhizal and arbuscular mycorrhizal	550 umol mol^{-1}	6x 22 m diameter plots, 3 CO_2 treatment, 3x control	Saplings planted at start of experiment	Lagomarsino et al., 2007 Godbold et al., 2006 Lukac et al., 2003	Neutral (unmodified soil) Neutral (mycorrhiza) Mixed (mycorrhiza)
Web-FACE/ SCC FACE	Basel, Switzerland	2000	2014	Beech (*Fagus sylvatica*), Sessile oak (*Quercus petraea*), Norway spruce (*Picea abies*)	Ectomycorrhizal	520 µmol mol^{-1}	14 trees CO_2 treatment, 14 control	80-120 years old	Keel et al., 2006	N/A (isotopic analysis)
Stillberg FACE	Stillberg, Switzerland	2001	2009	Larch (*Laris*) & Pine	Ectomycorrhizal	+200 ppm (550 ppm)	40x hexagonal 1.1 m^2 plots. 20x CO_2	30 years old	Dawes et al., 2013	Neutral (modified soil)

				(*Pinus*) treeline			treament, 20x control			
Bangor FACE	Bangor, Wales	2005	2008	Alder (*Alnus glutinosa*), Silver birch (*Betula pendula*), Beech (*Fagus sylvatica*)	Ectomycorrhizal and arbuscular mycorrhizal	+200 ppm (580 ppm)	8 octagonal plots, 4x CO_2 treatment, 4 control	Saplings planted at start of experiment	Godbold et al., 2015	Positive (sporocarps) Neutral (mycorrhiza)
EucFACE	Richmond, Australia	2012		*Eucalyptus* spp.	Ectomycorrhizal and arbuscular mycorrhizal	550 ppm	6x 25 m diameter plots, 3x CO_2 treatment, 3x control	80-100 years old	Castañeda-Gómez et al., 2021 Castañeda-Gómez et al., 2020	Mixed (modified soil) Neutral (modified soil)
BIFoR FACE	Staffordshire, England	2014		English oak (*Quercus robur*) and Hazel (*Corylus avellana*)	Ectomycorrhizal and arbuscular mycorrhizal	+150 ppm (550 ppm)	9x 25 m diameter plots, 3x CO_2 treatment, 3x infrastructure control, 3x control	150 years old	Baird et al., 2021	Neutral (airborne spores)

2.2 Systematic review of fungal research at forest FACE experiments

The forest FACE experiments listed used a variety of methodologies to study fungi. Table 2-i lists all the published fungal research from forest FACE experiments that directly investigated the effects of eCO_2 on fungal biomass, diversity, or composition, totalling 23 papers. In keeping with the goals of this thesis, it does not include studies investigating fungal community function such as extracellular enzyme assays, although these types of studies would be a natural follow-on from much of the work reported in the table, and in this thesis.

2.2.1 Fungal fruiting under eCO_2

Five of the forest FACE experiments (excluding the BIFoR FACE work reported in this thesis) completed some investigation of fungal fruiting under eCO_2, however these studies were often focussed on the transport of labelled carbon through the ecosystem, rather than a direct investigation of fungal ecology.

Aspen FACE and Bangor FACE both directly investigated sporocarp production. At AspenFACE, eCO_2 significantly increased ECM sporocarp biomass, and also altered sporocarp community composition, although these effects decreased as the age of the tree stands increased (Andrew and Lilleskov, 2009). Sporocarps were also larger (both in size and weight) under eCO_2 at BangorFACE, although these results come from only three fungal species, as no other sporocarps were found (Godbold et al., 2015).

Due to the small number of studies, it is difficult to know if these results would be replicated at other woodland FACE experiments. Additionally, as both studies focussed on ECM fungi (excepting a single species at Bangor FACE), it is impossible to know whether the results would be replicated across all fungi, or whether different functional groups or even different fungal species would respond in similar ways. Both Aspen FACE and Bangor FACE had a mixture of arbuscular and ectomycorrhizal fungi associated tree species, so it is unclear whether forest systems with different mycorrhizal types would respond in a different way (for example, Duke FACE was an obligate ectomycorrhizal system so may have responded differently).

At DukeFACE and WebFACE sporocarp collections were used to study the movement of carbon isotope ^{13}C through the woodland, tracking the carbon originating from the CO_2 used for fumigation. At DukeFACE all functional groups of fungi showed a decreased ^{13}C signature in the eCO_2 treatment plots (indicating that the carbon in the eCO_2 enrichment moved through the carbon cycle to the fungi). ECM fungi showed on average a stronger decrease in ^{13}C signal than saprotrophs, despite responses varying between species. However; outside of the fumigation periods, saprotrophic fungi were the only functional group which retained the lowered ^{13}C signature. (Chen et al., 2016; Hobbie et al., 2014). At WebFACE, amongst the 85 fungal species fruiting, mycorrhizal fungi also showed a significantly lower ^{13}C value under eCO_2, however, unlike at DukeFACE, no ^{13}C labels were found in saprotrophic fungi, even after four years of eCO_2 fumigation (Keel et al., 2006). These studies both demonstrated that the mycorrhizal fungi have a lower ^{13}C signature under eCO_2 compared with ambient conditions ,and often have a lower ^{13}C signal than other functional groups of fungi. Carbon cycling through saprotrophic fungi does not seem to be equally consistent between the existing FACE studies, and it is unclear whether this is due to different forest types, or varying individual responses of fungal species in the communities at the different forests. Mycorrhizal fungi may be demonstrating a lower ^{13}C signal earlier in the FACE experiments as they receive carbon directly from their symbiotic trees. This is likely quicker than the labelled carbon reaching saprotrophs decomposing dead plant and fungal material. It is unclear whether the lower ^{13}C signal in mycorrhizal sporocarps compared with saprotrophs would continue across a longer duration of eCO_2 fumigation, or whether the signal would become similar across both functional groups as more of the labelled carbon moves through the carbon cycle.

2.2.2 Mycorrhizal colonisation of roots under eCO_2

Another common method used to study fungi was measuring mycorrhizal fungal colonisation of roots. A variety of methods were used including: isolating roots and mycelium from soil samples and ingrowth bags, minirhizotron imagery, and root box imagery. At BangorFACE there was no significant effect of eCO_2 on ECM colonisation of roots isolated from soil, however, there were more non-mycorrhizal root tips under eCO_2 (Godbold et al., 2015). At EuroFACE, they used leaf litter decomposition bags, and examined fine roots for mycorrhizal colonisation. There was no consistent response of mycorrhizal colonisation of roots to eCO_2 treatment, with the different *Populus* species in the experiment showing different degrees of colonisation and eCO_2 responses (Godbold et al., 2006). In a separate study at EuroFACE where root ingrowth cores were used, there was also a varied response depending on the *Populus* species, whereby eCO_2 significantly increased AM colonisation of *Populus alba* and *Populus nigra* and significantly increased ECM colonisation of *P.alba*. The third *Populus* species in the experiment (*Populus euramericana*) did not experience a significant increase in colonisation by either AM or ECM fungi (Lukac et al., 2003).

In contrast, at DukeFACE, eCO_2 significantly increased ECM root colonisation, but there was no impact of eCO_2 on AM root colonisation, or hyphal length of any mycorrhizal species (Garcia et al., 2008). These DukeFACE soil sample results were reinforced by a minirhizotron dataset investigating *Cenococcum geophilum* ectomycorrhiza (McCormack et al., 2017). This minirhizotron dataset showed a large increase in *C. geophilum* ECM production under eCO_2, constrained mainly to the top 16 cm of soil. A separate root box experiment at DukeFACE showed that fungal cord lengths significantly increased (alongside root lengths) in the upper soil layer under eCO_2 (Meier et al., 2014). A final DukeFACE dataset, analysing minirhizotron data for all mycorrhizae, found that there was no effect of eCO_2 on mycorrhizal tip length and diameter. eCO_2 treatment did increase the longevity of mycorrhizal tips, and there was an eCO_2 by depth interaction, whereby mycorrhizal tips produced in shallow soil where thicker than tips in deeper soil under eCO_2 (Pritchard et al., 2014). The conflicting results between these four DukeFACE studies could be due to varying responses between individual fungal species. It is possible that *C. geophilum* could have displayed a strong positive response to eCO_2, but other ECM species were decreasing in response, producing an overall neutral effect when both were taken into consideration. Across the FACE experiments which investigated mycorrhizal colonisation, there was no consistent response to eCO_2. The differing responses could be due to the variation in experimental methodologies (for example, roots examined from soil samples versus litter bags). There could also be variation between FACE experiments due to differences in the dominant mycorrhizal type. However, as the EuroFACE study demonstrates, even within the same forest system, and using the same methodology, eCO_2 did not cause a consistent response in mycorrhization. Differing responses were seen between ECM and AM fungi, and between different plant species.

This high level of variation highlights the importance of studying the effects of eCO_2 in a complex system including multiple tree species and multiple fungal species. The variability of fungal responses to eCO_2 also demonstrates that results from a single species study (e.g. the DukeFACE *C.geophilum* study) should not be used to simply extrapolate as the eCO_2 response for the whole system, certainly not without serious consideration of the roles those fungi play in ecosystem and their abundances.

2.2.3 Fungal communities in modified soil and other substrates under eCO_2

Another methodology used to study fungal communities included using DNA sequencing, Phospholipid Fatty Acid Analysis (PLFA), and microbial biomass measurements, on modified soil or other modified substrates. Substrates tested included litter decomposition bags, sand ingrowth bags, and soil ingrowth cores (often with different mesh sizes to exclude different soil components). The two fungal studies completed at the 2nd generation FACE experiment, EucFACE, fall under this category. Firstly, a mesh bag study, where bags containing either sand, root litter, or soil were installed for one year with the contents then analysed using PLFA and internal transcribed spacer (ITS) fragment DNA sequencing. The number of fungal species was unaffected by eCO_2, but community composition was significantly affected by eCO_2, with the strongest effect seen in the root litter bags. The impact of the eCO_2 on the relative abundance of fungi was dependent on the substrate. For example in the soil bags, ECM fungal biomass increased in response to eCO_2, but there was no response by AM fungi (Castañeda-Gómez et al., 2020). In a later study, using modified root ingrowth soil cores with different mesh sizes, there was no effect found of eCO_2 on relative abundance of any fungal functional groups, and no effect of eCO_2 on fungal biomass (Castañeda-Gómez et al., 2021). At StillbergFACE, they also saw no effect of eCO_2 on the biomass of mycelium colonising sterile sand bags (Dawes et al., 2013). Finally, a Duke FACE study using sand ingrowth bags and PLFA and DNA sequencing did not detect a net increase in mycelial biomass under $eCO_{2.}$ There were also no effects of eCO_2 on total richness or diversity of fungi detected, however there were community level differences between ambient and eCO_2 plots (Parrent and Vilgalys, 2007).

Between the four studies there was considerable variation in the response to eCO_2. There was variation in the methodologies used between experiments, which makes it challenging to separate any variation due to ecology versus variation in methodologies. As highlighted by a study at Rhinelander FACE, there can be considerable variation in the responses of soil fungi to eCO_2 dependent on the soil depth studied (Dunbar et al., 2014). The soil depth, or soil horizons, studied were rarely discussed in results or conclusions across the forest FACE literature, and may be partly responsible for variation between papers.

None of the studies demonstrated a change in fungal biomass under eCO_2 and only one of the four studies demonstrated a change in relative abundance of any fungal groups. The two studies which investigated community level differences did see changes in community composition under eCO_2, however the overall number of fungal species remained the same.

However, although there did not seem to be an effect of eCO_2 on fungal biomass or the total number of fungal species, there were community level differences between the eCO_2 treatment and control plots. This change in community composition could then cause a resultant effect on fungal community functioning, for example different types or quantities of extracellular enzyme produced.

2.2.4 Fungal communities in unmodified soil under eCO_2

The final methodology was the use of DNA sequencing, PLFAs, or microbial biomass on unmodified soil samples to investigate below-ground fungal communities. There were four studies from the DukeFACE site. In an initial study sequencing mycorrhizal root tips, and referencing with a site-specific fruit body DNA library, there were more rare fungal species. The relative frequency of some ECM species under eCO_2 but at plot level there was no effect of eCO_2 on fungal community richness or diversity (Parrent et al., 2006). When running PLFAs on soil samples, there was no effect of eCO_2 on fungi (Feng et al., 2010). From DNA sequencing triplicate soil cores across all plots no effect of eCO_2 on phylum composition was detected (Weber et al., 2012). Finally, when analysing soil cores from a single pair of plots (one eCO_2, one aCO_2) using quantitative PCR (qPCR) of fungal small subunit rRNA genes, they found no effect of eCO_2 on fungal biomass or fungal community richness, but the community composition was affected (Weber et al., 2013). At PopFACE, there was also no effect of eCO_2 on the fungal community after five years of eCO_2 treatment in unfertilised soil, but they did see a combined nutrient by eCO_2 effect on community composition (Lagomarsino et al., 2007). Finally at Aspen FACE, they did not demonstrate any significant increase in fungal relative abundance, fungal biomass, fungal community composition, or fungal beta diversity with eCO_2 treatment (Chung et al., 2006; Dunbar et al., 2014).

As with the four studies from Section 2.2.3, none of the seven studies investigating fungi in unmodified soil demonstrated an effect of eCO_2 on fungal biomass. This seems fairly conclusive that at least in the 1st generation FACE experiments, there was no effect of eCO_2 on net soil fungal biomass. This is an interesting result as many of the papers hypothesized that the increase in plant photosynthesis would also result in an increase in fungal biomass. One possible explanation is that fungal biomass is limited by another factor, for example nutrient availability. There may also be multiple contrasting pressures on fungi, for example, resulting in no net changes. For example, eCO_2 could be increasing mycorrhizal fungal biomass, however concurrently increased decomposition rates could be causing decreases in fungal biomass, resulting in no change in the net biomass.

Six out of these seven studies in unmodified soil substrates demonstrated no effect of eCO_2 on fungal communities, with the exception of the DukeFACE qPCR study. This is somewhat surprising given that other methodologies have seen changes in fungal communities with eCO_2.

2.2.5 Effects of eCO_2 on fungal pathogens

There has only been a single fungal plant pathology study completed in a forest FACE experiment to date. The study at DukeFACE investigated the severity of fungal pathogen, *Phyllosticta minima* disease severity on *Acer rubrum* saplings growing under

eCO_2. Under eCO_2 treatment, fewer saplings were infected, fewer leaves were infected per plant, and disease severity decreased (McElrone et al., 2005).

2.2.6 Summary and conclusions

Due to the limited number of studies, varying methods, and samples taken at varying times during the fumigation duration of experiments, it is difficult to draw clear conclusions from the available studies – something which was also highlighted by Norby and Zak (2011). It was common in the above research for studies within the same FACE experiment to show different (and potentially conflicting) results. Some of these differences could be due to different sampling years and duration of eCO_2 fumigation, and the differing methodologies focussing on different fungal sub-groups (particularly for attempting to draw conclusions between ECM only studies and studies investigating all fungi). There were also differences between the 1st generation FACE experiments regarding tree age at start of fumigation, and the land-use history of the site. Both of these could significantly affect fungal communities.

However, even within the same experiment, there were still widely varying responses to eCO_2. This highlights the importance of controlling as many variables as possible within the current FACE experiments (EucFACE, BIFoR FACE, and Amazon FACE), in particular the eCO_2 fumigation duration, and experimental methodologies. By controlling these further, it would eliminate a number of the questions regarding the variability of results between the 1st generation FACE experiments, and enable easier comparison of results between the 2nd generation of FACE sites.

However, there are some patterns emerging from the previous research. Signals from ^{13}C tagged eCO_2 are shown in fungal sporocarps during fumigation periods, and the eCO_2 treatment may increase the weight and size of sporocarps. All studies focussing on mycorrhizal colonisation of roots were undertaken in ECM-dominated systems, where reported results were inconsistent across (and even within) the sites studied. It seems likely that effects of eCO_2 on mycorrhizal colonisation of roots may be species-specific, and may not be a consistent response, even within the same fungal functional group. In-growth bags and cores showed that across multiple sites, fungal community richness remained the same under eCO_2, but the community composition was altered. However, this effect of eCO_2 on community composition does not seem to have been replicated across studies looking at unmodified soil samples, where no forest FACE studies so far have reported changes to the fungal community composition (or community richness) under eCO_2. Across both modified and unmodified soil samples, all studies reported no effect of eCO_2 on soil fungal biomass, an important result, particularly due to the consistency across methodologies and between FACE experiments. There was only one study on a single fungal species investigating the impact of eCO_2 on plant pathogens, so it is therefore impossible to draw any broad conclusions of the effect of eCO_2 on tree pathogen dynamics.

The experiments within this thesis aimed to address some of the research gaps identified in the above literature. There have only been three published studies on fungi at the 2nd generation forest FACE experiments, with the research within this thesis representing the first research on fungi in the BIFoR FACE facility. This therefore provides an important contribution to the literature on the impacts of eCO_2 on mature forests. Another research gap identified is concurrent studies at the same FACE facility using different methodologies. Previous FACE experiments frequently used different methodologies to study fungi at different years within the eCO_2 fumigation period. This makes it challenging to separate effects of differing fumigation lengths with differences due to experimental methodology. Within this thesis concurrent experiments were run using three methodologies, aiming to capture the fungal community during the same time period. Many of the previous studies also focussed results on a sub-set of fungal species, generally ECM fungi, but sometimes an even smaller sub-set focussing on an individual species or genera. As clearly demonstrated in the previous literature, fungal species can have completely contrasting responses to eCO_2. In the studies presented in this thesis, all functional groups of fungi were studied, allowing more conclusions to be drawn about how different fungal functional groups respond to eCO_2.

2.3 BIFoR FACE

All experiments within this thesis were conducted at the BIFoR FACE facility. The BIFoR FACE facility is located in a 19.1 hectare mature oak forest in Staffordshire. 150-year-old English oak (*Quercus robur*) is the dominant tree species, with previously coppiced hazel (*Corylus avellana*) forming a distinct understory layer. Other frequent tree species in the woodland include sycamore (*Acer pseudoplanatus),* common hawthorn (*Crataegus monogyna),* and holly (*Ilex spp*.). Major ground plant species include bramble *(Rubus fruticosus),* beech fern *(Phegopteris connectilis*), ivy (*Hedera sp*.), bluebell (*Hyacinthoides non-scripta*), and grasses where the canopy has been opened for access rides (G. Platt, private communication, 2019). Hanging and fallen deadwood is left in place except where it poses a direct health-and-safety risk. The soil is a non-calcareous sandy loam, with a pH of around 4.5 in the 0-10 cm depth, and around 5.5 in the 10-37 cm depth (Hollis and Jones, 2021). The landscape surrounding BIFoR FACE is predominantly young tree plantations of mixed broadleaves and conifers (MacKenzie et al., 2020).

The CO_2 enrichment system is described in detail by Hart et al., 2019 and MacKenzie et al., 2020. In brief, the system consists of nine roughly circular “arrays” of three types, each array being approximately 30 m in diameter (Figure 2-i). Arrays 1, 4, and 6 are fumigated with additional CO_2, increasing the atmospheric CO_2 by 150 ppm above ambient (to approximately 550 ppm). The CO_2, pre-mixed with air, is released into the tree canopies, using pipes running the height of 25 m tall towers around the perimeter of each array. Arrays 2, 3, and 5 have the same tower infrastructure, but fumigate with air at ambient eCO_2 levels (approximately 400ppm). Arrays 7, 8, and 9 are non-infrastructure control arrays. Arrays 1–6 are grouped into three treatment pairs, based on pre-fumigation vegetation and soil biochemistry analysis, each with a single elevated CO_2 and single ambient CO_2 array. The array pairs are as follows: 1 and 3; 2 and 4; 5 and 6. Arrays 1 – 6 only were used for all analyses in this thesis. Elevated CO_2 treatment occurs during the oak growing season, approximately 1st April to 31st October during daylight hours, with the first fumigation year occurring in 2017, and eCO_2 treatment continuing for a minimum duration of 10 years.

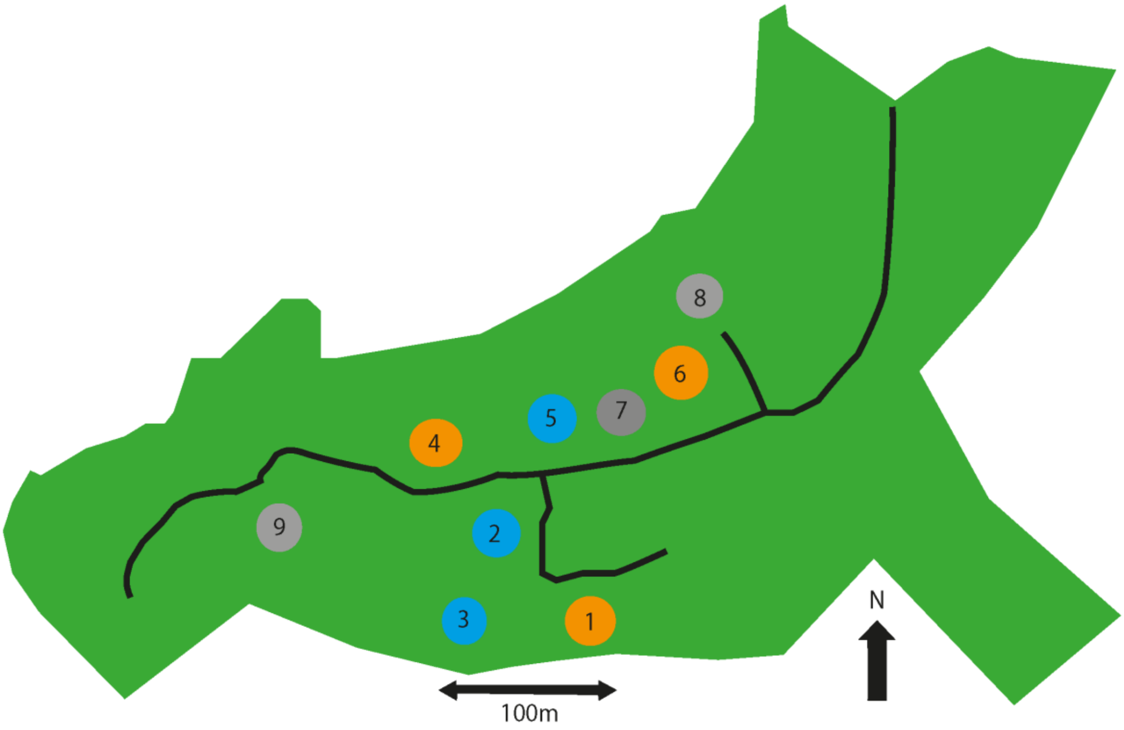


Figure 2-i Conceptual map of the BIFoR forest and FACE facility located in Staffordshire (UK) adapted from Hart et al., 2019. The green area shows the whole 19.1 ha Mill Haft woodland. The access road is shown as a thick black line. Circles represent scientific research "arrays". Orange circles 1, 4, and 6 are eCO_2 treatment arrays, blue circles 2, 3, and 5 infrastructure control arrays, and grey circles 7, 8, and 9 non-infrastructure control arrays.

2.4 Overview of thesis research aims

The broad overall aims of the studies present in this thesis were to characterise fungal communities and their dispersal at the BIFoR FACE site and assess the impact of eCO_2 on these communities. Three different methods were used to assess fungal communities at different stages of their life cycle, as detailed in the below chapters:

Chapter 3: The impact of two years of elevated CO_2 treatment on mass concentrations of woodland bioaerosols

Chapter 4: The impact of three years of elevated CO_2 treatment on mass concentrations of woodland bioaerosols

Chapter 5: Woodland fungal fruiting under elevated CO_2 treatment

Chapter 6: Using DNA metabarcoding to investigate woodland soil fungal communities at BIFoR FACE: A pilot study

Chapter 7: A five year study of soil fungal communities in a mature woodland FACE experiment

Chapter 8: Investigating the impacts of season and elevated CO_2 treatment on woodland soil fungal communities

Chapter 9: Conclusions and future work

CHAPTER 3: THE IMPACT OF TWO YEARS OF ELEVATED CO_2 TREATMENT ON MASS CONCENTRATIONS OF WOODLAND BIOAEROSOLS

3.1 Introduction

Aerobiology investigates the production, transport, and removal of airborne biological particles such as bacterial, fungal, and viral microbes, plant pollen, and soil and plant debris. Fungal spores form a large proportion of the bioaerosol community, with ground level concentrations typically 10,000–50,000 spores m^{-3} (compared with 1000–2000 pollen grains m^{-3}) (Sesartic and Dallafior, 2011, and references therein). Modelling suggests that fungal spores form approximately 23% of bioaerosol mass globally (Heald and Spracklen , 2009). Understanding the dispersal of fungal spores is not only essential for understanding fungal ecology and their direct relevance to forest plant and soil communities, but also for understanding human disease and allergens, plant and crop health, livestock health, as well as the role played by bioaerosols in ice nucleation and cloud condensation (Pöschl, 2005; Reinmuth-Selzle et al., 2017; Sesartic and Dallafior, 2011). The significant influence of airborne fungal particles on forest biology is often overlooked.

Spores are produced by fungi as propagules during asexual and sexual reproduction (Læssøe and Petersen, 2019). Fungal spores vary in shape and size, with typical diameters of 1–30 µm, although their diameters can vary from 1–1000 µm (Fröhlich-Nowoisky et al., 2009; Halbwachs and Bässler, 2015; Jones and Harrison, 2004). Spore dispersal is a complex process, affected by size and shape of the spores, meteorological and ecological factors, and the life history of the fungus. Fungi which produce below-ground fruit bodies, and some mycorrhizal fungi without sporocarps, rarely have spores in significant concentrations in the air, and are more commonly spread by animals. Above-ground fungal fruiting bodies with active release mechanisms or tall stipes may release spores higher into the air (Biedermann and Vega, 2020; Dressaire et al., 2016; Halbwachs and Bässler, 2015; Horton, 2017; Kivlin et al., 2014; Lilleskov and Bruns, 2005; Stephens and Rowe, 2020). Spore size, shape, and ornamentation influence their dispersal, with smaller and less dense spores able to travel further, although even very large spores may be dispersed by the air (Chaudhary et al., 2020; Norros et al., 2014, 2012). In addition to the spore and fungal characteristics, the meteorology and ecology of the environment both significantly influence spore dispersal (Halbwachs and Bässler, 2015; Oneto et al., 2020). Mature forests often have complex, multi-layered canopies which affect airflow through the forest, in addition to altering temperature and relative humidity (RH) variability (Bannister et al., 2021; Gilbert and Reynolds, 2005; Norros et al., 2014).

Meteorological factors have also been shown to impact airborne fungal communities significantly. Several studies have measured concentrations of airborne fungal spores in UK (largely in cities), investigating diurnal and seasonal variables as well as meteorological variables on fungal spores. The effects are almost always species-specific, with a broader seasonal trend of fungal spore concentrations being highest in late summer and autumn (Hollins et al., 2004; O'Connor et al., 2014; Sadyś et al., 2016a, 2014). Larger European studies (e.g. Grinn-Gofroń et al., 2019) and reviews have shown that the two most influential meteorological variables for airborne fungal spore concentrations are temperature and RH (Jones and Harrison, 2004; Moore-Landecker, 2011).

As well as being affected on shorter time scales by meteorological factors, airborne fungal concentrations can also be affected by climate change, both due to increases in CO_2 concentrations as well as the corresponding increase in temperature, although effects on fungi may vary depending on the species (Burge, 2002). Wolf et al. (2003)

tested the response of 11 arbuscular mycorrhizal (AM) fungi to eCO_2 at the BioCON FACE grassland experiment, with only a single *Glomus* species producing additional spores in the soil. Wolf et al. (2010) demonstrated an increase in spore production from *Alternaria alternata* (a common airborne allergen) under eCO_2 in a growth chamber. Similarly, in an aspen (*Populus tremuloides*) open-topped chamber eCO_2 experiment, Klironomos et al. (1997) found that airborne fungal spore concentrations increased, which they suggested was due to corresponding increases in spore concentrations in the leaf litter.

In addition to direct changes in spore production, it is possible that fungal sporocarp production, and therefore spore production, could also increase due to climate change. Ecological measurements show the autumn fungal fruiting seasons in Europe have on average become longer over the last 45 years. The average fungal fruiting season starts earlier and finishes later due to climate change, as well as more fungi having an additional spring fruiting season (Gange et al., 2007; Kauserud et al., 2012). This climate-induced phenology change has also been demonstrated in a corresponding increase in the airborne fungal spore season (Sadyś et al., 2016b). These large changes in the fungal season were mainly attributed to temperature increases; however, at the Aspen FACE experiment, it was found that ectomycorrhizal fungi sporocarp production increased under eCO_2, so it seems possible that CO_2 impacts fungal sporocarp production independently of temperature (Andrew and Lilleskov, 2009). All of these demonstrated changes in fungal phenology, sporocarp production, and sporulation suggest that bioaerosol concentrations are also likely to change under eCO_2. Even if these findings are fungal species-specific, they have potentially wide-ranging effects for forested habitats.

The studies described above demonstrate that fungi are likely to change spore and sporocarp production under eCO_2 ranging from 192–600 ppm, in addition to the significant changes witnessed in fungal growing seasons under the current anthropogenic increases and broader climate effects. Even if these findings are fungal species-specific, they indicate that fungal bioaerosol concentrations can be expected to change under eCO_2, with potentially wide-ranging effects for forested habitats (Baird and Pope, 2022). However, none of the above studies were completed in complex mature woodlands, with the experiments being completed in laboratories, open-topped chambers or, in the case of Aspen FACE, a young plantation forest. Mature and ancient woodlands represent a more complex and diverse environment, which are likely to respond differently to eCO_2 than the plantations of young trees on agricultural soil studied in earlier FACE experiments (Norby et al., 2016). There are also few studies of forest airborne spore concentration responses to eCO_2, with the majority of studies focussing on sporocarp production.

To study airborne forest fungal bioaerosols under eCO_2 in a mature temperate woodland, we installed low-cost Optical Particle Counters (OPCs) into the BIFoR FACE experiment during Autumn 2018. We assumed that bioaerosols can be represented by measured difference between PM_{10} (particulate matter less than 10µm) and PM_1 (particulate matter less than 1µm) mass concentrations, as detailed in the methods section.

Our hypotheses were:

A) Hourly fungal bioaerosol concentrations will correlate with hourly weather conditions (wind, RH, temperature).
B) Fungal bioaerosol concentrations will increase in woodland patches treated with eCO_2 for two years.

3.2 Methods

3.2.1 BIFoR FACE

The BIFoR FACE experiment methodology is described in detail in Section 2.3. In brief, the BIFoR FACE experiment allowed the investigation of the direct and indirect effect of eCO_2 on bioaerosols in a minimally disturbed forest environment. FACE arrays 1–6 only were used, measuring in each pair of arrays consecutively.

In order to focus on the fungal bioaerosol component, measurements were taken during the autumn, when pollen and non-fungal spore counts from the dominant vegetation were likely to be low. For example, the pollen counts are highest in spring for the two dominant tree species at BIFoR FACE. UK oak pollen season can range from March to July and lasts for 4–8 weeks, with peak concentrations usually occurring in May (Grundström et al., 2019). Hazel pollen season falls earlier in the year, starting as early as January, peaking in February or March (National Pollen and Aerobiology Research Unit, 2012). For ground cover plant species, the grass pollen season peaks in the summer months, sometimes extending into early September (National Pollen and Aerobiology Research Unit, 2012), and *P. connectilis* sporulation peaking mid-July until mid-September (Page, 1997). None of these pollen and fern sporulation seasons coincided with the measurement period. The measurement period coincided with the fungal fruiting season at BIFoR (see below), as well as previously measured peaks in UK fungal spore concentrations (Davies et al., 1963).

Monthly macro-fungi surveys completed during 2018 demonstrated that the annual peak of fungal fruiting in 2018 occurred across September and October at BIFoR FACE, with high levels of fungal fruiting continuing into November. A variety of species were present, with the majority of species fruiting producing spores capable of being measured by the 1-10 µm of the OPCs.

3.2.2 Instrumentation

In this study, OPC-N2 sensors were used (Alphasense, Essex), which count and size particles between 0.3 µm and 10 µm in diameter. The sensors are described in detail by Crilley et al. (2018) and Sousan et al. (2016). In brief, the OPCs count the number of particles, and use particle light scattering to determine particle size using Mie scattering approximations (an de Hulst, 1957). To calculate particle mass concentration, particles were assumed to be of uniform spherical shape, the density is assumed to be 1.65 g cm^{-3} and the refractive index is assumed to be 1.5 + i0. The values for density and refractive index were the taken from the standard settings of the OPC. The choice of particle density and refractive index has implications for the derived mass concentrations, however comparison between measurements taken within the woodland should be self-consistent. It is noted that fungal spores, the target of this study, are often ellipsoidal in shape and can be defined by a short and long axis diameter. Different fungal species produce spores with different shapes, sizes, and density. The assumptions used by the OPC are typically wrong on a single particle (or bioaerosol) basis but should approach reality on an ensemble averaged basis.

The sensors do not explicitly discriminate between particle types, so in order to discriminate between fungi and other smaller particles (bacteria and anthropogenic aerosols), data was excluded from particles smaller than 1 µm in diameter, measuring from 1 µm up to the maximum 10 µm measuring capacity of the OPCs. This size discrimination, in conjunction with the experimental location and seasonal timing of the experiment make it highly likely the majority of bioaerosols being captured were predominantly of fungal origin.

One of the key concerns raised for the sensing of any aerosol type using low-cost OPCs is that they can report artificially high readings under high RH conditions. Hygroscopic aerosols take up water from the surrounding gaseous environment as a function of RH, with greater water uptake at higher RH. To compare the abundance of aerosol under different environmental conditions, it is preferable to use a ‘dry’ aerosol concentration, with the hygroscopic growth due to water removed. To parameterise particle hygroscopicity, κ-Köhler theory is used, which describes the particle hygroscopicity using a single hygroscopicity parameter kappa (κ) (Petters and Kreidenweis, 2007). Pope (2010) details how κ-Köhler theory can be used to link particle mass to hygroscopicity by Eq. (1).

$$a_w = \frac{\left(\frac{m}{m_0} - 1\right)}{\left(\frac{m}{m_0} - 1\right) + \frac{\rho_w \kappa}{\rho_p}} \tag{1}$$

a_w is equilibrium RH expressed as a decimal, m is the wet aerosol mass, m_0 is the dry aerosol mass, and ρ_w and ρ_p the density of water and the dry particulate, respectively. The value of κ can be found by curve fitting of the pollen humidograms (m/m_0 versus a_w), although this was not explicitly measured for this study.

Biological particles such as fungal spores and pollen have been demonstrated to be hygroscopic, although they typically have a low kappa (κ) value, with pollen κ typically around 0.05–0.1 as opposed to a κ of approximately 0.3 for average anthropogenic aerosols (Griffiths et al., 2012; Pope, 2010). Due to the hygroscopicity of fungal aerosols, high relative humidity can result in a high mass concentration reading in sensors, such as the low-cost OPCs, which do not warm or dry the air. This hygroscopic effect has been explored using low-cost OPCs in urban environments by Crilley et al. 2020 and Crilley et al. 2018, where they used a calibration factor to correct for RH effects.

3.2.3 Instrument installation, bias-checking, and calibration

We used two low-cost OPCs to study bioaerosols at BIFoR FACE. The two OPCs used were controlled to take measurements every 60 seconds using a Raspberry Pi computer (Crilley et al., 2018, based on Hagan, 2017). Between 9th November and 13th December 2018, one OPC was installed in an eCO_2 array, with the other OPC in an aCO_2 array, rotating around the pairs of arrays (Table 3-i). The air inlet for each Optical Particle Counter was orientated towards the south-west in order to face the predominant wind direction for the site (Hart et al., 2019). The air flows through the instrument at a rate of 5.5 L/min.

Two Tinytag Plus 2 TGP-4500 (Gemini data loggers, Chichester, UK) units were used to measure RH and temperature alongside the low-cost OPCs. The Tinytag units measure temperature from -25°C to +85°C using an internally mounted thermistor, with the manufacturers stating an accuracy of 0.01°C or better. Under field conditions, the TinyTag sensors closely followed temperature measurements taken by a weather station located at 2 m height in Array 1, with the mean temperature measured by the TinyTags measuring within 0.4°C of the weather station. The Tinytags measure RH using an externally mounted capacitive sensor, from 0–100% RH, with a manufacturer stated accuracy of +/- 3.0% RH at 25°C. Tinytag units were placed on the top of the OPCs, with the RH sensor facing in the same direction as the OPC inlet.

Above-canopy wind speed was measured using two-D ultrasonic anemometers (WMT700, Vaisala) approximately 1 m above the canopy (25 m height) on the northernmost tower of Array 1. Below-canopy wind speed and direction (2 m height) were measured using 03002-L Wind Sentry set (Campbell Scientific, Loughborough, UK) located in Array 1. The manufacturer stated minimum threshold wind speed was 0.8 m s^{-1} for the Campbell anemometer, with 2 m height wind speeds below this minimum threshold considered as zero for the purposes of this experiment.

Table 3-i Dates and locations of equipment installation across the 2018 experimental duration at BIFoR FACE.

Date range (All dates in 2018)	Tiny Tag unit	OPC unit	Array number	Treatment
9th Nov to 21st Nov	A	1	A2	aCO_2
	B	2	A4	eCO_2
21st Nov to 30th Nov	A	1	A6	eCO_2
	B	2	A5	aCO_2
5th Dec to 13th Dec	A	1	A1	eCO_2
	B	2	A3	aCO_2

Before the main measurement period, both the pair of OPCs and TinyTags were installed for a six-day side-by-side intercomparison period at 1.5 m height in Array 1 of BIFoR FACE from 30th November 2018 until 05th December 2018. Using the data from this intercomparison week, bias correction factors were calculated to ensure inter-instrument consistency.

No bias factor was applied to the temperature data from the TinyTag units. RH data from the TinyTags was first filtered to remove any measurements lower than 50% and greater than 99%, and a bias correction factor of 1.03 applied to TinyTag Unit B. A similar calibration was performed on the particulate matter (PM) data collected by the OPCs, with the data from OPC 1 being increased by a bias factor of 1.45. Bias factors were calculated from the six-day side-by-side period, with the factor applied to the data from the full measurement period of 37 days.

For both the TinyTag and OPC pairs, the consistency between the two instruments, rather than absolute calibration, was deemed more important to allow for observation of differences between two different locations. Hence, one instrument of each pair was bias-corrected to the other instrument of the pair. However, a comparison outside of the forest environment was completed between the low-cost OPCs and a TSI 3330 (a reference grade OPC), with the low-cost OPCs measuring within 25% of the TSI without any humidity corrections, which is considered a good performance for the low-cost sensors (Crilley et al., 2020, 2018).

3.2.4 Spore dispersal model

A conceptual model was developed to help interpret the OPC results from BIFoR FACE. Within dense forests, the mean horizontal wind speed *U* inflects around the tops of the trees, increasing approximately exponentially with height below the inflection, and logarithmically with height above it (Bannister et al., 2021; Cionco, 1965; Finnigan, 2000; Raupach et al., 1996). Mean horizontal wind velocity *U* at each height *z* inside the canopy can, therefore, be approximated via Eq. (2):

$$U(z) = U_{h_c} e^{-a\left(1-\frac{z}{h_c}\right)} \tag{2}$$

where h_c is the mean height of the trees and U_{h_c} is *U* at h_c. The attenuation coefficient, *a*, accounts for the flow's response to the density of the forest and tends to increase with increasing leaf area index (LAI) and flexibility of the plant elements (Cionco, 1978; Kaimal and Finnigan, 1994) The exponential relationship in Eq. (2) does not hold in forests with a sparse trunk space and open edges, e.g. many pine plantations, for which a secondary wind velocity maximum occurs below the main crown (Baldocchi and Meyers, 1988). However, it provides a reasonable first approximation for a forest such as that at the BIFoR FACE site, with extensive understorey growth and closed edges during the leaf-on season.

We adapted the model of Nathan et al., 2002 by using this exponential velocity profile to obtain a rough estimate of the horizontal distance over which spores disperse in a dense forest, *D.* We consider spores evenly distributed within small air parcels within the canopy airspace. *D* equals the distance an air parcel carrying spores travels between the time of release ($t_0 = 0$) and the time at which the spores in the parcel settle on the ground (t_1). As a simplification, here we consider spore 'release' to include both detachment by the mean wind and the point at which spores begin to settle back to the ground after being swept upwards by short intense gusts (Aylor, 1978). For a spore falling at an average settling velocity v_s, $t_1 = h_r/v_s$, where h_r is the height of spore release. The vertical position of spores within an air parcel during settling at time *t* is $z(t) = h_r - v_s t$. Substituting this expression into Eq. (2), we generate Eq. (3):

$$U(t) = U_{h_c} e^{-\frac{a}{h_c}(h_c - h_r + v_s t)} \tag{3}$$

The horizontal distance over which the spores disperse is therefore:

$$D = \int_0^{t_1} U(t)dt \tag{4a}$$

which, taking $t_1 = h_r/v_s$ and $U(t)$ from Eq. (3), gives:

$$D = \frac{U_{h_c} \cdot h_c}{a v_s}\left[e^{-a}\left(e^{\frac{a h_r}{h_c}} - 1\right)\right] \tag{4b}$$

This model assumes the mean wind profile has already adjusted to the presence of the forest, for example, after passing into the forest from surrounding rural areas (see below).

We specified the mean height of the trees as 25 m to reflect those at the BIFoR FACE site. Reported values of a for forests fall within a relatively narrow range of around 1.5–4.8 (Amiro, 1990; Cionco, 1978; Su et al., 1998). We took $a = 2.5$, as a reasonable assumption for autumn conditions at BIFoR FACE, and measured for forests of similar LAI. LAI measurements at BIFoR FACE peak at 7 – 8 in summer, and drop to below 2 in winter (Bannister et al., 2023; Su et al., 1998). Sensitivity testing (not shown) indicated that using higher and lower values of *a* respectively decreased and increased the occurrence of spores travelling long distances, but had little effect on the modal value of *D.* i.e. using a lower a thickened the right tail of the probability density function of *D* but affected its peak relatively little.

We performed a stochastic ensemble of model runs (*n* = 1,000,000) in R (version 4.0.3, http://cran.r-project.org) with low (0–2 m s^{-1}), low-medium (*med-l*) (2–3 m s^{-1}), high-medium (*med-h*) (3–4 m s^{-1}), and high (4–6 m s^{-1}) mean wind speeds at the top of the canopy, U_{h_c}. We considered only spores in the bottom 10 m of the forest airspace, i.e. h_r in the range 0–10 m. The model was run (n = 1,000,000) for spore release heights (h_r) of 2 m, 5 m, and 10 m. Strong turbulent fluxes of momentum, and scalar quantities such as spores, occur in the upper region of forest canopies (Belcher et al., 2012; Finnigan, 2000), which this simplified model cannot capture. Mean settling velocity, v_s, was specified in the range 0.001–0.005 m s^{-1}, taking reported values for fungal spores in the size range 1–10 µm (Di-Giovanni et al., 1995; Tesmer and Schnittler, 2007). For each model run, for we specified a random value of U_{h_c}, h_r and v_s from a uniform distribution within each of these ranges (using the *runif* function in R).

3.2.5 Data processing, visualisation, and analysis

All data analysis was completed in R version 4.0.3 (R Core Team, 2020), with figures created using *openair* and *ggplot* (Carslaw and Ropkins, 2012; Wickham, 2016). Relationships between PM_{10}-PM_1 concentrations and RH, temperature, and wind speed were visualised used scatter plots and smoothed *loess* curves, generated in *ggplot*. Box plots with mean PM_{10}-PM_1 (and interquartile ranges) were generated to visualise differences in bioaerosol concentrations between eCO_2 and aCO_2 arrays. Scatter plots with regression lines were generated for Figure 3-vi.

Kruskal-Wallis tests were used to test for statistically significant differences between the eCO_2 and aCO_2 arrays in Section 3.3.2.

3.3 Results

3.3.1 Hypothesis A: Hourly fungal bioaerosol concentrations will correlate with hourly weather conditions (wind, RH, temperature).

During the experimental period, there was a total of 46.3 mm of precipitation, with a median temperature of 7.7°C within the forest. The lowest recorded temperature was -1.8°C, and the highest 15.8°C. The average daily temperature peak (and the lowest RH) occurred at approximately 13:00 local time. RH throughout the measurement period was typically high, with the median and mean measured between 89–91% RH across the duration of the experiment, with the lowest recorded measurement at 63% RH. Wind speed patterns followed each other closely above and below the canopy, with below canopy measurements significantly lower. The mean wind speed above the canopy was 2.7 m s^{-1}, whereas below the canopy it was 0.4 m s^{-1}.

From 75 – 90% RH there was no effect on bioaerosol concentrations, however at RH greater than 90% there was a rapid increase in concentrations (Figure 3-i A). This sudden increase in particle size at very high humidities demonstrated a clear hygroscopic effect; we therefore performed a correction factor to the data from Crilley et al., 2018, using a κ values of either 0.3 or 0.1 (Griffiths et al., 2012; Pope, 2010). Before correction, the median hourly PM_{10}-PM_1 concentration under eCO_2 conditions was 15.7 µg m^{-3} and 16.7 µg m^{-3} under ambient conditions (Figure 3-i A). After the application of the Crilley et al. correction factor using a κ of 0.3, this decreased to a median of 5.3 µg m^{-3} under eCO_2 and 5.9 µg m^{-3} at ambient CO_2 concentrations (Figure 3-i B), and using a κ of 0.1, the median was 9.3 µg m^{-3} under eCO_2, and 10.1 µg m^{-3} under ambient conditions (Figure 3-i C). The ratio between the mean concentrations obtained from the eCO_2 and aCO_2 plots depended upon whether the Crilley correction factor was used and what value was used for κ. The values were 94%, 90%, and 92% for the uncorrected ratio, the ratio using κ = 0.3 and the ratio using κ = 0.1, respectively (Figure 3-i D). Due to the decrease in PM concentrations with respect to RH shown using a κ of 0.3, the data corrected using a κ of 0.1 was used for all further analyses, as this was deemed more appropriate given the likely particle composition (low hygroscopicity bioaerosols). After correction to remove the instrument artefacts, there was no effect of RH on bioaerosol concentrations. If the observed median concentrations of PM_{10}-PM_1, using the Crilley correction with a κ of 0.1, were solely composed of idealized spherical fungal spores with radius of 3mm and density of 1.65 g/ml this would equate to spore concentration of approximately 50,000 spores m^{-3}, which falls at the high end of Sesartic and Dallafior (2011) estimate of ground level spore concentrations of 10,000 – 50,000 m^{-3}.

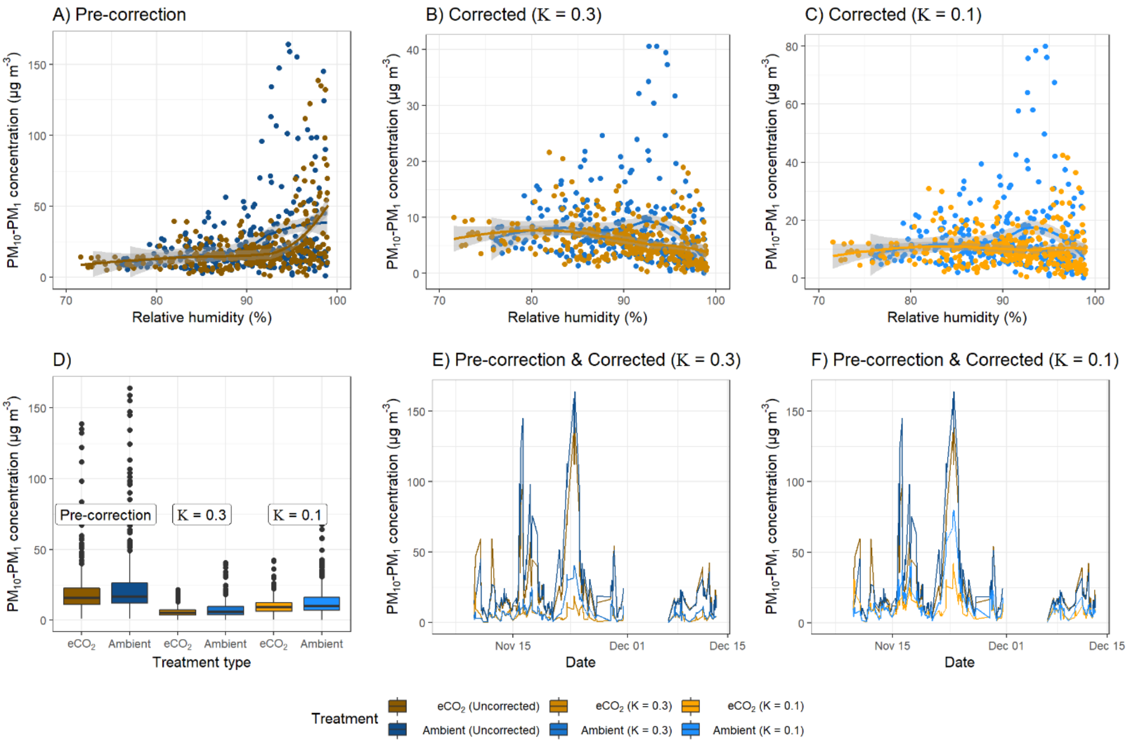


Figure 3-i Relative humidity and bioaerosol concentrations (PM_{10}-PM_1) at BIFoR FACE in Autumn 2018. All the plots show PM_{10}-PM_1 concentrations under aCO_2 (Arrays 2, 3, and 5) in blue colours, and eCO_2 (Arrays 1, 4, and 6) in orange colours. Data pre-correction is shown is dark orange and blue, corrected data using a κ of 0.3 mid-blue and mid-orange, and the corrected data using a κ of 0.1 uses the light blue and orange. Subplots are: (A) PM_{10}-PM_1 concentrations without any correction applied, (B) and (C) PM_{10}- PM_1 concentrations with a correction factor from Crilley et al., 2018. (B) used a κ of 0.3, and (C) a κ of 0.1 for the correction

factor calculation. (D) PM_{10}-PM_1 concentrations for each correction type, and (E) and (F) timeseries of PM_{10}-PM_1 concentrations.

Below-canopy wind speed measurements broadly followed the trend of those above the canopy, but measured significantly lower speeds at the lower height (Figure 3-ii A). Above the canopy, the hourly wind speed never dropped below 0.8 m s^{-1}, whereas recorded speeds of 0 m s^{-1} (i.e. below anemometer threshold) were common below canopy. The maximum hourly wind speed above canopy was 7.05 m s^{-1}, compared with 2.59 m s^{-1} below canopy.

Bioaerosol concentrations demonstrated high variability under changing wind speeds; however, a possible U-shaped curve was observed, with higher bioaerosol concentrations at the lowest and highest wind speeds measured (Figure 3-ii B and C). This effect was shown using the wind speeds taken from both above (25 m) and below (2 m) the canopy. Figure 3-iii shows the effect of wind speed and direction on bioaerosol concentrations using *openair* polar bivariate plots (Carslaw and Ropkins, 2012). Observed wind speed patterns follow broadly similar trends between each pair of arrays, with the majority of medium concentration (green-yellow) bioaerosol activity occurring in the SW and SE quadrants, and only low (blue) concentrations under northerly winds. Peaks in bioaerosol concentration (presumed high sporulation events) are visible in red, with some events being replicated across both eCO_2 treatment and control (e.g. the SW quadrant event in A5 and A6), and other high PM events only occurring in a single array (e.g. the SE quadrant event in A4). Detecting high PM events in a single array at a distinct time indicates that the OPCs can detect differences between the BIFoR FACE arrays.

To investigate the relationship between spore dispersal and wind speed further, we used the model outlined in Section 3.2.4 to investigate the horizontal distance over which spores disperse, *D*. Figure 3-iv shows the probability density functions of *D* under four wind speed scenarios: low, low-medium, medium-high, and high. As would be expected, generally, the lower the spore release height and wind speed, the shorter the overall distance travelled by spores. Most wind conditions experienced at BIFoR FACE fell under the “low” wind speed scenario (black lines), for which the modal distance travelled by spores was less than 20 m for all release heights (Figure 3-iv). With the arrays having diameters of 25–30 m, this means that under typical conditions, we could expect spores to stay within the array they were released in or, at least, be very unlikely to be transported into neighbouring arrays (i.e. a spore released under eCO_2 is unlikely to be measurable in an aCO_2 array in low wind conditions). However, at higher wind speeds, a heavy tail was present on each of the plots, indicating that spores are more likely to travel much greater distances at higher wind speeds, potentially causing mixing between the arrays.

There was a small linear positive relationship between temperature and bioaerosol concentrations, with PM_{10}-PM_1 concentrations of around 5 µg m^{-3} at the lowest temperatures (1-3 °C), increasing up to 10 µg m^{-3} at the highest temperatures of 12-13 °C.

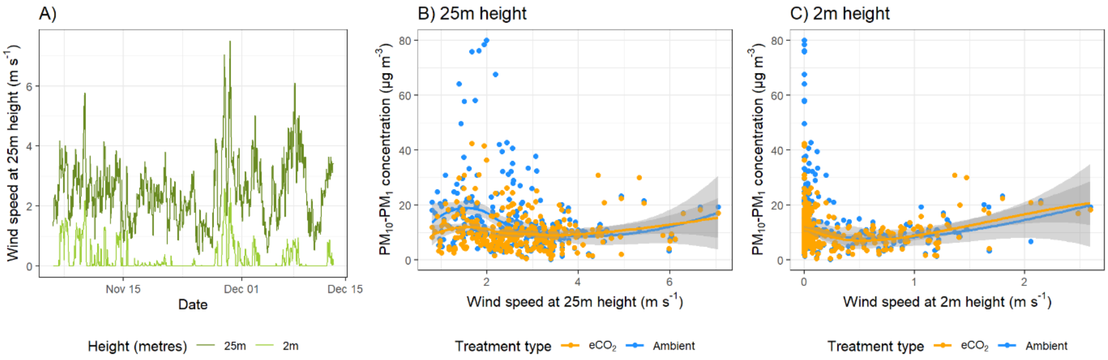


Figure 3-ii Wind speed and bioaerosol concentrations at BIFoR FACE in Autumn 2018. Data from aCO_2 arrays (Arrays 2, 3, and 5) in blue, and data from eCO_2 arrays (Arrays 1,4, and 6) in orange. Subplots are: A) Hourly average wind speeds above (25 m height) and below (2 m height) the canopy with wind speed data at 25 m height is in dark green, and data from 2 m height in light green. Subplot B) compares hourly average PM_{10}–PM_1 concentrations with hourly average wind speeds measured at 25 m height, and C) the same PM_{10}–PM_1 concentrations using wind speeds measured at 2 m height.

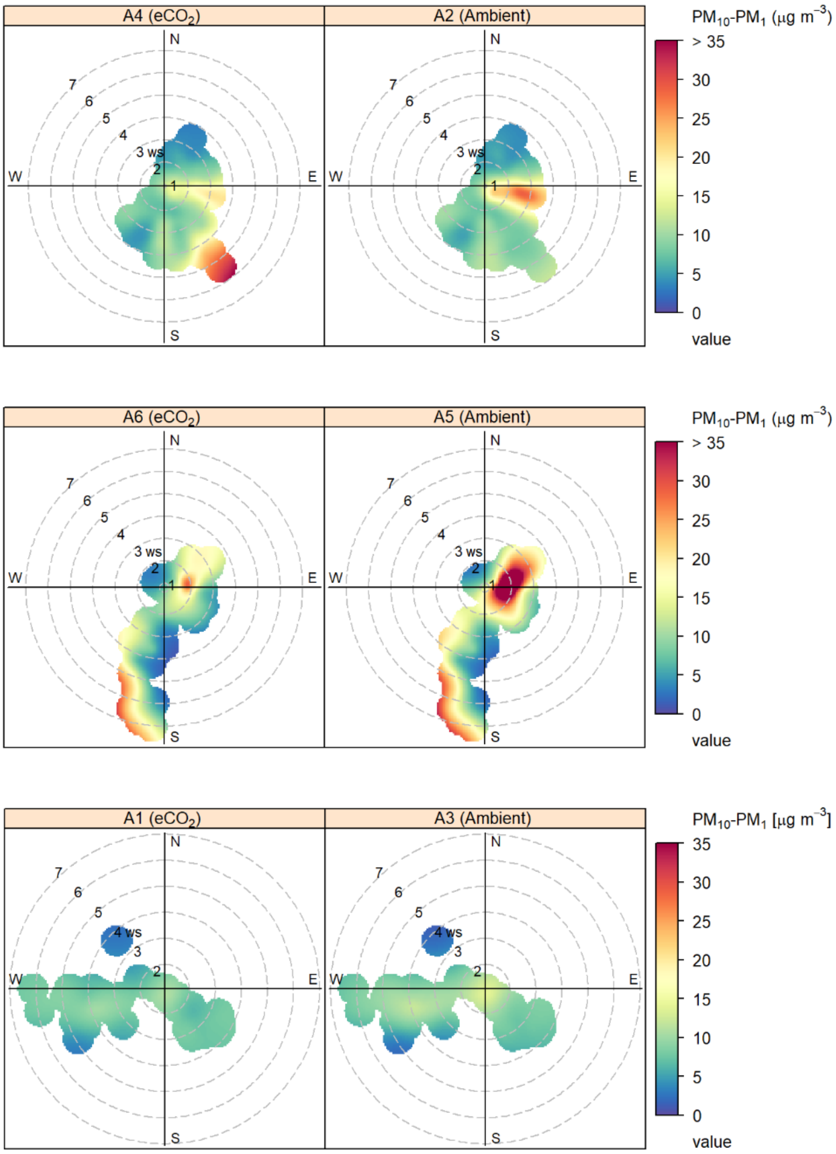
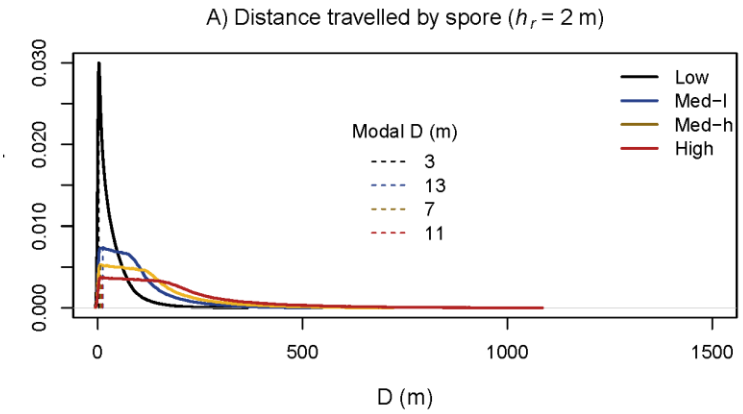
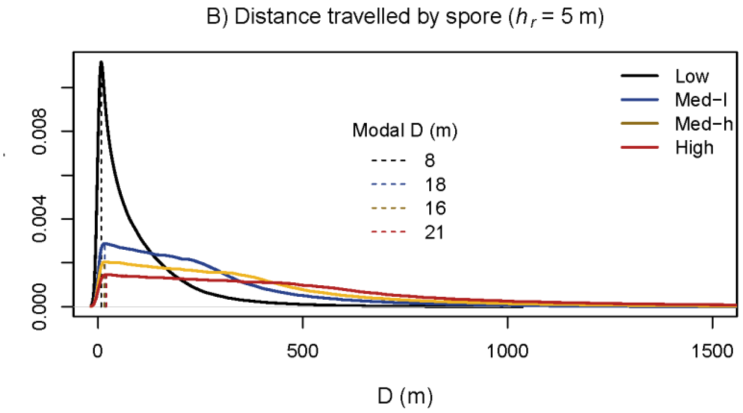


Figure 3-iii. Openair polar bivariate plots showing bioaerosol concentrations (PM_{10}-PM_1) with wind speed and direction across the Autumn 2018 experimental duration at BIFoR FACE (Carslaw and Ropkins, 2012). Subplots are displayed in the three array pairs (one eCO_2, one aCO_2) in which the pair of OPCs were located. Dates of OPC installation in each array pair are detailed in Table 3-i. Colour gradients display the concentrations of bioaerosols detected, with low concentrations shown in blue/green colours, mid concentrations in yellow, and high concentrations in orange/red.


A) Distance travelled by spore ($h_r = 2$ m)


B) Distance travelled by spore ($h_r = 5$ m)

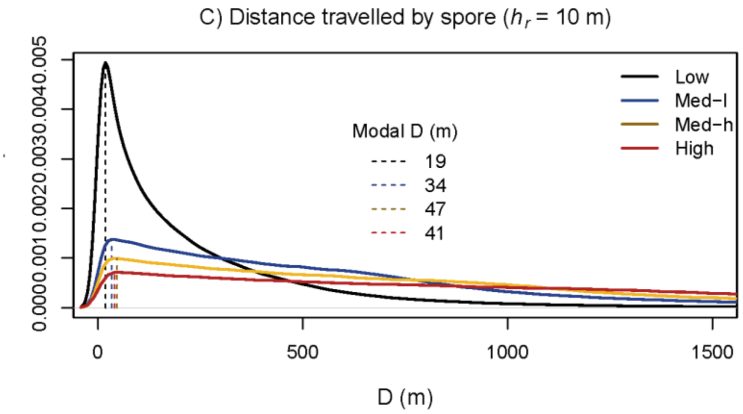

C) Distance travelled by spore ($h_r = 10$ m)

Figure 3-iv. Model outputs of probability density functions for distance travelled by spores in a forested environment. Subplots A), B), and C) use spore release heights (h_r) of 2, 5, and 10 m respectively. Solid lines are in different colours for each of the low (black), low-medium (blue), medium-high (yellow) and high (red) $\boldsymbol{U_{h_c}}$ (wind speed) cases. Dashed lines show modal D (distance) travelled by the spores. N = 1,000,000 for each case.

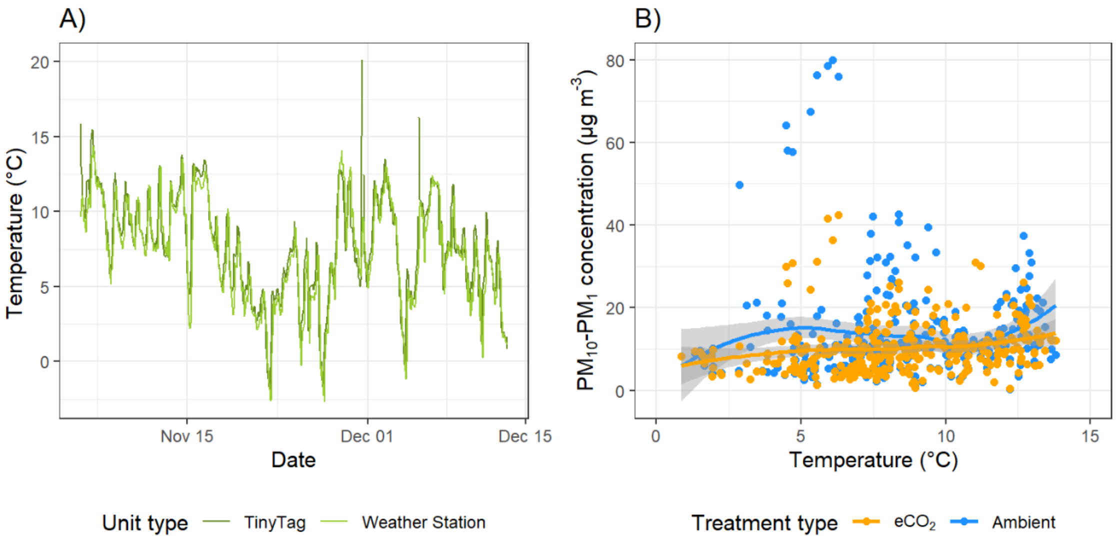


Figure 3-v Temperature and bioaerosol (PM_{10}-PM_1) concentrations across the experimental duration at BIFoR FACE. Subplots are: A) Temperature data, TinyTag data shown in dark green, Array 1 weather station data in light green, B) compares temperature data with bioaerosol concentrations, with data from aCO_2 arrays (Arrays 2,3,and 5) in blue, and data from eCO_2 arrays (Arrays 1,4, and 6) in orange.

3.3.2 Hypothesis B: Fungal bioaerosol concentrations will increase in woodland patches treated with eCO_2 for two years.

Bioaerosol concentrations matched extremely closely between the two treatment groups (Figure 3-vi B). The small difference in the overall medians (Figure 3-vi A) can be largely attributed to the PM_{10}-PM_1 concentration difference between the eCO_2 and aCO_2 during the largest bioaerosol event around the 22nd of November (during the second measurement period, shown shaded in grey), where the control array (shown in blue) measures higher than the eCO_2 array in orange.

Although a small difference was visible between the mean bioaerosol concentrations in eCO_2 versus aCO_2 conditions (Figure 3-vi A) there was no significant difference ($p = 0.489$) between two groups, however a heavy tail was present in the ambient arrays.

To investigate this relationship further, we separated out the data into low bioaerosol concentration conditions (<10 µg m^{-3}) and high bioaerosol concentrations (>= 10 µg m^{-3}) (Figure 3-vi D, E, and F). When bioaerosol concentrations were low (Figure 3-vi D and E), there was no significant difference between the eCO_2 and ambient arrays ($p = 0.689$). These low concentrations likely represent background levels of aerosols that are consistent throughout the forest. However, when selecting for high bioaerosol concentration events, there was a significant effect seen, whereby eCO_2 treatment suppressed concentrations of high bioaerosol events ($p = 0.023$) (Figure 3-vi D and F).

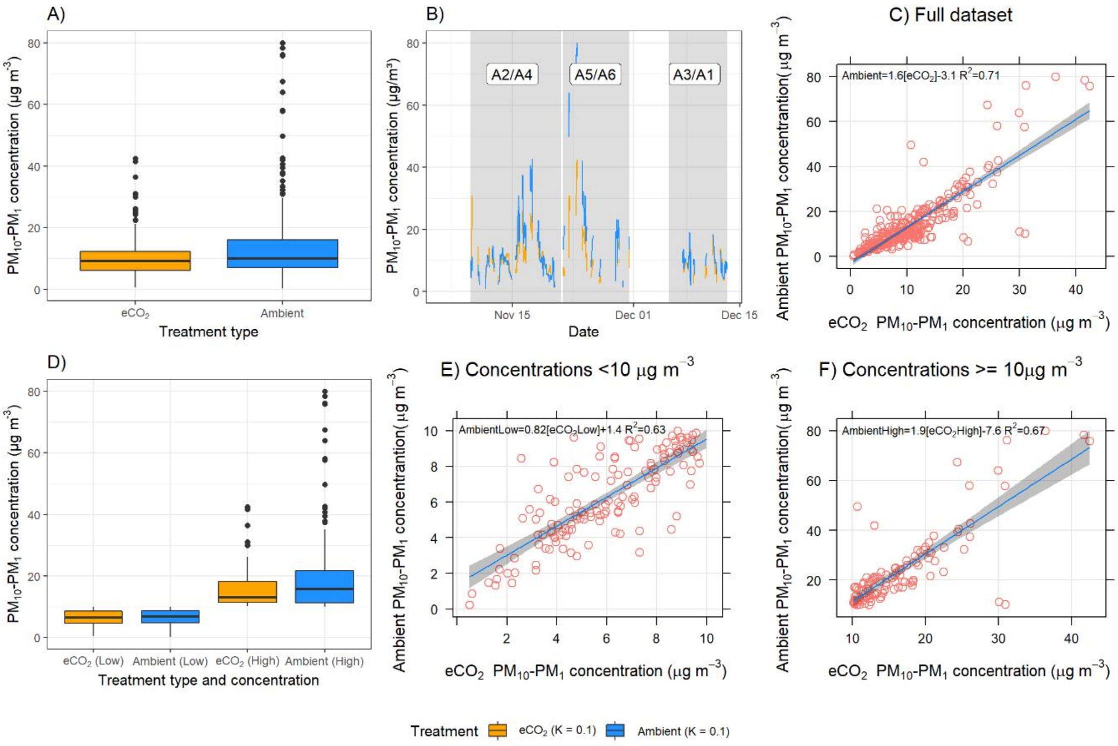


Figure 3-vi. Bioaerosol concentrations across the experimental duration in ambient (Arrays 2,3, and 5) and eCO_2 (Arrays 1, 4, and 6) arrays. eCO_2 treatment is shown in orange, ambient

control in blue. Subplots A, B, and C include the full dataset. Subplots D, E, and F split the dataset into low (<10 µg m^{-3}) and high (>=10 µg m^{-3}) bioaerosol concentrations.

3.4 Discussion

In hypothesis A, we suggested that hourly bioaerosol concentrations would correlate with hourly weather conditions. In support of hypothesis A, we found that various meteorological variables affected bioaerosol concentrations. The RH before correction was of particular importance, especially as the low-cost OPCs do not have any warming or drying capacity, and therefore are susceptible to recording higher mass concentrations of aerosols under high humidity conditions as particles swell with additional water (Crilley et al., 2018). The RH % threshold for significant particle swelling was 90–95%, which is a much higher RH value than would be expected for anthropogenic aerosols, which typically contain more hygroscopic components, and therefore provides evidence that the measured PM_{10}–PM_1 fraction represents a predominantly biological source. This result matches others from the literature, where significant swelling of fungal spores was seen, but only at very high humidities (greater than 90%) (Reponen et al., 1996). We believe this threshold for particle swelling further demonstrates that we are recording a biological source. This hygroscopic evidence is in addition to the ecological and phenological evidence for spores being the dominant source within the PM_{10}-PM_1size fraction during the measurement period.

After correction, we did not see any effect of RH on fungal spore concentrations. The evidence in the literature for the relationship between spore concentrations and RH is mixed, probably largely due to species-specific effects. Some fungal species are dependent on high RH to develop spores (and then release spores by rain droplets), whereas other species release spores in response to a drop in RH (Jones and Harrison, 2004; Li and Kendrick, 1995). The high diversity of fungal species at BIFoR (each with possibly varying responses to RH) could result in this flat line. Another possible explanation is that the fungi never experience low RH conditions due to the consistently high RH at BIFoR FACE, potentially with the RH never dropping low enough to trigger a burst of spore release. Finally, it is important to mention that although the Crilley correction is an essential part of using low-cost sensors in a high-humidity environment, the high swelling threshold of fungal spores means that the κ value of 0.3 (or even 0.1) may be inappropriately high for biological particles such as these. As shown in Figure 3-i B and C, after correcting the data, the PM_{10}–PM_1 concentrations decrease after 95% RH, although this effect is smaller when using a κ of 0.1. For future studies, calibrating the low-cost OPCs against a reference grade instrument in the experimental environment would enable a more thorough investigation of the RH dependence, provide a calibrated κ-value, and therefore improve the accuracy of the Crilley correction for bioaerosols (Crilley et al., 2020).

The data showed approximately U-shaped curve of bioaerosol concentrations in response to wind speed, with the highest PM_{10}–PM_1 concentrations being displayed at low and high wind speeds. This likely indicates a balance between spore release mechanisms and mixing of low-aerosol air. At low wind speeds, there is minimal movement of air through the forest, so any spores released do not travel significant distances. At high wind speeds, there is much higher movement of air, which decreases concentrations by carrying spores further; however, spore release by wind could also be increased (Dressaire et al., 2016). At medium wind speeds, a balance between these two effects occurs, maintaining bioaerosol concentrations at a lower level (Jones and Harrison, 2004).

Our model demonstrated that under low wind-speed conditions, spores are most likely to travel a short distance (less than 20m), suggesting that between-array mixing was unlikely under the meteorological conditions experienced during the measurement period. Additionally, a comparison between the regional EAC4 bioaerosol concentrations and BIFoR FACE bioaerosol concentrations did not show any correlation. Hence, we believe the measured PM_{10}-PM_1 is local to woodland, likely local to an individual array, and not representative of the regional air mass. Both the model results and the EAC4 comparison match with the large body of literature which shows that the majority of fungal spores travel only a short distance, with only a very small percentage travelling longer distances (for example: Galante et al., 2011; Money, 2016; Norros et al., 2012).

Particle release height (h_r) had a significant impact on the distance travelled by a particle, which is an important consideration given the source of bioaerosols. For example, fungal spores are likely to have a significantly lower h_r than pollen which, based on our model, would significantly impact the distance travelled by the different bioaerosol types. Due to the complexity of the forest environment, there are a number of aspects which the model did not capture which could alter the distance travelled by a spore. Complex wind dynamics such as ejections (infrequent but strong upward gusts of air) and turbulence around forest edges could increase the distance travelled by a spore, as well as varied spore release dynamics between fungi (Dressaire et al., 2016; Norros et al., 2014). The model also assumes a spatially representative wind speed profile, which does not account for local velocity effects induced by gaps, clearings, and changes in canopy density. These local effects may be important near the ground, where wind speeds are generally low. However, the distance travelled could also be shorter than modelled, for example, the model does not capture spore deposition onto forest surfaces other than the ground, spores being swept downwards by gusts, and changes with wet versus dry deposition.

The data demonstrated a decrease in bioaerosol concentrations at lower temperatures, which is the expected response within the range of temperatures we observed in a woodland of this type (Sadyś et al., 2016b, 2016a). The number of fungal sporocarps present across the duration of the experiment decreased, and it is therefore logical to expect that spore production would also consequently decrease. For future studies, it would be interesting to begin measurements earlier in the fungal season, allowing us to capture the full duration of the main sporocarp season, as well as the end of the active eCO_2 fumigation at the FACE experiment.

For Hypothesis B, we predicted that two years of eCO_2 would increase fungal bioaerosol concentrations. Regarding this hypothesis, to our knowledge, this is the first assessment of bioaerosols in any forest FACE experiment to date, and therefore provides valuable data contributing to the understanding of the forest environment at BIFoR FACE as well as in the broader context of other FACE experiments, and woodlands more widely. We did not see a significant effect of eCO_2 on total aerosol concentrations. However, when data were split into low and high aerosol concentrations, two differing responses were demonstrated. Under low concentrations, there was no significant effect of eCO_2 treatment, we therefore think these low concentrations represent the background concentrations of aerosols present both inside and in the wider environment around the forest. However, when high concentrations of bioaerosols were detected, eCO_2 treatment resulted in a suppression of PM_{10}-PM_1 concentrations, suggesting that fungal spore production was lower under eCO_2. This was demonstrated in a number of larger sporulation events during the experimental period, for example the large sporulation event peaking on 22nd November, where although both arrays showed the sporulation event, the magnitude of this event was significantly different between eCO_2 and aCO_2.

This evidence, as well as the eCO_2 dispersal data from Hart et al. (2019) and the modal spore travel distances shown in our model suggests to us that this experimental set-up is capable of detecting differences between the eCO_2 and aCO_2 arrays. It is apparent that at background concentrations these differences are not present between arrays, however there may be a response to eCO_2 during large sporulation events. This could be for a number of reasons, the primary reason being that the experiment was in the very early stages of fumigation (year two of ten total years planned), and there might be a lag expected in fungal responses, as they are most likely responding indirectly to eCO_2 via changes in leaf litter and soil. For example, competition between autotrophs and heterotrophs for nutrients, or an increase in leaf litter production resulting in an increase the population of decomposer fungi. There is evidence for increased autotrophic productivity under eCO_2 at BIFoR FACE in the form of increased leaf-scale photosynthesis (Gardner et al., 2020) and fine root production (Ziegler et al., 2021). Whether this increased autotrophic productivity then triggers a subsequent increase in fungal activity requires further work. Continuing to monitor bioaerosol concentrations throughout the 10+ year experimental duration of the BIFoR FACE experiment, along with monitoring in other FACE experiments, will be key in understanding how eCO_2 affects bioaerosol concentrations long term.

Another possible reason for not picking up an eCO_2 response in fungal bioaerosols in the full dataset is that the maximum diameter of the particles that can be detected by the OPCs is 10 µm, which does exclude several fungal species, including those known to be present in the BIFoR forest during the experimental duration. However, we note that many common woodland spore species are smaller than 10 µm, including a variety of species observed fruiting during the bioaerosol measurement period. In addition to the observed species, many spores commonly observed to be airborne in the UK, including *Cladosporium*, *Ganoderma*, and *Aspergillus* species, have diameters less than 10 µm (Sadyś et al., 2016). If a response to eCO_2 is species-specific, then it is possible that we are missing an effect in fungi with larger spores. It is also possible that although the total and background aerosol concentration was stable under eCO_2, the aerosol composition could have been different (e.g. altered ratios of fungal species present), which we were not able to detect using the OPCs. Different fungal community composition or abundances between the aCO_2 and eCO_2 arrays could therefore be responsible for the differing response we detected during sporulation events. In order to determine definitive particle composition, other techniques such as detectors using fluorescence, or DNA sequencing of biological material is required (Fröhlich-Nowoisky et al., 2016; Gosselin et al., 2016; Healy et al., 2012). Finally, our experimental measurement period occurred outside of the eCO_2 fumigation season, and therefore the main growing season of the trees, which could have reduced any direct effects of the eCO_2, although, given the likely cumulative effect of the eCO_2 treatment over the growing season, and the fact that our measurements were focused on the period of peak sporulation, it seems more likely that our measurements were well-timed to observe any treatment effects.

3.5 Conclusions

We have demonstrated that low-cost OPCs are suitable for measuring PM_{10}-PM_1 concentrations in forests, or other high-humidity environments. We demonstrated that the PM_{10}-PM_1 metric is a good proxy for bioaerosols because of the bioaerosol representative size range, the location of the study site (a woodland in a rural location), the field measurement taking place during the season of peak fungal activity, and the low hygroscopicity of the particles measured. Through comparison with the EAC4 estimate of PM_{10}-PM_1 we highlighted that the woodland measurements do not follow regional air pollution trends and that the observed PM_{10}-PM_1 concentrations are likely from woodland sources. The findings from this study have consequences for other research into the aerobiology of forests, and also opens up the bioaerosol research field to a wider array of locations and researchers. The low-cost sensors measured significant swelling in PM_{10}-PM_1 sized particles at very high RH, which we corrected for using a calibration factor from Crilley et al. (2018). For future work, generating a κ value using a reference-grade instrument in situ would improve accuracy (Crilley et al., 2020). Temperature, wind speed, and wind direction were all shown to affect bioaerosol concentrations; however, we did not see any effect of RH (post-correction). Elevated CO_2 may repress concentrations of bioaerosols during high sporulation events ($p = 0.023$), however this significant difference was not detected across all concentrations of aerosols ($p = 0.489$). Therefore, further investigation later in the 10+ year experimental duration is warranted, as well as investigating forest bioaerosols in other forest FACE experiments globally. The use of the PM_{10}-PM_1 metric as a proxy for bioaerosols, in woodland and other settings, should be further evaluated through future experiments that unambiguously measure bioaerosol concentrations.

CHAPTER 4: THE IMPACT OF THREE YEARS OF ELEVATED CO_2 TREATMENT ON MASS CONCENTRATIONS OF WOODLAND BIOAEROSOLS

4.1 Introduction

As discussed in Chapter 3, the influence of bioaerosols on forest biology is often overlooked, despite the important roles that fungal spores, and bioaerosols more widely play in woodlands. In forest FACE experiments, bioaerosols have been even further overlooked, with the study described in Chapter 3 being the only published work on bioaerosols from a forest FACE experiment to date (Baird et al., 2021).

In this previous study, a clear local woodland source of bioaerosols could be detected. The bioaerosol concentrations were affected by temperature, wind speed, and wind direction, with a possible decrease in bioaerosol concentrations in high sporulation events with eCO_2 treatment. In this chapter, we aimed to build on previous work, providing further evidence regarding hypotheses, as well as addressing some of the remaining queries from the 2018 study.

A key element of the previous study was the use of low-cost Optical Particle Counter (OPC) sensors to measure bioaerosol concentrations. As demonstrated, these sensors can be used very effectively to measure bioaerosols, however a corrective factor must be applied to account for high-humidity particle swelling (Baird et al., 2021; Crilley et al., 2020, 2018). Post-correction, no effect of RH on particle concentrations was detected. To specifically test this finding further in this new study, airborne particle sensors with drying capacities were installed to measure bioaerosol concentrations in Autumn 2019.

The sensors differed from the OPCs used in Chapter 3, as the particles were passed through a stream of heated air, drying the particles before sizing takes place. This makes results from these instruments less susceptible to particle hygroscopicity affecting the mass concentration readings at high humidity levels.

Additionally, in the previous study, the measurement period started part way through the main fungal fruiting, and therefore sporulation, season. The measurements also commenced after eCO_2 fumigation had ended for the 2018 season. The timing of this measurement period left questions regarding the effects of active eCO_2 fumigation on fungal bioaerosols, as well as the fungal species which fruit earlier in the season in September and October. To address these queries, the measurement period was extended for the 2019 study, starting earlier in the fungal fruiting season, and capturing aerosol concentrations both during active eCO_2 fumigation and once the FACE system was switched off at the end of the 2019 season.

We tested similar hypotheses to Chapter 3, specifically:

A) Hourly fungal bioaerosol concentrations will correlate with hourly weather conditions (wind, RH, temperature).
B) Fungal bioaerosol concentrations will increase in woodland patches treated with eCO_2 for three years.

4.2 Methods

4.2.1 BIFoR FACE

This chapter uses a modified methodology from Chapter 3. As with the previous study, BIFoR FACE was used as the experimental site. The duration of this study was extended to capture more of the fungal fruiting and sporulation season, starting in early October. Despite the earlier start to the measurement period, it remains unlikely that significant quantities of plant pollen were airborne at this time. As before, monthly sporocarp surveys were completed during the measurement period (Chapter 5), confirming that significant quantities of fungal spores were present in the woodland during the measurement period.

4.2.2 Instrumentation

For this measurement campaign, two aerosol sensors, specifically a TSI 3330 Optical Particle Counter and a TSI Dusttrak light-scattering laser photometer (TSI Inc., Minnesota, USA) were used. These sensors do not explicitly discriminate between particle types, so as previously, PM was used as a proxy for bioaerosols.

Due the slight differences in measuring technologies between the two aerosol sensors used, they had different sensitivities to the smallest particle sizes, meaning there was not good agreement of PM_1 mass concentrations during the side-by-side intercomparison period. However, agreement at a PM_{10} scale was good. Therefore, for the purposes of this chapter, PM_{10} was used for all analyses (as opposed to the PM_{10}-PM_1 used in Chapter 3).

Above-canopy wind speed averages were measured using sensors located at the top of four towers located on the edges of the woodland, with below-canopy wind speed measured using a 03002-L Wind Sentry set located in Array 1. For the array specific wind speed data, above-canopy wind speed was measured using two-D ultrasonic anemometers (WMT700, Vaisala) ~1 m above the canopy (~25 m height) on the northernmost tower of Arrays 1, 4, and 6. Tiny Tag sensors were not used for this measurement campaign. RH and temperature data for the experimental duration were measured using sensors located near the bottom of the four towers located on the woodland edges.

4.2.3 Instrument installation, bias-checking, and calibration

The aerosol sensors were initially installed side-by-side in BIFoR FACE Array 1 for a 24-hour intercomparison period on 07th October, before being installed in a pair of

BIFoR arrays (one eCO_2 and one aCO_2) at a time from 09th October 2019 – 03rd December 2019 (as detailed in Table 4-i).

Table 4-i The dates and locations of instruments across the 2019 measurement campaign at BIFoR FACE. Sensor 3 refers to the TSI 3330 and Sensor 4 refers to the TSI Dusttrak.

Measurement period	Dates (all dates in 2019)	Sensor	Array number	Treatment
1	09th October – 22nd October	3	1	eCO_2
		4	3	aCO_2
2	22nd October – 05th November	3	4	eCO_2
		4	2	aCO_2
3	05th November – 19th November	3	5	aCO_2
		4	6	eCO_2
4	19th November – 03rd December	3	1	eCO_2
		4	3	aCO_2

A bias factor calibration was used to ensure the two aerosol sensors were measuring consistently. The data from Sensor 4 was increased by a bias factor of 1.06. This bias factor was calculated from the 24-hour side-by-side intercomparison period, with the factor then applied to the data from the full measurement period.

Both sensors use heated air to dry particles before measuring PM concentrations, therefore the correction factor from Chapter 3 to correct for particle hygroscopicity and RH interactions was not used.

4.2.4 Data processing, visualisation, and analysis

All data analysis was completed in R version 4.0.3 (R Core Team, 2020), with figures created using *openair* and *ggplot* (Carslaw and Ropkins, 2012; Wickham, 2016). Relationships between PM_{10} concentrations and RH, temperature, and wind speed were visualised used scatter plots and smoothed *loess* curves, generated in *ggplot*. Box plots with mean PM_{10} (and interquartile ranges) were generated to visualise differences in bioaerosol concentrations between eCO_2 and aCO_2 arrays.

Kruskal-Wallis tests were used to test for statistically significant differences between the eCO_2 and aCO_2 arrays.

4.3 Results

4.3.1 Hypothesis A: Hourly fungal bioaerosol concentrations will correlate with hourly weather conditions (wind, RH, temperature).

Across the 56 day measurement period, there was a total of 96.74 mm of precipitation, and the mean temperature in the forest was 6.6°C. The lowest recorded temperature was -3.9°C, and the highest was 14.5°C. Similarly to the previous study, RH was very high throughout the experiment, with a mean of 93.0%, minimum 64.2%, and max of 99.9%.

Despite using sensors with active particle drying mechanisms, there was a large increase in the PM_{10} mass concentrations measured above 99% RH. Data points occurring at humidities above 99% were removed for the purposes of all other analyses in this chapter. Under both eCO_2 and aCO_2 conditions, a small increase in PM_{10} concentrations was measured as RH increased. In particular in eCO_2 conditions there was a large increase in bioaerosol concentrations when RH was greater than 97% (Figure 4-i A and B).

On average, the temperature decreased from October – December, however there was a peak in temperatures in late-November (Figure 4-ii A). In aCO_2 arrays there was a subtle unimodal distribution, where PM_{10} concentrations were higher at median temperatures, and lowest at the temperature extremes of 0 and 15°C (Figure 4-ii B). Under eCO_2 conditions, the relationship between temperature and PM_{10} was more complex, however bioaerosol concentrations significantly decreased at temperatures above 10°C (Figure 4-ii C).

As expected, and similarly to the data presented in Chapter 3, wind speeds were significantly lower below the canopy (below 25m), with a very limited number of dates where wind speed was above the 0.8 m s^{-1} threshold detectable with the Campbell anemometer (Figure 4-iii A). The highest PM_{10} concentrations occurred at the lowest wind speeds (<1 m s^{-1} at 25 m height, and <0.25 m s^{-1} at 2 m height), and the effect seemed to be stronger in eCO_2 arrays (Figure 4-iii).

For all the measurement periods, there was bioaerosol activity at low concentrations under all wind directions. For the first measurement period (Array 1 and Array 3), there were limited differences in concentrations measured between the two arrays, however in the final two measurement periods, there were considerably higher concentrations in the eCO_2 arrays, particularly in the south-east for Array 6, and north-west and south-east for Array 1 (Figure 4-iv). This reflects the much higher bioaerosol concentrations that were detected in the eCO_2 arrays during these measurement periods (Figure 4-v C).

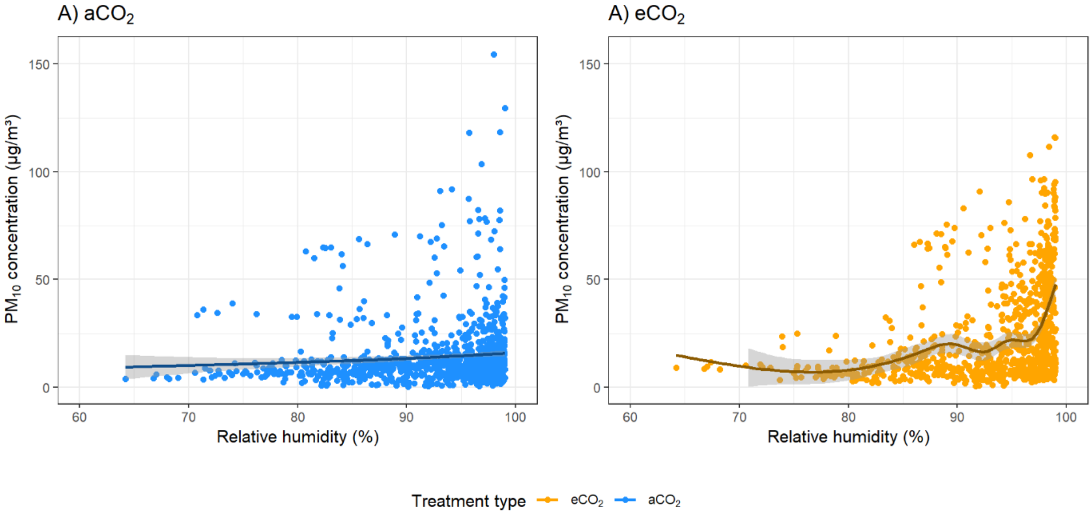


Figure 4-i Relative humidity and bioaerosol concentrations (PM_{10}) at BIFoR FACE in Autumn 2019. Subplots show: A) PM_{10} concentrations under aCO_2 conditions (Arrays 2, 3, and 5) in blue colours, and B) eCO_2 conditions (Arrays 1, 4, and 6) in orange colours.

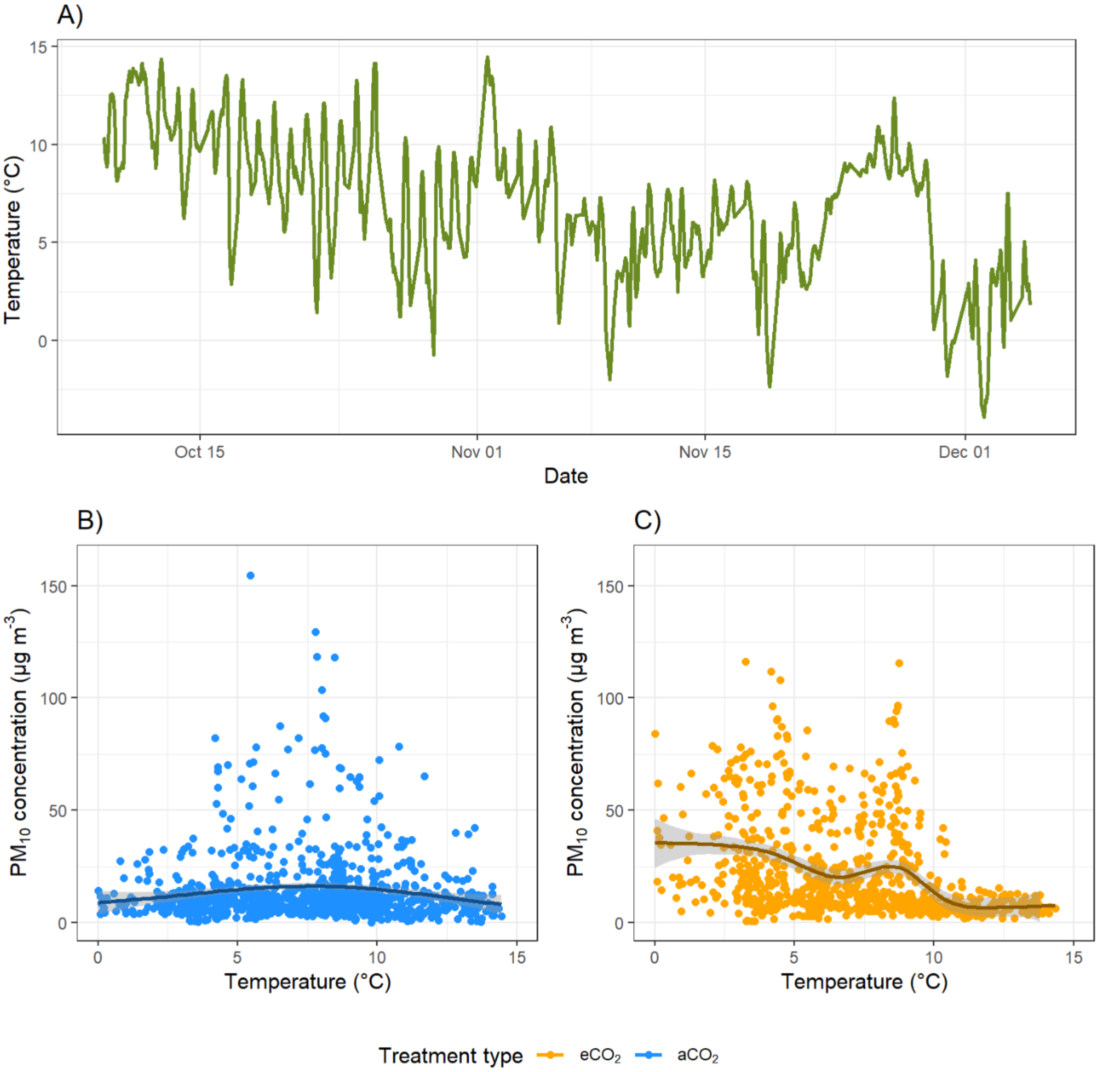


Figure 4-ii Temperature and bioaerosol (PM$_{10}$) concentrations across the experimental duration at BIFoR FACE. Subplots are: A) Temperature (°C), B) PM$_{10}$ concentrations with temperature under aCO$_2$ shown in blue, C), PM$_{10}$ concentrations with temperature under eCO$_2$ shown in orange.

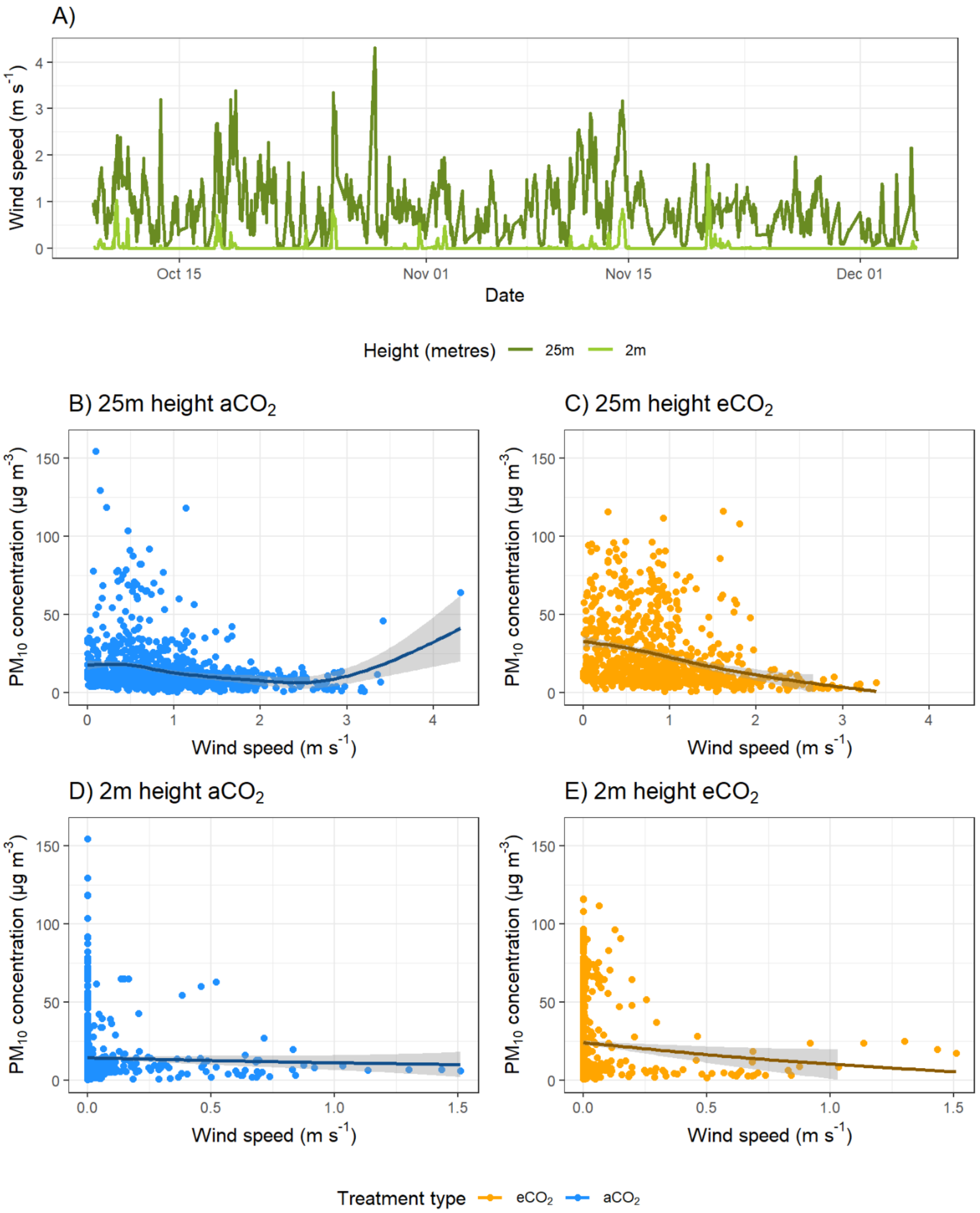


Figure 4-iii Wind speed and bioaerosol concentrations at BIFoR FACE in Autumn 2019. Subplots are: A) Wind speeds above (25m height) and below (2m height) the canopy, in dark green and light green respectively. Subplots B – E all show PM_{10} concentrations under B) aCO_2 at 25m height, C) eCO_2 at 25m height, D) aCO_2 at 2m height, and E) eCO_2 at 2m height. Data from aCO_2 arrays (Arrays 2, 3, and 5) is shown in blue, and data from eCO_2 arrays (Arrays 1,4,

and 6) in orange. Note the x-axes are different between subplots showing data from 25m height and 2m height.

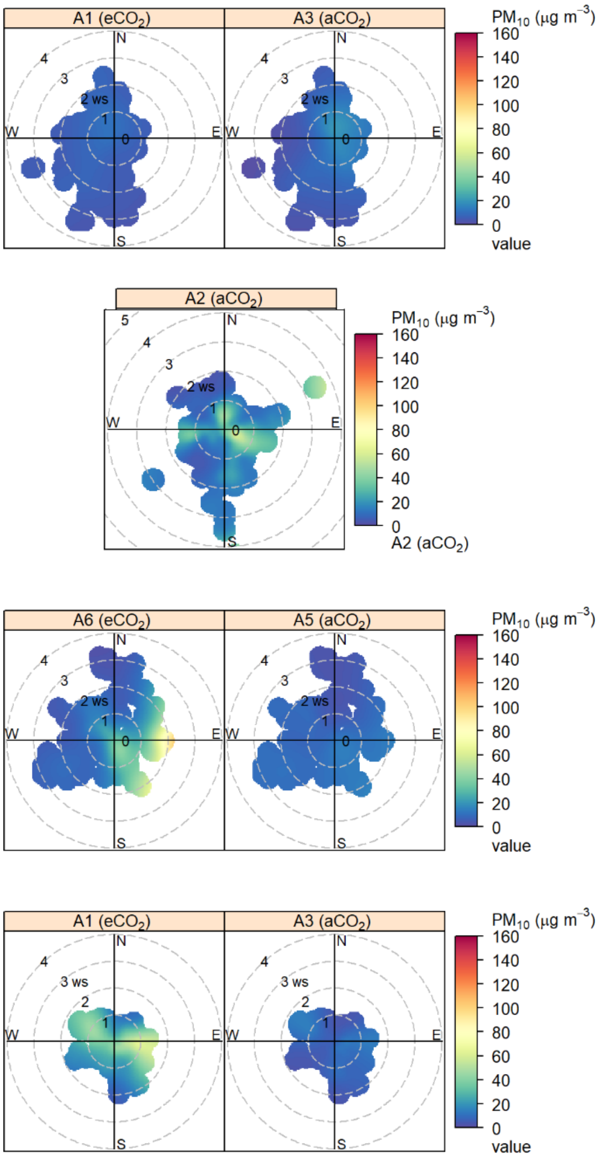


Figure 4-iv Openair polar bivariate plots showing bioaerosol concentrations (PM_{10}) with wind speed and direction (Carslaw and Ropkins, 2012). Subplots show each of the four measurement periods vertically, showing the data from each pair of arrays (eCO_2 and aCO_2) measured during each measurement period. Only aCO_2 data is shown from measurement period 2 due to instrument failure in the corresponding eCO_2 array. Colour gradients display the concentrations of bioaerosols detected, with low concentrations shown in blue/green colours, mid concentrations in yellow, and high concentrations in orange/red.

4.3.2 Hypothesis B: Fungal bioaerosol concentrations will increase in woodland patches treated with eCO_2 for three years.

For the full measurement period, there was no significant difference in the mean PM_{10} concentrations between aCO_2 and eCO_2 arrays (p-value 0.347) (Figure 4-v A). However, the upper inter-quartile range of the eCO_2 data was much higher than aCO_2. This was largely due to the third and fourth measurement periods (early-November onwards), where the bioaerosols concentrations were much higher under eCO_2 treatment (Figure 4-v C).

Figure 4-v B shows two apparent groups of aerosols, one occurring at higher levels in aCO_2 arrays (the peak at 10 $\mu g\ m^{-3}$ on the x-axis), and one higher in eCO_2 arrays (the main signal). In order to analyse these differences further, the data was split into the four measurement periods (Figure 4-vi). At the start of the experiment, in measurement period 1, PM_{10} concentrations were higher in aCO_2 arrays. However, later during the experiment duration during period 3 and 4, bioaerosol concentrations were higher under eCO_2 treatment. During period 3, there was a notable population of aerosols occurring at concentrations below 15 $\mu g\ m^{-3}$, where PM_{10} concentrations were very similar under eCO_2 and aCO_2.

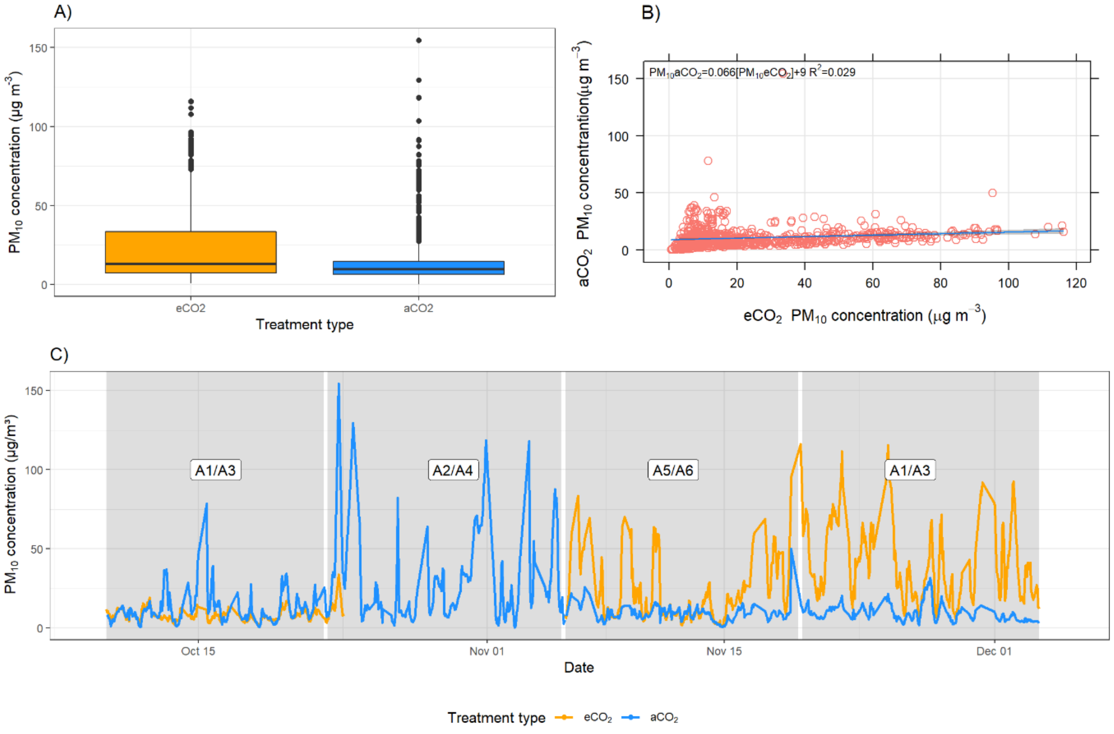


Figure 4-v PM$_{10}$ concentrations from the full experimental duration. Subplots are: A) Boxplot comparing average PM$_{10}$ concentrations in aCO$_2$ and eCO$_2$ arrays, B) PM$_{10}$ concentrations in eCO$_2$ arrays (x-axis) and aCO$_2$ arrays (y-axis), C) PM$_{10}$ concentrations timeseries. Data from aCO$_2$ arrays is shown in blue, and eCO$_2$ arrays in orange.

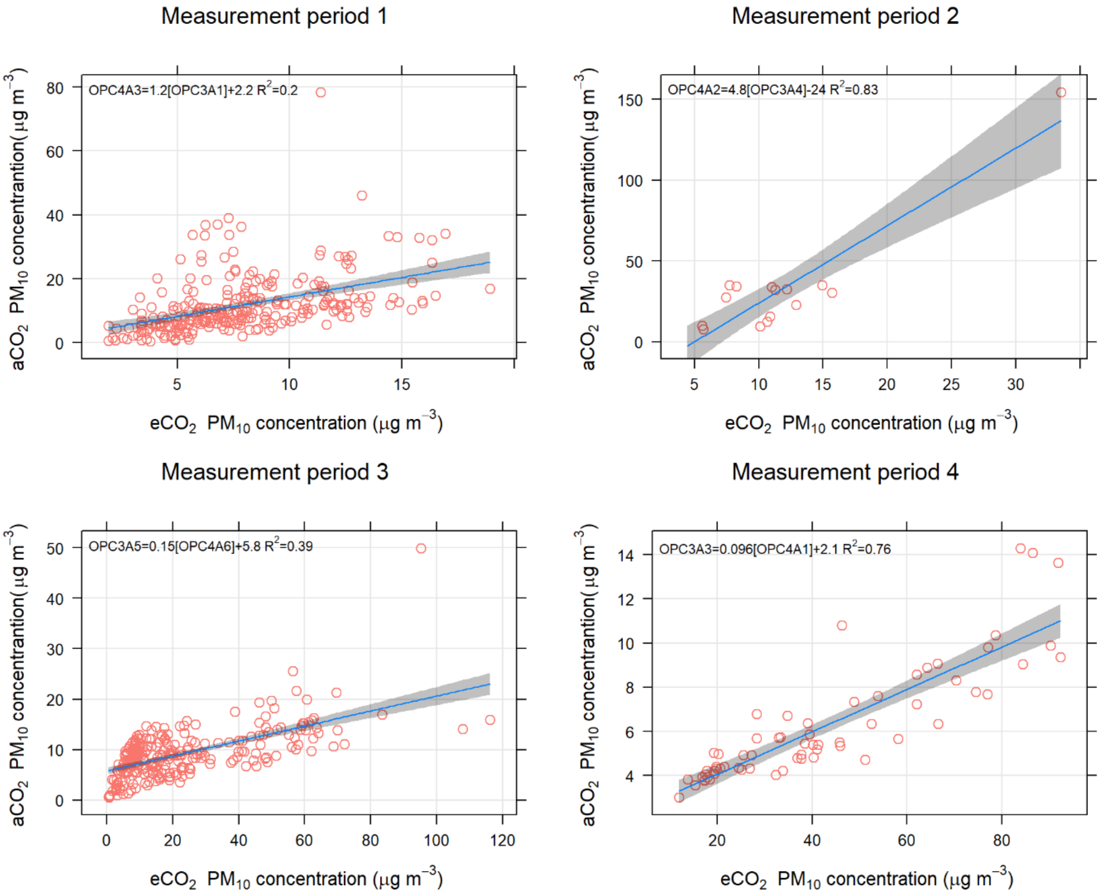


Figure 4-vi. PM_{10} concentrations across each of the four measurement periods in Autumn 2019. Each measurement period refers to a single pair of arrays at a time The measurement periods are numbered consecutively, starting at the earliest dates (measurement period 1) to the latest dates (measurement period 4).Note the x and y axis differ between each of the subplots.

4.4 Discussion

As in Chapter 3, there were two main hypotheses: A) bioaerosol (PM_{10}) concentrations would correlate with weather conditions, and B) bioaerosol concentrations would increase in arrays treated with eCO_2.

Unlike in Chapter 3, the instrumentation used in 2019 actively dries particles before sizing them, minimising any effect of particle hygroscopicity at high humidities. In Chapter 3, after applying the Crilley correction to the data we did not find any response of bioaerosol concentrations to RH. In this 2019 study, there was a small effect of RH on bioaerosol concentrations, particularly at very high humidity in eCO_2 arrays. This response could be due to the instrumentation not performing perfectly under field conditions, and some moisture remaining in the particles, particularly as the woodland is a very high humidity environment. As there was a small increase in particle concentrations in aCO_2 arrays, some effect of RH on particle size seems likely. However, as the effects were stronger in eCO_2 arrays, it seems likely there was also an interaction between eCO_2 and RH, whereby either higher total levels of fungal sporulation, or higher numbers of particular species of fungi occur as a result of the eCO_2 treatment, and these fungi are triggered to release spores under high RH levels.

This may also suggest that the calibration performed in the previous study may be over-correcting for RH effects, and masking biological changes (Baird et al., 2021; Crilley et al., 2020, 2018). However, the previous study also used PM_{10}-PM_1 (compared with PM_{10} used in this study). It may be that the population of aerosols smaller than 1µm may be more hygroscopic, or may respond differently to eCO_2 than larger particles. To test the RH/eCO_2 interaction further, future study could include a wider range of side-by-side intercomparison between instruments, to further assess the performance of the equipment in high-humidity conditions. It would also be useful to directly collect bioaerosols, and use DNA sequencing to definitively identify the bioaerosol population. This would provide further confirmation of whether PM_{10}, or PM_{10}-PM_1, are suitable proxies for studying fungal spores during the peak sporulation season, and enable the study of whether different populations of fungal spores are present in eCO_2 arrays versus aCO_2 control arrays. Different species of fungi may produce spores which react differently under RH, which may explain some of the differences observed.

Under aCO_2 conditions, PM_{10} concentrations were highest at median temperatures, and dropped slightly when the temperatures were at their lowest and highest. This contrasts with the results from 2018, where although there was a similar temperature range experienced, PM concentrations increased at the highest temperatures observed during the autumn experimental duration. This difference could be due to the earlier start-point of this experiment, with potentially different fungal species fruiting across the experimental duration. The overall effects of temperature on PM concentrations in aCO_2 arrays were subtle in both 2018 and 2019, and so the differences between years may simply be due to annual variation.

Under eCO_2, PM_{10} concentrations showed a different response to temperature, suggesting a possible interactive effect. There was a drop in PM_{10} concentrations in eCO_2 arrays at temperatures greater than 10°C. Higher bioaerosol concentrations were measured in eCO_2 arrays in the 2nd half of the experiment, when temperatures were lower, so the response may be coincidental. However, there may be an eCO_2/temperature interaction where other eCO_2-induced changes in the woodland (for example, increased tree photosynthesis, increased fine root growth) triggered higher fungal fruiting later in the year, and therefore at lower temperatures than they would normally be seen at (Gardner et al., 2022; Ziegler et al., 2023). Under both eCO_2 and aCO_2 conditions, future studies with a longer measurement period (to capture a wider temperature range) and repeating the study as the BIFoR FACE experiment continues would allow further investigation of the effects of temperature on bioaerosol concentrations.

The relationship between wind speed and PM_{10} concentrations was very similar between 2018 and 2019, which further evidences the accuracy of the model in Chapter 3 (Figure 3-iv). A peak in bioaerosol concentrations occurred at low wind speed, with the concentrations dropping as wind speeds increased. However, the overall wind speeds were lower in 2019 compared with 2018, and therefore no peak at high wind speeds was observed. In 2018, the increase in PM_{10} concentrations with high wind speeds was only observed when above-canopy wind speeds reached above 5 m s^{-1}, speeds which were not reached during the 2019 measurement campaign. It is therefore likely that wind speeds were not high enough below-canopy to cause an increase in spore release and dispersal. In order to further study the response of bioaerosol concentrations to high wind speeds, it may be necessary to measure over a much longer duration, to capture enough high wind speed days.

In 2018, in the full dataset, we did not detect a significant difference between eCO_2 and aCO_2 arrays with respect to bioaerosol concentrations. We observed that PM_{10}-PM_1 concentrations were similar between eCO_2 and aCO_2 arrays at low concentrations, however during high concentration events, bioaerosol concentrations were higher in aCO_2 arrays. In 2019, although the overall dataset results were similar, with no significant difference between the concentrations measured in eCO_2 versus aCO_2 conditions, the relationship between eCO_2 treatment and bioaerosol concentrations was extremely varied across the experimental duration.

During the first measurement period (in Arrays 1 and 3), for the majority of the duration, concentrations were very similar between the eCO_2 and aCO_2 arrays, however the peaks in PM_{10} concentrations were higher in Array 3 (aCO_2). This is the same as the pattern we observed during Autumn 2018. However in the latter half of the 2019 experiment, this pattern changes dramatically, with concentrations significantly higher in eCO_2 arrays, across both measurement period 3 and 4. As detailed in Table 4-i, the instruments were swapped between eCO_2 and aCO_2 arrays between periods 3 and 4, i.e. Sensor 3 was located in A5 (aCO_2) during period 3, and located in A1 (eCO_2) during period 4, which makes it highly unlikely the effects seen are due to instrument artefacts. One possible explanation is that the eCO_2 treatment has caused a shift in the fungal species present in eCO_2 arrays, or delayed the end of the fungal fruiting season, causing an increase in the sporulation in eCO_2 arrays during this latter half of the season. Previous studies have demonstrated an increase in the average fungal fruiting season with climate change, which had previously been predominantly attributed to temperature changes. However, two previous forest FACE experiments demonstrated changes to fungal fruiting under eCO_2 (Andrew and Lilleskov, 2009; Godbold et al., 2015). It therefore seems plausible that changes to the fungal fruiting season, or fruiting population could have occurred with eCO_2. Other possibilities include that eCO_2 altered the spore productivity of the fruit bodies, causing more spores to be released per sporocarp. There may also have been cumulative effects of eCO_2, as this study started at the end of the third year of fumigation (compared with two in the previous study). The increase in tree photosynthesis and fine root growth demonstrated at BIFoR FACE may have increased availability of carbon in the soil, and this effect could increase with the experimental duration, resulting in different responses between years two and three of eCO_2 treatment (Gardner et al., 2022; Ziegler et al., 2023).

The increase in PM_{10} concentrations also occurred after the eCO_2 treatment was switched off for the end of the tree growing season (switch-off occurred on 31st October 2019), so it is possible that there was an effect of the eCO_2 fumigation that had an effect on fungal sporulation, potentially suppressing it, or causing changes in the air flow throughout the woodland. However, given this was not observed in 2018 (where the measurements occurred after the eCO_2 was switched off for the end of the season), and that aCO_2 arrays did not have higher PM_{10} concentrations than under eCO_2 during the fumigation period, this seems unlikely.

To investigate the change in the PM_{10} concentrations across the season, we divided the data into each of the four measurement periods (Figure 4-vi). On the whole, the diagrams reflect the relationships shown in the PM_{10} time series, however there is a population of aerosols in measurement period 3 that are very similar between eCO_2 and aCO_2 arrays. This further suggests there may be two (or more) groups of bioaerosols that each respond differently to the eCO_2 treatment. These could be different fungal species, or different types of bioaerosol. Further work using microscopy or DNA-based methods to investigate the types of bioaerosol present, and the different species of fungi present, would likely help to investigate these differing bioaerosol com further.

4.5 Conclusions

The results from this second bioaerosol measurement campaign at BIFoR FACE provide further valuable woodland bioaerosol data, and to date, BIFoR FACE remains the only woodland FACE experiment to investigate bioaerosols.

There were strong similarities in the response of PM_{10} concentration to changes in wind speed between 2018 and 2019, with concentrations peaking at very low wind speeds, and dropping as wind speeds increased. Responses of bioaerosol concentrations to temperature and RH were not the same in the 2018 and 2019 datasets, therefore further measurements are required to evaluate the relationship between these parameters.

The response of bioaerosols to eCO_2 treatment was starkly different across the experimental duration, and between 2018 and 2019. This may be due to the additional year of eCO_2 treatment causing further biological changes to either fungal community composition or fruiting dynamics, and may also be due to the different unit use (PM_{10} versus PM_{10}-PM_1). This highlights the need for further measurements across the duration of the BIFoR FACE experiment, particularly investigating the composition of bioaerosols within the woodland, as this 2019 work appears to highlight varying responses in different bioaerosol communities.

CHAPTER 5: WOODLAND FUNGAL FRUITING UNDER ELEVATED CO_2 TREATMENT

5.1 Introduction

Fungal communities form a vital part of woodland biodiversity, with the UK home to a number of internationally rare fungal habitats, including temperate rainforests, and significant numbers of veteran trees. Since the advent of reliable and cheap DNA based methods, fungal sporocarp surveys are becoming a less common method for studying fungi in the field. In addition to a significant skills shortage in field mycologists, field surveys are often criticised for being biased towards fungi with large fruit bodies, with micro-fungi and fungi without fruit bodies, recorded less or not at all (van der Linde et al., 2012). In addition, surveys are frequently only completed once, which is very likely to miss fruiting of some fungal species and therefore provide an inaccurate measure of fungal populations (Halme and Kotiaho, 2012).

Despite these criticisms, fungal fruiting surveys can still be an important and useful method for studying fungal communities. Several studies have demonstrated that repeated surveys, timed appropriately for the habitat, are a good method for measuring fungal diversity (Cannon, 1997; Halme and Kotiaho, 2012; Tóth and Barta, 2010). The popularity of field mycology and fungal recording in the UK has resulted in long-term datasets over several decades, which can provide important insights into the impacts of climate change on fungi in real time.

Several large studies across Europe have identified major changes in the phenology of fungal fruiting patterns in response to climate change, with the average autumn fungal fruiting season both starting earlier and ending later (Andrew et al., 2018c, 2018b; Boddy et al., 2014; Gange et al., 2007; Kauserud et al., 2012, 2010). Gange et al. (2007), where data collection occurred in southern England, also identified that changes in fungal fruiting were habitat-dependent, with larger shifts in fruiting dates associated with fungi in deciduous woodlands compared with coniferous woodlands.

In addition to seasonal changes in fungal fruiting patterns, the geographical patterns and host ranges of fungi in the UK have altered as a result of climate change. Changes in latitudinal abundance of fungal fruiting depending on their functional group have been reported. ECM fungal fruiting has become more common in northern regions of the UK, and decreased in the south, and saprotrophic fungal abundances have declined in abundance across both the northern and southern areas (Gange et al., 2018). Additionally, the host ranges of fungi have been demonstrated to change. For example, the Wood Ear *Auricularia auricula* was previously only found growing on elder (*Sambucus nigra*), however since 1979, its host range has significantly expanded, with it now commonly found fruiting on 16 tree host species (Gange et al., 2011).

Although we have extensive datasets on the impacts of climate change on fungal fruiting in real time, we lack data in climate manipulation experiments (Bidartondo et al., 2018). Changes to fungal fruiting in previous studies have largely been attributed to temperature and precipitation changes, however it is challenging to unpick the interacting effects of climate change, and make predictions of how this may change with future climate extremes.

Two previous woodland FACE experiments investigated the impacts of eCO_2 on fungal fruiting. At AspenFACE, eCO_2 was shown to significantly increase the mass of ECM sporocarps (Andrew and Lilleskov, 2009). ECM sporocarps were also significantly larger under eCO_2 at BangorFACE during their collections in autumn 2006, however the increase in sporocarp weight under eCO_2 was not statistically significant (Godbold et al., 2015).

To study the effects of eCO_2 treatment on fungal fruiting patterns at BIFoR FACE, we completed monthly fungal sporocarp surveys from March 2018 – February 2021, including sporocarp sampling for the surveys completed from October 2018 onwards. We hypothesized that eCO_2 fumigation would cause an extension of the fungal fruiting season, as well as increasing the mass of sporocarps collected under eCO_2.

5.2 Methods

5.2.1 BIFoR FACE

The BIFoR FACE facility design and methodology is described in detail in Chapter 2.3; in brief the facility is located in an ancient deciduous woodland, dominated by mature oak (*Quercus robur*) with a previously coppiced hazel (*Corylus avellana*) understory. The eCO_2 system installed in the woodland is comprised of three arrays fumigated with 150ppm CO_2 above ambient (approximately 550ppm), and three infrastructure control arrays which are maintained at ambient CO_2 concentrations (approximately 400ppm). Fungal fruiting surveys were conducted monthly in Arrays 1 - 6 at the BIFoR FACE facility. Each array has raised metal walkway from which research can be conducted, that approximately forms a transect in the arrays from the edge entrance, passing the centre point, and continuing on to an opposing edge. Fruiting surveys were conducted from this walkway, in the area 0 – 1 m from the walkway perimeter.

5.2.2 Fruit body survey methodology and sample processing

The survey area (from ground level to 2 m height on any vegetation or deadwood) inside each of the six arrays was visually inspected for fungal fruit bodies monthly from March 2018 until February 2020, totalling 24 surveys completed. All arrays were surveyed on each date, excepting 24th October 2018 when the survey was completed in arrays 1 – 4 only. All six arrays were surveyed on a single date, except the September, October, and November surveys in 2019 which were completed across two, three, and two days respectively.

The mean number of days between surveys was 29, ranging from the minimum survey gap of 14 days, and a maximum of 42. In all surveys, all visible sporocarps within the survey areas were photographed in detail and descriptive notes taken. From October 2018 – February 2020, samples were also taken of all species found. Some additional fungal species were found within the woodland but outside the array survey areas, and also during some early additional surveys; these species were not included in the analyses for this chapter, but are included in the full list of fungal records for BIFoR, available in supplementary data (Appendix, Section 11.1).

After collection of fruit bodies on site at BIFoR FACE, samples were immediately sealed into individual zip-lock bags before being frozen at -20˚C within 10 hours of collection. Samples were stored at -20˚C for up to two years before being freeze dried in batches in a similar manner to the methods described in Davies, 1962 and Haskins, 1960. Freeze drying times varied depending on the size of the specimens, larger sporocarps required a significantly longer drying time. After drying, samples were placed individually in sealed plastic bags with a sachet of silica gel before being stored long term at room temperature. Dried samples were weighed with their accompanying plastic bags and silica gel, with the average weight of silica gel removed from the final weights for each sample, before the data was further processed.

Samples were identified to the highest taxonomic level possible, largely using literature from Volumes 1 and 2 of Læssøe and Petersen, 2019, but additionally Hugill and Lucas, 2021, Ing, 2020, and Kibby, 2021, 2020a, 2020b.

5.2.3 Sanger sequencing

Sanger sequencing was completed at the University of Birmingham by Rachel Calder. Of a total of 398 fruit body samples collected, a subset of 88 samples were sequenced to test for field ID accuracy using the below method.

DNA from fruit body samples was extracted using the Qiagen DNeasy PlantPro DNA extraction kit. 1 in 10 dilutions of the resulting DNA were used as input material for PCR, using ITS1 and ITS4 primers from White et al. (1990). 20 µl PCRs were run using Phusion High-Fidelity DNA Polymerase (Thermo Scientific), 0.5 µM primers, and 2 µl of the diluted sample DNA. Thermocycling conditions were 98°C for 30 seconds, followed by 34 cycles of 98°C for 10 seconds, 57°C for 10 seconds, and 72°C for 15 seconds, with a final extension of 72°C for 5 minutes. PCR products were purified using AMPure XP beads (Beckman Coulter Life Sciences) according to the manufacturer's instructions. The resulting product was sent for Sanger sequencing at Genewiz (Azenta Life Sciences). 76 of the 88 samples sent for sequencing returned a usable sequence. Sequences were trimmed as appropriate based on visual assessment of chromatograms using Chromas 2.6.6 (Technelysium). Trimmed sequences were then entered into the BLAST tool of the UNITE database, and the highest-scoring match for each query was taken forward (Abarenkov et al., 2021).

Of the 76 samples sequenced, a total of 29 samples had a matching identification between field ID and Sanger sequencing to genus level or higher. A further five samples had matching identifications, but field ID was only to family level, whereas sequencing increased resolution to species level. 24 samples were not identified in the field beyond phylum level, but were identified using DNA. Four samples had conflicting IDs between sequencing and field identification.

5.2.4 Data analysis

Data analysis was completed in R (R Core Team, 2020). Species counts were normalised against the size of survey areas, as these were not identical between arrays. Factors were generated to normalise counts relative to the shortest array transect (Array 3), as listed in Table 5-i. Non-normalised data was used for species lists and sporocarp weights (which were listed as average weights per sample). Kruskall-

Wallis tests (a form of t-test for non-parametric data) were used to test for differences in the mean number of fungal species and the mean sporocarp weight in eCO_2 treatment versus aCO_2 control arrays. *FUNGuild* was used to assign ecological guilds (functional groups) to fungi, using *FUNGuildR* version 1.1 (Furneaux and Song, 2021; Nguyen et al., 2016). All figures were generated in *ggplot2* version 3.3.5 (Wickham, 2016).

Table 5-i. Fungal survey normalisation factors per array

Array	Survey area length (m)	Factor of shortest length walkway
A1	92.46	0.56
A2	55.92	0.93
A3	52.00	1.00
A4	101.3	0.51
A5	86.32	0.60
A6	73.88	0.70

5.3 Results

A total of 861 fungal individuals were recorded across the two years of monthly surveys. Using field identification, 523 individuals were identified to family level, and 437 to genus level. Species were recorded once per array, per sampling date, with some species being recorded many times. Common *Ascomycetes* included *Hypoxylon fuscum*, and *Xylaria hypoxylon*; with the most frequently recorded *Basidiomycetes* including *Stereum hirsutum*, *Lactarius quietus*, *Hypholoma fasciculare*, and *Auricularia auricula*.

Species in a total of 44 genera were identified. Most identified species belonged to the *Basidiomycota* phylum, representing 77% of species found, with *Ascomycota* making up 15%. Of the fungi identified to family level, the five most common families were *Mycenaceae*, *Xylariaceae*, *Auriculariaceae*, *Stereaceae*, and *Russulaceae*. After unassigned fungi, saprotrophs were the most common functional group, followed by pathotroph-saprotroph-symbiotrophs, pathotroph-saprotrophs, and symbiotrophs.

An average of four fungal species were recorded per array per sampling date; although this was highly variable across the year, with peaks of 12 – 15 species per array in the autumn months (Figure 5-i C). Species from 35 genera were found in eCO_2 arrays, and 36 genera in aCO_2 arrays, with significant overlap in species found across the treatment types. A total of 27 genera were shared between treatment and control, corresponding to many of the species commonly found fruiting, for example species in the *Mycena*, *Lactarius*, *Stereum*, and *Russula* genera. Species more rarely found fruiting were most likely to be found in either treatment or control, for example *Gymnopus fusipes* was only found fruiting in eCO_2 arrays, and *Ascocoryne sarcoides* only in aCO_2 arrays. Eight genera were only found in eCO_2 arrays and nine in aCO_2 arrays.

The number of species fruiting per array matched very closely between treatment and control arrays, with peaks and troughs in fruiting occurring in similar months, although the peak in fruiting was slightly later in 2018 in aCO_2 arrays, but this was not replicated in 2019 (Figure 5-i C). There was no significant difference in the number of fungal species fruiting per array per date in eCO_2 arrays compared with aCO_2 arrays ($p = 0.41$, Figure 5-i A).

The mean sporocarp weight per month (Figure 5-i D) was very similar across autumn 2018 and spring 2019, however became more variable across the latter half of 2019, with sporocarps broadly heavier in aCO_2 arrays. Across the 18 months of samples, there was no significant difference in mean sporocarp weight in eCO_2 treatment versus aCO_2 control arrays ($p = 0.45$, Figure 5-i B).

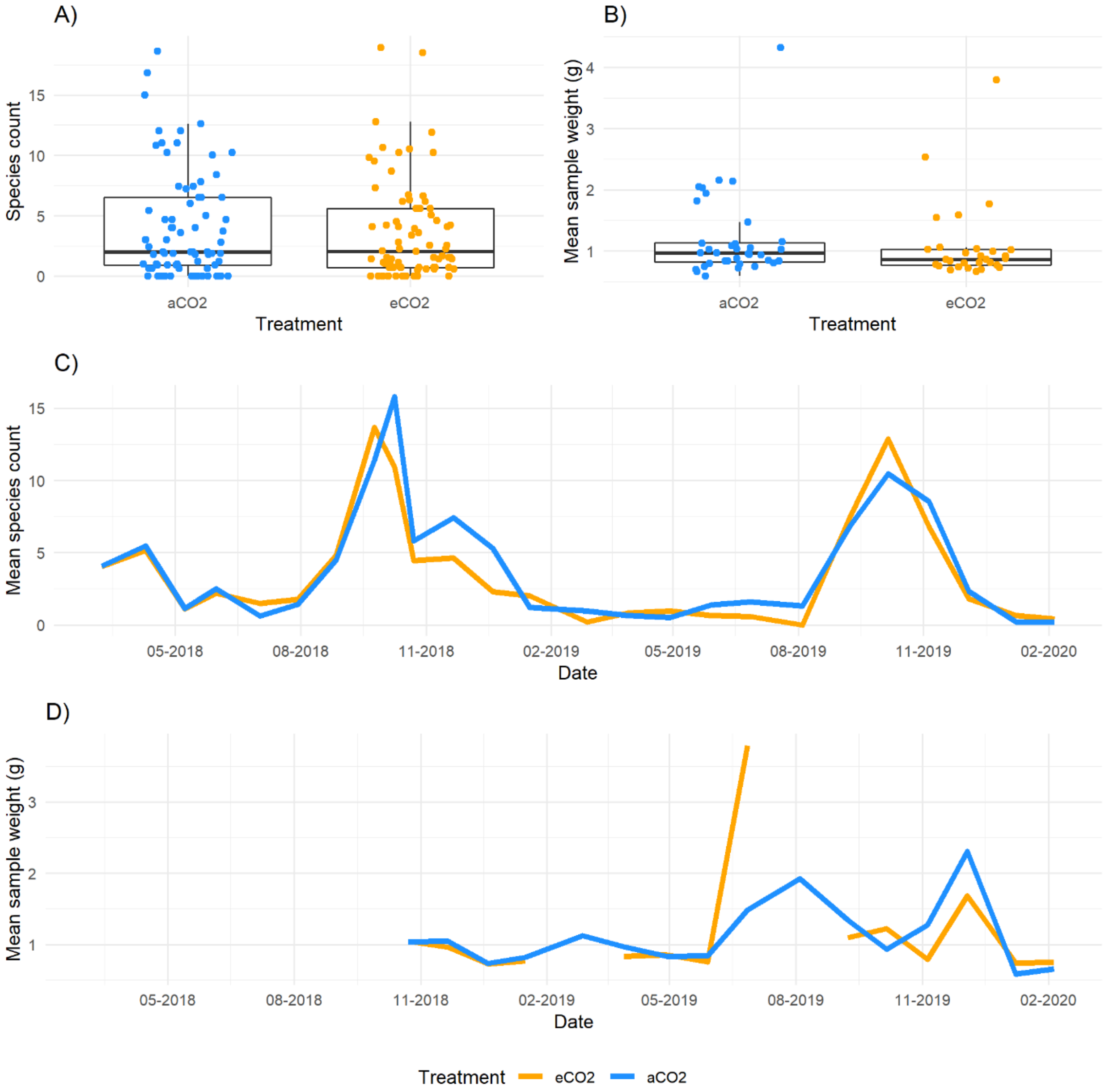


Figure 5-i Fungal fruiting in eCO_2 *treatment and* aCO_2 *control arrays. Subplots show: A) Corrected species counts, B) Mean sample weights, C) Time series of mean species counts, D) Time series of mean sample weights.*

5.4 Discussion

Although studies on fungal fruiting are relatively commonplace, we are internationally lacking datasets on fungal fruiting within climate manipulation experiments (Bidartondo et al., 2018). Although many of the previous woodland FACE facilities have collected data on fungi (see Chapter 2.2), only two investigated the impacts of eCO_2 on woodland fungal fruiting (Andrew and Lilleskov, 2009; Godbold et al., 2015). Across the two-year duration of this experiment, we completed a total of 24 fungal surveys at the BIFoR FACE facility, investigating the impacts of eCO_2 treatment on fungal fruiting in a mature temperate woodland.

A broad range of fungal species were found, across a total of 44 genera. Fungi in the *Basidiomycota* phylum dominated. Although demonstrating a relatively diverse fungal community in the BIFoR FACE woodland, there are likely considerably more fungal species which were not recorded. For example Gange et al. (2019) detected 2319 species across a 65 year time scale in Wiltshire, 30 years of surveys in a Japanese oak forest revealed 668 fungal species (Sato et al., 2012), and Büntgen et al. (2012) identified 273 species across 40 years of surveys in a Swiss nature reserve. Although all these studies demonstrate considerably longer timescales of surveys, they highlight the extremely high levels of diversity present, particularly in already highly biodiverse sites such as ancient woodlands. This highlights the value of surveys across a long-time period on sites such as this, particularly when fungi frequently do not fruit every year, or even at the same time points each year (Halme and Kotiaho, 2012). Continuing to complete surveys, including covering a larger survey area in each array, would most likely continue to increase the species found at BIFoR FACE, and would likely allow more robust analysis of differential abundance of species between eCO_2 and aCO_2 arrays. Additionally, considering the possibility of using different surveyors, both to utilise the personal expertise of individuals, and account for survey bias would be valuable. In particular, utilising expertise on micro-fungi, *Ascomycota*, and non-soil dwelling fungi (e.g. polypores) that are less well identified in metabarcoding studies would further increase the value of sporocarp surveys, and complement soil metabarcoding work (Frøslev et al., 2019).

Additionally, identification of around 15% of the fungal sporocarps found was not possible. This reflects the challenging nature of identifying fungi, but some modifications could be made to future survey methodologies to enable more time for identification when fungi were freshly collected. Modifying survey methodology for future samples to decrease the survey area per date in the autumn (e.g. surveying a pair of arrays per date), and prioritising spore collection and microscopy prior to long-term storage could enable increased identification rates. Although DNA sequencing was used to check the accuracy of field IDs for a sub-set of samples, identification rates (and accuracy) could be improved by incorporating sequencing of fruit body DNA for all samples included within this study, and for any future surveys. Using both microscopy and DNA sequencing for sample identification is increasingly becoming the gold standard, particularly for unusual samples, or those which do not have well resolved taxonomy (Truong et al., 2017).

As would be expected for a site of this type, the main peak in fungal fruiting occurred during the autumn months (September – November), with the highest number of samples and species recorded during these months. Contrary to our hypothesis, there were no significant differences in the number of fungal species fruiting under eCO_2, or the changes to the seasonality of fruiting. Previous climate studies have demonstrated clear shifts in fruiting season with climate change, including more species fruiting in spring, however these changes were predominantly attributed to temperature changes (Boddy et al., 2014; Gange et al., 2007). Mean sporocarp weight was also not affected by eCO_2 treatment, although there was a decrease in sporocarp weights under eCO_2 in winter 2019. This contrasts with work at two previous FACE experiments, where fruit body weight increased under eCO_2, however these two previous studies were undertaken solely on ectomycorrhizal sporocarps, and in newly established woodlands (Andrew and Lilleskov, 2009; Godbold et al., 2015).

Further survey work looking at the composition of fungi fruiting, the seasonality of fruiting, and the weight of sporocarps is warranted as the eCO_2 fumigation at BIFoR FACE continues, and other ecological changes occur in the woodland. 2018 and 2019 were only the second and third years of eCO_2 fumigation respectively, and therefore it is possible that changes occurring in the soil and trees may not yet be reflected in fungal fruiting patterns. In addition to using fungal fruiting surveys to investigate fungal ecology, several previous FACE studies have used samples collected in fungal fruiting surveys tracked the movement of ^{13}C used in the FACE fumigation into fungi (Chen et al., 2016; Hobbie et al., 2014; Keel et al., 2006). This would be a valuable addition to the BIFoR FACE experiment, and could provide data for the overall carbon balance of the woodland, as well as inputting into wider carbon and climate modelling.

5.5 Conclusions

In conclusion, despite the technical challenges of a survey of this type, this dataset provides an important dataset to long-term fungal records in the UK, and in temperate deciduous woodlands as a whole. A wide variety of fungi were found and identified; however, it is highly likely that a large number of species remain undiscovered within the woodland.

In the second and third years of eCO_2 fumigation at the BIFoR FACE experiment, there were no significant effects of eCO_2 on the number of fungal species fruiting or the weight of sporocarps. However, there were some early signs that there may be differences, particularly for sporocarp weight.

It is therefore clear that further fungal survey work at BIFoR FACE is warranted, both to uncover further fungal diversity, and to investigate the relationship between eCO_2 and fungal fruiting further. Based on these two years of data, modifications could be made to future survey methodology to increase the accuracy and ease of surveying.

CHAPTER 6: USING DNA METABARCODING TO INVESTIGATE WOODLAND SOIL FUNGAL COMMUNITIES AT BIFoR FACE: A PILOT STUDY

6.1 Introduction

Temperate woodlands are a vital habitat, contributing significantly to global carbon stores and biodiversity. UK woodlands form an important part of the carbon store, as well as containing globally rare habitats and habitat features such as temperate rainforests and ancient and veteran trees (Ellis, 2016; Harris et al., 2021; Nolan et al., 2020).

Fungi are an essential group of organisms in temperate woodlands, forming a key part of the woodland carbon cycle, and providing extremely high biodiversity value. Despite this, there is a lack of information on how woodland fungi will be affected by climate change, and what impact this could have on our woodland biodiversity and national and international carbon mitigation targets. Long term studies demonstrating the impact of climate change on fungal fruiting suggest that elevated temperatures are a key factor affecting fungal fruiting patterns (Andrew et al., 2018b; Gange et al., 2007). In addition, changes to plant growth in response to elevated CO_2 (increased photosynthetic rates, increased fine root production, increased leaf litter production) all increase carbon inputs into the soil, which is likely to affect fungi (Gardner et al., 2022; Ziegler et al., 2023).

Although there are large datasets documenting the effects of climate change on fungal fruiting in the UK (for example Gange et al., 2007), we are lacking in similar data on the impacts of climate change on soil fungal communities. Soil fungal communities (and soil microbes communities more widely) have historically been extremely challenging to study, however the rapid expansion and decreasing cost of high throughput DNA sequencing technologies has been a huge scientific step forward (Tedersoo et al., 2022).

Even with the advent of high throughput DNA sequencing methods, there are few studies investigating soil fungal communities in the UK. The Global Fungi database , which collates a worldwide database of fungal studies using ITS1 or ITS2 rRNA fungal subunit barcoding, to date highlights only three studies undertaken in the UK, with a cumulative total of 72 samples from woodland or forest soils (George et al., 2019b; Jarvis et al., 2015; Tedersoo et al., 2014; Větrovský et al., 2020). Other international studies (for example, Anthony et al., 2022; Suz et al., 2014) include samples from other English woodlands, however typically these have only focussed on ectomycorrhizal fungi.

Concurrently, there is a lack of fungi datasets either collected across an extended time period, or collected in climate manipulation experiments for mature temperate woodlands. Early woodland FACE experiments internationally have included data collected on soil fungal communities in young trees and woodlands, which have demonstrated mixed responses of soil fungal community composition to eCO_2, and limited evidence for effects of eCO_2 on soil fungal richness (Chung et al., 2006; Dawes et al., 2013; Dunbar et al., 2014; Feng et al., 2010; Lagomarsino et al., 2007; Parrent et al., 2006; Parrent and Vilgalys, 2007; Weber et al., 2013, 2012). Although these data are mixed, they highlight the need for further study, particularly in mature woodland systems, and for longer durations (Norby and Zak, 2011).

We used DNA metabarcoding to investigate soil fungal communities at the BIFoR FACE experiment, collecting important data on the early effects of eCO_2 on soil fungi in mature temperate woodlands as well as contributing to the overall database of UK soil fungal studies, and worldwide temperate woodland climate manipulation studies.

Our hypotheses were:

A) Fungal community composition and richness will be significantly different between soil horizons.
B) Fungal communities will be affected by one year of eCO_2 treatment.

6.2 Methods

6.2.1 BIFoR FACE

The BIFoR FACE experiment methodology is described in detail in Chapter 2.3. In brief, the BIFoR FACE experiment allowed the investigation of the direct and indirect effect of eCO_2 on soil fungal communities in a minimally disturbed mature temperate woodland environment. Samples were collected from Arrays 1 – 6. The soil at BIFoR FACE is a non-calcareous sandy loam, with a pH of around 4.5 in the 0-10 cm depth, and around 5.5 in the 10-37 cm depth; with pH consistent across the sampling locations (Hollis and Jones, 2021). The predominant tree species is oak (*Quercus robur*), which forms associations with ectomycorrhizal fungi. There is also a dominant hazel (*Corylus avellana*) understory, which forms associations with both ectomycorrhizal and arbuscular mycorrhizal fungi (Brundrett and Tedersoo, 2020).

Soil samples were collected in March 2017 and March 2018. Spring 2017 had typical weather conditions for the time of year, with 9.6mm precipitation and a mean temperature of 6.4°C on the week preceding sample collection. In contrast, spring 2018 had a period of extreme cold weather occurring in February 2018. On the week preceding the 2018 sample collection, there was a total of 18.6mm precipitation and a mean temperature of 0.2°C.

6.2.2 Soil sampling, processing, and storage

Three soil samples were collected from each of the BIFoR FACE Arrays 1 – 6 on the 27th March 2017 and 06th March 2018, totalling 36 cores. Samples were collected using a metal soil corer (Van Walt Ltd) with 50 mm diameter, and 300 mm length, with the core lined with a new plastic liner for each sample. Samples were stored at 4°C for up to a week before processing. Cores were divided into soil horizons before being sieved, and removing stones, roots, and soil microfauna by hand. Sieved soils were aliquoted into a centrifuge tube before being frozen at -70°C to -80°C.

The O (organic) and A (surface) soil horizons were extracted for this study, totalling 72 samples. The DNA extracted from these 72 samples was sequenced for the ITS1 fragment of the fungal genome as a marker for fungal communities.

6.2.3 DNA extraction

0.25 g defrosted soil was aliquoted per soil sample before being extracted using the Qiagen DNeasy PowerSoil kit. Blank extractions were completed as negative controls. A modified method was used for step two of the protocol, where a Precellys 24 bead beater (Stretton Scientific) was used as an alternative to the vortex step. Samples were run at 6500 rpm for two periods of 20 seconds, with a 20 second rest in between spin periods. After homogenisation, samples were cooled on ice before proceeding with step three of extraction. An initial check for DNA quality and quantity was performed using a Nanodrop 1000 Spectrophotometer (Thermo Scientific) before the DNA was frozen at -20°C for storage before sequencing. Samples were transported to the sequencing facility on ice within six hours of removal from the freezer.

6.2.4 DNA sequencing

Sequencing was completed by the NERC Biomolecular Analysis Facility (NBAF) at the University of Liverpool. The following primers were used to amplify the ITS region of fungi:

F:

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNGGAAGTAAAAGTCGTAACAAGG

R:

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGAGATCCGTTGTTGAAAGTT

1 µL of DNA was used as input material into a first round PCR with conditions of 10 seconds at 98°C, 30 seconds at 55°C, 30 seconds at 72°C, for 15 cycles, then a 2 minute extension at 72°C. First round PCR products were purified using AMPure SPRI Beads before entering the second PCR using Nextera XT sequencing primers containing indexes (i5 and i7) for sample identification. 15 cycles of PCR were performed using conditions: 30 seconds at 95°C, 30 seconds at 55°C, 30 seconds at 72°C, for 15 cycles, then a 5 minute extension at 72°C. Samples were purified using AMPure Beads before being quantified using Qubit and assessed using the AATIFragment Analyzer. These final libraries were pooled in equimolar amounts using the Qubit and Fragment Analyzer data, and the pool subsequently size selected on the Sage Pippin Prep at 300-600 basepairs (bp). The quantity and quality of the size selected pool was assessed by Bioanalyzer and subsequently by qPCR using the Illumina Library Quantification Kit from Kapa on a Roche Light Cycler LC480II (Roche Diagnostics Ltd, Burgess Hill, UK) according to manufacturer's instructions. Briefly, a 20 µl PCR reaction (performed in triplicate for each pooled library) was prepared on ice with 12 µl SYBR Green I Master Mix and 4 µl diluted pooled DNA (1:1000 to 1:100,000 depending on the initial concentration determined by the Qubit® dsDNA HS Assay Kit). PCR thermal cycling conditions consisted of initial denaturation at 95°C for 5 minutes, 35 cycles of 95°C for 30 seconds (denaturation) and 60°C for 45 seconds (annealing and extension), melt curve analysis to 95°C (continuous) and cooling at 37°C, using

the Light Cycler LC480II. The template DNA was denatured for 5 minutes at room temperature using freshly diluted 0.1 N sodium hydroxide (NaOH) and the reaction was subsequently terminated by the addition of HT1 buffer. Following calculation of the molarity using qPCR data, template DNA was diluted to a loading concentration of 10 pM using the HT1 buffer. The RNA libraries were sequenced on an Illumina MiSeq platform with version 3 chemistry using sequencing by synthesis (SBS) technology to generate 2 x 300 bp paired-end reads. To help balance the complexity of the amplicon library 15% PhiX was spiked in.

6.2.5 Data pre-processing

Read trimming was completed by the NBAF at the University of Liverpool. The raw Fastq files were trimmed for the presence of Illumina adapter sequences using *cutadapt* version 1.2.1 (Martin, 2011). The option -O 3 was used, so the 3' end of any reads which match the adapter sequence for 3 bp. or more was trimmed. The reads were further trimmed using *Sickle* version 1.200 (Joshi and Fass, 2011) with a minimum window quality score of 20. Reads shorter than 20 bp after trimming were removed. Trimmed files were imported into R for the remainder of the processing and analysis.

Excepting the trimming completed at the NBAF, all analyses were conducted in R version 4.0.3 (R Core Team, 2020). ITS rRNA amplicon sequence processing was completed in *DADA2* version 1.18 (Callahan et al., 2016) and *phyloseq* version 1.34 (McMurdie and Holmes, 2013).

6.2.6 Data processing, visualisation, and analysis

Operational Taxonomic Units (OTUs) with less than 0.0001% abundance were removed. Samples were screened to remove those with less than 500 reads, but this filtering did not remove any samples for this experiment. After pre-processing and processing, a total of 4797603 trimmed reads, and a median of 58161 reads per sample (range of 20482 to 97689) were generated, totalling 2721 OTUs.

For richness, evenness, and relative abundance plots, data was rarefied to even depth using the minimum library size present (20482 reads) using *phyloseq* version 1.34 (McMurdie and Holmes, 2013). Observed richness (number of OTUs) was also calculated using *phyloseq* version 1.34. Relative abundance plots were generated from rareified data, with read counts transformed to a ratio of total read counts per sample. To test for statistical differences in richness between groups, a two-way anova test was used, incorporating soil horizon, date, and eCO_2 treatment variables.

Differences in community composition between groups were visualised using principal component analysis (PCA) ordination plots, with each sample shown as a single point. Before plotting ordination plots, data was normalised using a centred log-ratio (CLR) transformation using *microbiome* version 1.12.0 (Gloor et al., 2017; Lahti and Shetty, 2012). PCA calculations were completed using *phyloseq* version 1.34 (McMurdie and Holmes, 2013). Ordination plot ellipses were drawn for each group using the *stat_ellipse* function in *ggplot2*, using the default ellipse type which assumes a multivariate t-distribution (Wickham, 2016). To test for statistical differences in community composition, a Euclidean distance matrix was generated from the CLR-transformed data, and significance tested using the *adonis* test in *vegan* version 2.5-7, analogous to a PERMANOVA test (Oksanen et al., 2020). All figures were generated in *ggplot2* version 3.3.5 (Wickham, 2016).

FUNGuild was used to assign ecological guilds (functional groups) to fungal OTUs, using *FUNGuildR* version 1.1 (Furneaux and Song, 2021; Nguyen et al., 2016).

Data from aCO_2 (control) arrays only was used for Section 6.3.2.

6.3 Results

6.3.1 Baseline data

Across the 72 samples in the experiment, a total of 2721 OTUs were detected. Using the UNITE database, 97% of OTUs were allocated to at least phylum level, and 79% to at least genus level. A total of 104 genera were identified across the samples. After normalisation, *Russulaceae* was the most dominant family, making up 35% of OTUs. Species in the *Mortierellaceae* family were the second most abundant group, comprising just under 15% of OTUs. Richness was highly variable between samples, varying from 19 to 122 species per sample (Figure 6-i A).

In the baseline samples collected in 2017, before eCO_2 fumigation began, there was no significant difference between the group of arrays allocated as aCO_2 arrays (arrays 2, 3, and 5), and the group allocated for future eCO_2 treatment (arrays 1, 4, and 6) ($p = 0.251$). However, there were high levels of variation between individual arrays ($p = 0.001$).

6.3.2 Effect of soil layer

There were significant differences in the fungal communities between the O and A soil horizons ($p=0.001$), with the communities forming distinct clusters in PCA analysis (Figure 6-i B). As would be anticipated for a complex woodland soil system, the PCA analysis revealed high levels of variation in the system, with the first two principle components each representing 5.6% and 4.9% of variation in the system respectively. There was not a significant difference in community richness between the O and A horizons ($p = 0.43$, Figure 6-i A).

Fungal communities in both soil layers were dominated by *Ascomycota* and *Basidiomycota*, however there was a higher relative abundance of *Basidiomycota* in the A horizon (Figure 6-i C). The dominant (highest relative abundance) families in both

O and A horizons were *Russulaceae*, making up 30% of the OTUs in the O layer, and 38% in A horizon. *Mortierellaceae* was the next most common family across both horizons, making up 18% of OTUs in O and 10% in A. Yeasts from groups such as *Piskurozymaceae* and *Trimorphomycetaceae* totalled over 15% OTUs in the A horizon, but were less abundant in the O horizon. Symbiotrophs were the most common functional group across the soil profile, as explained by the high prevalence of ectomycorrhizal fungi in the *Russulaceae* family (predominantly *Russula* and *Lactarius* species) (Figure 6-i D). Symbiotrophs had a higher relative abundance in the A horizon, with higher proportions of fungi with mixed functional groups (e.g. Saprotroph-Symbiotrophs) in the O horizon.

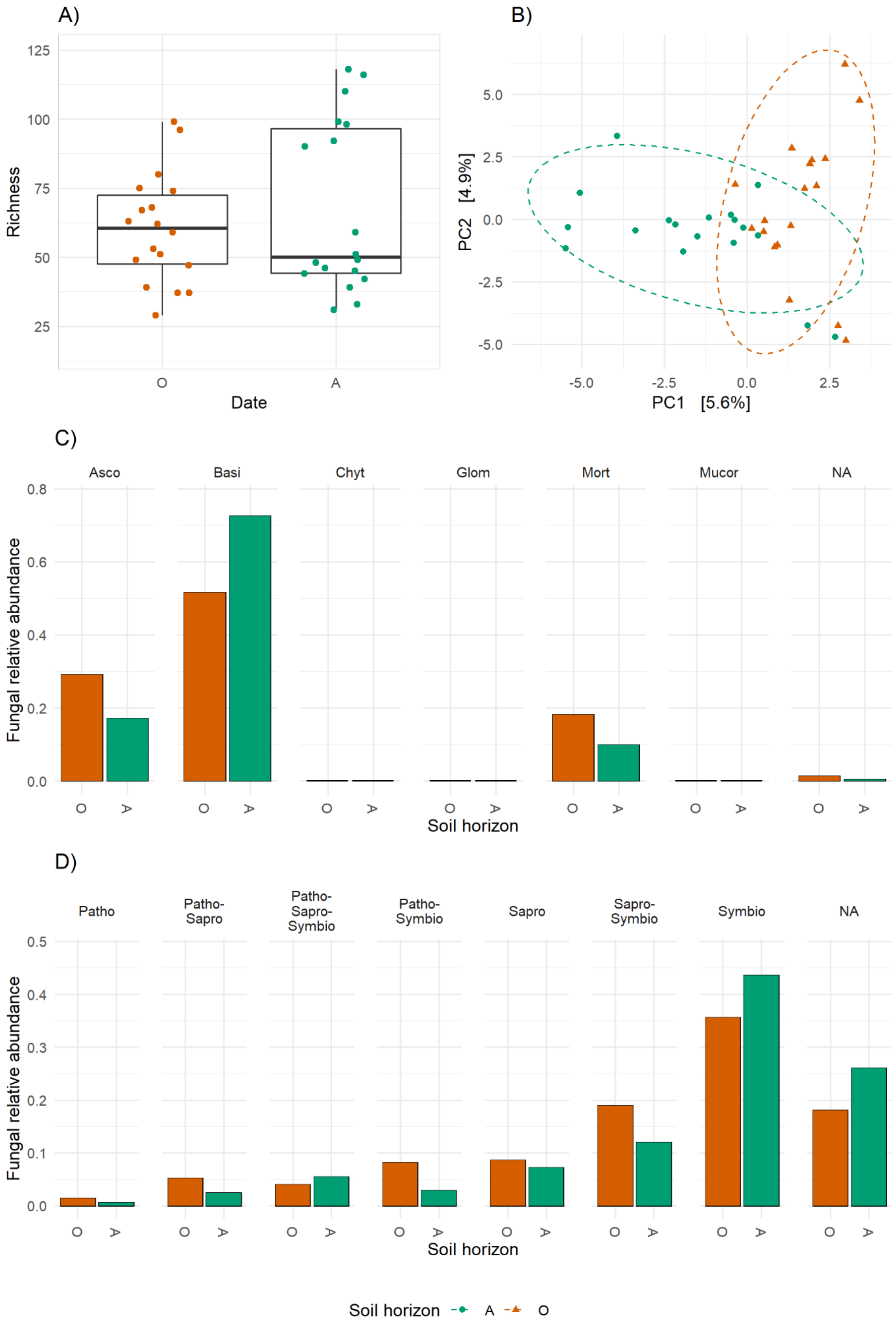

A)
Richness
125
100
75
50
25
O
A
Date
B)
PC2 [4.9%]
5.0
2.5
0.0
-2.5
-5.0
-5.0
-2.5
0.0
2.5
PC1 [5.6%]
C)
Asco
Basi
Chyt
Glom
Mort
Mucor
NA
Fungal relative abundance
0.8
0.6
0.4
0.2
0.0
O
A
Soil horizon
D)
Patho
Patho-Sapro
Patho-Sapro-Symbio
Patho-Symbio
Sapro
Sapro-Symbio
Symbio
NA
Fungal relative abundance
0.5
0.4
0.3
0.2
0.1
0.0
O
A
Soil horizon
Soil horizon
A
O

Figure 6-i Fungal communities in the O and A soil horizons. Subplots show: A) Fungal richness, B) PCA plot of fungal communities, C) Relative abundance of fungal phyla, and D) Relative abundance of fungal functional groups.

Fungal phyla in subplot C are as follows (left to right): Ascomycota (Asco), Basidiomycota (Basi), Chytridiomycota (Chyt), Mortierellomycota (Mort), Mucormycota (Mucor), Unassigned (NA). Fungal functional groups in subplot D are as follows (left to right): Pathotroph (Path), Pathotroph-Saprotroph (Patho-Sapro), Pathotroph-Saprotroph-Symbiotroph (Patho-Sapro-Symbio), Pathotroph-Symbiotroph (Patho-Symbio), Saprotroph (Sapro), Saprotroph-Symbiotroph (Sapro-Symbio), Symbiotroph (Symbio), Unassigned (NA).

6.3.3 Effect of eCO_2

At baseline (Figure 6-ii A), there was no significant difference in fungal community composition between aCO_2 and eCO_2 arrays ($p = 0.251$). After one year of eCO_2 treatment, there was a shift in fungal communities, causing a significant difference between the eCO_2 and aCO_2 control arrays ($p = 0.039$, Figure 6-ii B).

Richness was slightly higher in eCO_2 arrays than in aCO_2, both at baseline and after a year of eCO_2 treatment, however this difference was not statistically significant in either year ($p = 0.78$, and $p = 0.57$ for 2017 and 2018 respectively).

As identified in Section 6.3.2, fungal communities were dominated by fungi in the *Basidiomycota* and *Ascomycota* phyla, and this was consistent across eCO_2 and aCO_2 arrays (Figure 6-ii C and D). Symbiotrophic (mycorrhizal) fungi dominated, however there was a high proportion of species which could not be assigned to a functional group (Figure 6-ii E and F). There were high levels of variation in the relative abundances of fungi between years, soil horizons, with eCO_2 treatment, and within the aCO_2 control treatment group. Some differences are visible, for example in the O soil horizon, there was a decrease in the relative abundance of mycorrhizal fungi after one year of eCO_2 treatment. However, this trend was not present in the A soil horizon, where relative abundances of mycorrhizal fungi remained similar in the O and A horizons between 2017 and 2018.

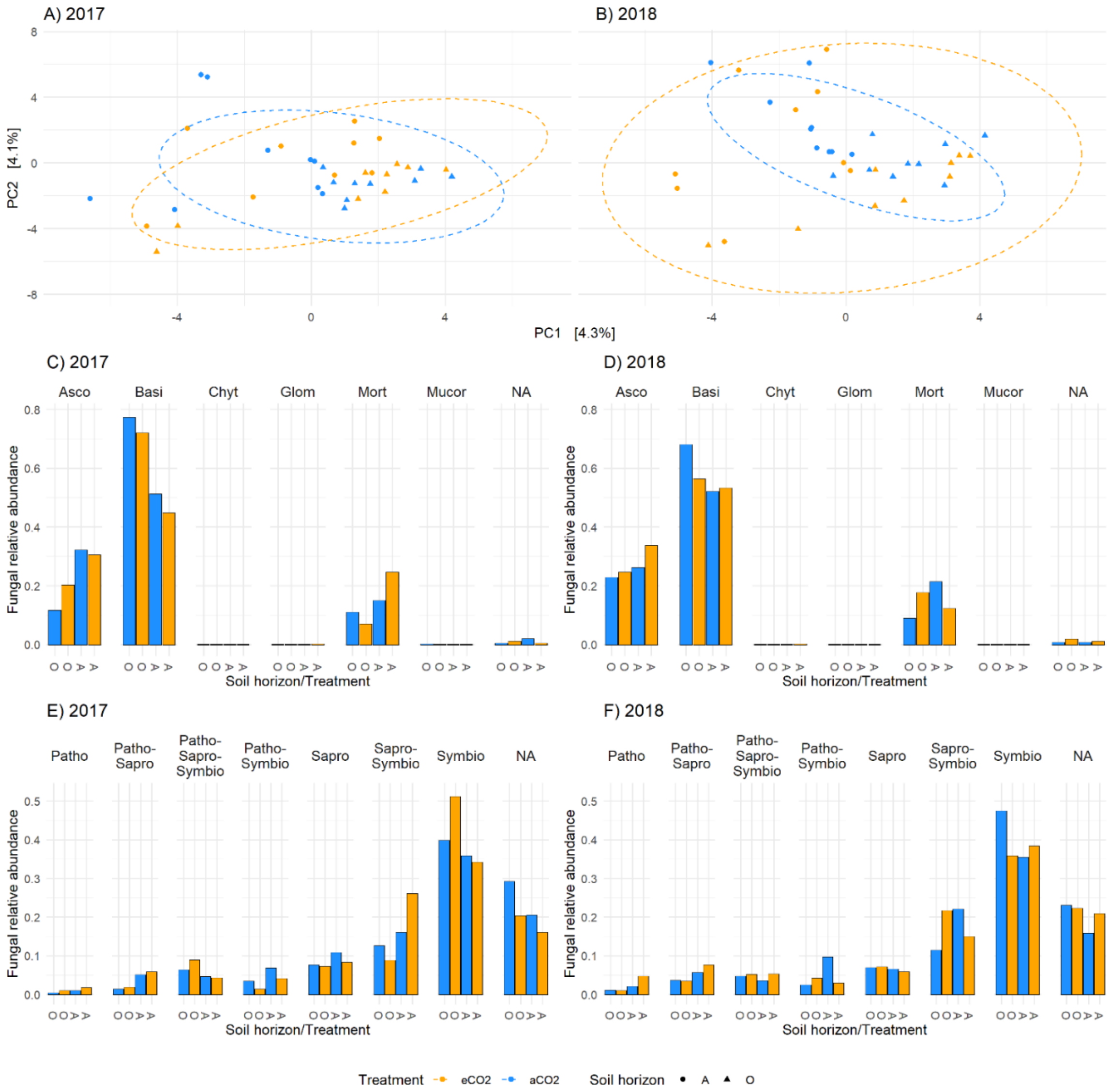


Figure 6-ii Fungal communities across eCO$_2$ and aCO$_2$ treatments in 2017 (baseline data) and 2018 (one year of eCO$_2$ treatment). Subplots show: A) PCA plot of fungal communities in 2017, B) PCA plot of fungal communities in 2018, C and D) Relative abundance of fungal phyla, E and F) Relative abundance of fungal functional groups.

Fungal phyla in subplots C and D are as follows (left to right): Ascomycota (Asco), Basidiomycota (Basi), Chytridiomycota (Chyt), Mortierellomycota (Mort), Mucormycota (Mucor), Unassigned (NA). Fungal functional groups in subplots E and F are as follows (left to right): Pathotroph (Path), Pathotroph-Saprotroph (Patho-Sapro), Pathotroph-Saprotroph-Symbiotroph (Patho-Sapro-Symbio), Pathotroph-Symbiotroph (Patho-Symbio), Saprotroph (Sapro), Saprotroph-Symbiotroph (Sapro-Symbio), Symbiotroph (Symbio), Unassigned (NA).

6.4 Discussion

Although the majority of the 1st generation of forest FACE experiments collected data on fungal communities, there have rarely been studies collecting data before eCO$_2$

fumigation started, the studies used a wide variety of different methods, and very few next-generation sequencing methodologies were used. These previous studies demonstrated widely varying effects of fungal communities to eCO_2, and comparing the results of these studies is particularly challenging due to the wide variety of experimental methodologies used.

In this study, we collected fungal soil metabarcoding data at the BIFoR FACE experiment before eCO_2 fumigation began, and after one year of fumigation, addressing two key hypotheses: A) Fungal community composition and richness will be significantly different between soil horizons, B) Fungal communities will be affected by one year of eCO_2 treatment.

It seems likely that the fungal species assemblage at BIFoR FACE is typical for a woodland of its type. However, it should be noted that there are limited datasets available for comparison. Richness was highly variable between individual samples, varying from 19 to 122 species, which is comparable to many of the lowland woodland fungal richness data reported in George et al., 2019b. *Russulaceae* was the most common family, followed by *Mortierellaceae*. Although focussing specifically on ectomycorrhizal fungi, other studies show *Russulaceae* to form a dominant and important part of the soil woodland mycobiome in oak woodlands (Courty et al., 2008; Suz et al., 2014; van der Linde et al., 2018). The high prevalence of *Mortierellaceae* may be due to the storage and processing of the soil samples, as species in the *Mortierellaceae* family have been identified as spoilage fungi in other fungal soil sequencing studies (Clasen et al., 2020). To further investigate the prevalence of *Mortierellaceae* in soil, future studies minimising the storage and processing time as far as possible would be a useful step. There was a notable lack of arbuscular mycorrhizal fungi identified in the data, with only a single *Glomeromycota* species listed. Although the woodland is likely to be dominated by ectomycorrhizal fungi, there are a number of tree species present at BIFoR FACE which are known to associate with arbuscular mycorrhizal fungi, namely *Corylus avellana* (hazel), and *Acer pseudoplanatus* (sycamore), in addition to high abundance of ground flora with AM fungi associations (for example, *Hyacinthoides non-scripta* (bluebell)). However, it is well documented in the literature that ITS1 and ITS2 primers do not typically identify species in *Glomeromycota* well, due to their highly variable ITS region (Lee et al., 2008; Lekberg et al., 2018; Tedersoo et al., 2022). In order to study this group in any detail, using combination of primers, potentially including other regions apart from ITS, or using primers specific to the whole ITS region may give better amplification of DNA from species in the *Glomeromycota*, and therefore give a more accurate representation of this group.

There were high levels of variation in fungal richness and species abundance between arrays, including in baseline data. Due to the strong heterogeneity in the BIFoR FACE forest, there are some vegetation differences between arrays. For example, all arrays have *Quercus robur* (oak), *Corylus avellana* (hazel), and *Acer pseudoplanatus* (sycamore); however, *Betula pendula* (silver birch) is only present in Arrays 1 and 3, and *Sambucus nigra* (elder) is present in Arrays 3, 4, 5, and 6 (K. Hart, private communication, 2020). These tree species differences may be driving distinct patterns in fungal community composition between arrays, which could be due to tree species-specific effects, or differences in tree traits (Prada-Salcedo et al., 2021; Urbanová et al., 2015). This seems particularly likely given that ectomycorrhizal fungi were the most abundant fungal functional group recorded across the experiments.

Hypothesis B stated that fungal community composition and richness would be significantly different between soil horizons. Despite the high levels of heterogeneity between samples, there was strong supporting evidence for differences in community composition between the O and A horizons, however there was no significant difference in richness.

This is consistent with other evidence in the literature which shows differences in soil fungal communities with depth or soil horizon (Courty et al., 2008; Hemkemeyer et al., 2019; Mundra et al., 2021; Šnajdr et al., 2008; Voříšková et al., 2014). Relative abundance of ectomycorrhizal fungi was higher in the A soil horizon compared with the organic layer, which had higher abundances of saprotrophic species. Other studies in temperate woodlands have also found that saprotrophs dominate in the organic layer, which is likely caused by a combination of abiotic factors changing with soil depth, but also competition between fungal functional groups (Bödeker et al., 2016; Carteron et al., 2020; Khokon et al., 2021). These results highlight the importance of studying multiple horizons within the soil profile, as fungal communities can be radically different with depth, and therefore may show different results with climate manipulation. To the best of our knowledge, none of the previous forest FACE experiments studied soil microbial communities across a range of soil depths, and there was large variation in the depths of soil samples used between and within different FACE experiments.

Across both the O and A soil horizons, there was no significant difference in fungal communities between the arrays assigned for eCO_2 treatment, and the control aCO_2 arrays at baseline. This is an important dataset for future fungal studies at BIFoR FACE, and one that is missing from many previous fungal studies at FACE experiments, which often started part way through the CO_2 fumigation treatments.

After one year of eCO_2 fumigation, there was a significant difference between fungal communities in the eCO_2 and aCO_2 arrays. This is a key finding, and provides evidence that eCO_2 fumigation is also altering non-photosynthetic species in the woodland in addition to the changes seen in plants, for example elevated photosynthetic rates (Gardner et al., 2022). However, there was no clear trend in fungal relative abundance with eCO_2, with responses differing between soil layers. In some previous similar woodland FACE studies, similar results have been seen, where overall richness has not been affected by CO_2, but the composition of fungal communities changed (Parrent et al., 2006; Weber et al., 2013). However, it is also important to acknowledge that other studies did not see any response from woodland soil fungal communities to eCO_2 (Chung et al., 2006; Dunbar et al., 2014; Feng et al., 2010; Weber et al., 2012).

Continuing to study fungal communities in mature woodland FACE experiments as a whole, and specifically further studies as fumigation continues at the BIFoR FACE experiment are important next steps to provide further data towards the hypotheses addressed in this study. Understanding long term shifts in fungal community composition and abundance is an essential part of the understanding the carbon cycle in FACE woodlands, and complements other work being done on woodland responses to eCO_2 at BIFoR FACE (Baird et al., 2021; Gardner et al., 2022; Kourmouli, 2022; MacKenzie et al., 2020; Roberts et al., 2022; Sgouridis et al., 2023).

6.5 Conclusions

In summary, the results from this dataset suggest that there are early responses of fungal communities to eCO_2, although these responses appear to be isolated to changes in the fungal community composition, with no response seen in overall richness. This is a vital piece of data for our understanding of responses of fungi in mature woodlands to eCO_2. Going forwards, further study is now warranted to investigate how these responses evolve as eCO_2 fumigation continues.

This chapter highlights that the observed soil fungal communities present at the BIFoR FACE site appear typical for a UK woodland of its type. Within the soil samples, there is a clear heterogeneity between soil horizons. This baseline dataset collected on fungal communities is an important part of any future studies across the duration of the FACE experiments. Additionally, it provides an important contribution to UK data on soil fungal communities, an area which is severely lacking in published datasets, particularly those not solely focussing on ectomycorrhizal communities.

CHAPTER 7: A FIVE YEAR STUDY OF SOIL FUNGAL COMMUNITIES IN A MATURE WOODLAND FACE EXPERIMENT

7.1 Introduction

Forests and woodlands globally store an estimated 861 ± 66 Pg of carbon (Pan et al., 2011). With global CO_2 levels reaching unprecedented levels (419ppm at the time of writing), research understanding the impacts of increased CO_2 on natural carbon sinks, such as woodlands, have never been more critical.

Free Air Carbon Dioxide (FACE) experiments form an important part of laboratory, field, and modelling work building our understanding of how eCO_2 affects trees and woodlands. The size of FACE experiments is much more suited to trees than smaller open-top chamber, greenhouse, and laboratory experiments, and the FACE design avoids many of the artefacts (for example, altered humidity levels) that are common in open-top chamber experiments (Norby and Zak, 2011).

The first generation of FACE experiments (for more detail see Chapter 2.1), studying predominantly young trees, demonstrated that eCO_2 causes significant effects across many components of the woodland ecosystem, including elevated net primary productivity and increased carbon storage, however many questions remained after these first experiments (Norby and Zak, 2011). A second generation of FACE experiments in mature woodlands are now underway, with experiments located in Australia (EucFACE), UK (BIFoR FACE), and under construction in Brazil (Amazon FACE). The goals of these experiments are to investigate the impacts of eCO_2 on mature woodlands across a wider range of woodland types, and to attempt to address many of the remaining questions from the first FACE generation, particularly the impacts of nutrient limitation and water stress, and the impacts of eCO_2 on biodiversity of woodlands (Norby et al., 2016).

As part of this wider programme of work investigating the impacts of eCO_2 on woodland biodiversity, we used the BIFoR FACE experiment in Staffordshire (UK) to research the effects of eCO_2 on soil fungal biodiversity. Several previous woodland FACE experiments have done work investigating fungal communities, however this work has typically not focussed on the biodiversity of the fungal communities, instead choosing to focus on the function of these communities (for example, changes in fungal biomass or mycorrhization of roots). Few experiments used fruit body surveys, and no FACE experiment to date has used DNA barcoding to investigate fungal communities. The research done into fungal biodiversity has shown varied responses to eCO_2, sometimes even within the same FACE experiment. For example, work at AspenFACE showed that ECM fungi fruit body community composition was significantly affected by eCO_2 treatment, however no effect of eCO_2 on soil fungal communities (composition, diversity, or relative abundance) was detected (Andrew and Lilleskov, 2009; Chung et al., 2006; Dunbar et al., 2014). Duke FACE showed varied responses across the soil fungal studies completed at the experiment, with several studies showing no impact of eCO_2 on fungal community composition or richness from soil samples (Feng et al., 2010; Parrent et al., 2006; Weber et al., 2012), however two other studies showed that community composition was altered under eCO_2 (Parrent and Vilgalys, 2007; Weber et al., 2013). At PopFACE, there was also no effect of eCO_2 on fungal communities after five years of eCO_2 treatment in unfertilised soil, but they did see a combined nutrient treatment by eCO_2 effect on community composition (Lagomarsino et al., 2007). In the second generation of FACE experiments, altered soil fungal community composition under eCO_2 has also been detected at EucFACE, however these studies used modified soil substrates (mesh bags and cores) (Castañeda-Gómez et al., 2021, 2020).

In addition to the variation between woodlands across the FACE experiments, the wide variety of experimental methodologies used to study fungal communities makes it challenging to draw conclusions on fungal biodiversity, even within a single experiment. For example, DukeFACE was the longest running FACE experiment, with 11 published papers related to the impacts of eCO_2 on fungi. However, the results from these studies vary significantly, and it is unclear whether the effects of eCO_2 on fungi changed across the duration of the experiment, or if the differing methodologies (for example, fruit body surveys, ingrowth bags, soil cores) revealed differing ways the fungal communities were behaving. These studies highlight the importance of gaining more information on the biodiversity in FACE experiments, but also using consistent methodologies across the duration of a FACE experiment.

Continuing from our pilot study (Chapter 6), we collected soil samples during the first five years of the BIFoR FACE experiment to investigate the impacts of eCO_2 on fungi in mature temperate woodlands, Using DNA metabarcoding, we investigated the impacts of eCO_2 treatment on soil fungal communities.

Our hypotheses were:

A) Fungal communities will be affected by eCO_2 treatment.
B) The effects of eCO_2 treatment on fungal communities will continue throughout the first five years of the BIFoR FACE experiment.

7.2 Methods

7.2.1 BIFoR FACE

Soil samples were collected annually in early spring over a five-year period (2017 – 2021) at the BIFoR FACE facility. The BIFoR FACE facility design and methodology is described in detail in Chapter 2.3, with further detail on soils, tree species, and mycorrhizal types described in Chapter 6.2.1.

7.2.2 Soil sampling, processing, and storage

Three soil cores were collected from each of Arrays 1 – 6 on the 27th March 2017, 06th March 2018, 25th March 2019, 09th March 2020, and 08th February 2021, totalling 90 cores. Samples were collected using a metal soil corer (Van Walt Ltd) with 50 mm diameter, and 300 mm length, with the core lined with a new plastic liner for each sample. Samples were stored at 4°C for up to a week before processing. Cores were divided into soil horizons before being sieved, and removing stones, roots, and soil microfauna by hand. Sieved soils were aliquoted into a centrifuge tube before being frozen at -70°C to -80°C.

Excepting two samples where there was inadequate material in the O horizon, DNA was extracted from the O horizons in each of the soil samples. These 88 samples were sequenced for the ITS1 fragment of the fungal genome as a marker for fungal communities.

7.2.3 DNA extraction

0.25 g defrosted soil was aliquoted per soil sample before being extracted using the Qiagen DNeasy PowerSoil Pro kit. Blank extractions were completed as negative controls. In order to maximise DNA extraction, a modified method was used for step two of the protocol, where a Precellys 24 bead beater (Stretton Scientific) was used as an alternative to the vortex step. Samples were run at 6500 rpm for two periods of 20 seconds, with a 20 second rest in between spin periods. After homogenisation, samples were cooled on ice before proceeding with step three of extraction. An initial check for DNA quality and quantity was performed using a Nanodrop 1000 Spectrophotometer (Thermo Scientific) before the DNA was frozen at -20°C for storage before sequencing. Samples were transported to their sequencing facility on ice within six hours of removal from the freezer.

7.2.4 DNA sequencing

DNA sequencing was completed by the University of Salford. To identify fungal communities, DNA for the ITS1F-2 rRNA gene was amplified using single index reverse primers and a modified protocol of Smith and Peay (2014) and Nguyen et al. (2015), as detailed in Griffiths et al. (2020). Duplicate PCRs were run using Solis BioDyne 5x HOT FIREPol® Blend Master Mix, 2 µM primers, and 1.5 µl of sample DNA. Thermocycling conditions were 95°C for 10 minutes, followed by 28 cycles of 95°C for 30 seconds, 52°C for 20 seconds, and 72°C for 30 seconds, with a final extension of 72°C for 8 minutes. PCR products were quality checked using a 2200 TapeStation (Agilent, USA). PCR replicates were combined into a single PCR plate and the products cleaned using HighPrepTM PCR clean up beads (MagBio, USA) according to the manufacturer’s instructions. To normalise the libraries, 1µl of each sample was combined, and a titration sequencing run with this pool using an Illumina v2 nano cartridge (paired end reads; 2 x 150bp) on the Illumina MiSeq at the University of Salford. Based on the percentage of reads sequenced per library, the volume required for the full sequencing run was calculated, and the samples pooled accordingly. ITS rRNA amplicon sequencing was conducted using paired-end reads (2 x 250bp) using an Illumina v2 cartridge on the MiSeq platform at the University of Salford. Extraction blanks were included as negative controls.

The samples from Chapter 7 and 8 were sequenced on the same sequencing run. The samples were also sequenced for bacterial communities using 16S primers, however the sequence quality and quantity were poor, and therefore this data was not included in these analyses.

7.2.5 Data pre-processing

All analyses were conducted in R version 4.0.3 (R Core Team, 2020). The ITS rRNA amplicon data was run through an initial trimming step using *cutadapt* (Martin, 2011), before amplicon sequencing processing was completed in *DADA2* version 1.18 (Callahan et al., 2016) and *phyloseq* version 1.34 (McMurdie and Holmes, 2013). Negative samples were sequenced, and no OTUs were detected in the negative extractions. OTUs with less than 0.0001% total relative abundance were removed. Samples with less than 1000 reads were removed, which resulted in only the negative control samples being removed. Chimeras (incorrectly joined sequences) were filtered and taxonomy assigned using the UNITE 8.3 database (Abarenkov et al., 2021). After processing, there were a total of 843400 reads, with a median of 9176 per sample (a range of 1081 to 19698 per sample), totalling 350 OTUs.

7.2.6 Data processing, visualisation, and analysis

For richness and relative abundance plots, data was rarefied to even depth using the minimum library size present using *phyloseq* version 1.34 (McMurdie and Holmes, 2013). Observed richness (number of OTUs) was also calculated using *phyloseq* version 1.34. Relative abundance plots were generated from rareified data, with read counts transformed to a ratio of total read counts per sample. To test for statistical differences in richness between groups, a two-way anova test was used, incorporating eCO_2 treatment and date variables.

Differences in community composition between groups were visualised using PCA ordination plots, with each sample shown as a single point. Before plotting ordination plots, data was normalised using a CLR transformation using *microbiome* version 1.12.0 (Gloor et al., 2017; Lahti and Shetty, 2012). PCA calculations were completed using *phyloseq* version 1.34 (McMurdie and Holmes, 2013). Ordination plot ellipses were drawn for each group using the *stat_ellipse* function in *ggplot2*, using the default ellipse type which assumes a multivariate t-distribution (Wickham, 2016). To test for statistical differences in community composition, a Euclidean distance matrix was generated from the CLR-transformed data, and significance tested using the *adonis* test in *vegan* version 2.5-7, analogous to a PERMANOVA test (Oksanen et al., 2020). All figures were generated in *ggplot2* version 3.3.5 (Wickham, 2016).

FUNGuild was used to assign ecological guilds (functional groups) to fungal OTUs, using *FUNGuildR* version 1.1 (Furneaux and Song, 2021; Nguyen et al., 2016).

7.3 Results

Similar to the pilot study in Chapter 6, this study also showed no significant difference in fungal community composition in baseline samples from 2017 ($p = 0.449$). Taken as a whole, soil samples from eCO_2 arrays across 2018 – 2021 had significantly different fungal communities than aCO_2 control arrays ($p = 0.001$, Figure 7-i B). However, not all years were significantly different when taken individually: 2018 ($p = 0.056$), 2019 ($p = 0.029$), 2020 ($p = 0.057$), 2021 ($p =0.035$).

The most abundant families occurred at similar proportions in both aCO_2 and eCO_2 arrays. Species in the *Russulaceae* dominated, making up 48% and 50% of OTUs in aCO_2 and eCO_2 arrays respectively. *Herpotrichiellaceae* was the next most abundant family in both control (14% of OTUs) and treatment (16%) samples, with differences occurring between aCO_2 and eCO_2 with the less abundant families. For example, *Hydnangiaceae*, and *Cordycipitaceae* were both more abundant in aCO_2 arrays, whereas *Amanitaceae* and *Boletaceae* were more abundant in eCO_2 arrays.

Richness varied from 18 to 61 OTUs per soil sample, with a median of 39 (Figure 7-i A). There was no significant difference in richness between aCO_2 and eCO_2 arrays at baseline ($p = 0.418$). After one, two, and three years of eCO_2 treatment, there was no apparent effect of eCO_2 on richness ($p = 0.496$, $p = 0.643$, $p = 0.194$ respectively). In 2021, there was a larger difference between the treatment and control arrays ($p = 0.071$), however it is unclear whether this is a cumulative effect of four years of eCO_2 treatment, or due to annual variation.

Across all five years of the experiment, *Basidiomycota* and *Ascomycota* remained the first and second most abundant phyla respectively (Figure 7-i C and D). Symbiotrophs (mycorrhizal fungi) were the most abundant functional group. The relative abundance of symbiotrophs at baseline (2017) was almost identical between eCO_2 and aCO_2 arrays, making up around 60% of OTUs. Over the following three years of the experiment, the relative abundance of this group dropped under eCO_2 and aCO_2, falling to around 50% in both treatment and control arrays. However, in 2021, the symbiotroph abundance in eCO_2 conditions dropped to 40%, which was not replicated in the control arrays, where abundance remained greater than 50% (Figure 7-i E and F).

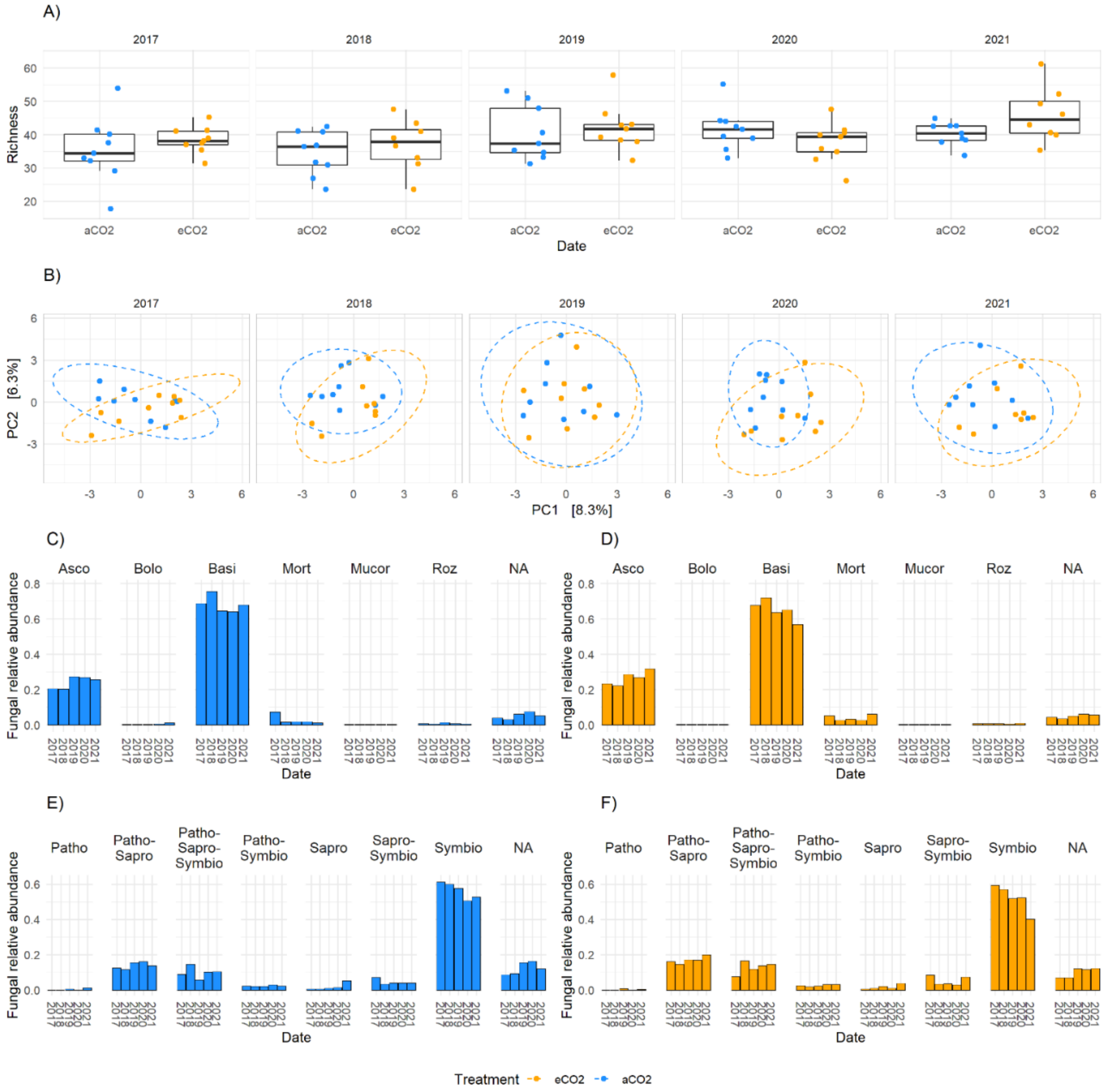


Figure 7-i Fungal communities in aCO$_2$ control arrays and eCO$_2$ treatment arrays across a five year period. Subplots show: A) Fungal richness, B) PCA plot of fungal communities, C) Relative abundance of fungal phyla in aCO$_2$ control arrays, D) Relative abundance of fungal phyla in eCO$_2$ treatment arrays, E) Relative abundance of fungal functional groups in aCO$_2$ control arrays, F) Relative abundance of fungal functional groups in eCO$_2$ control arrays.

Fungal phyla in subplots C and D are as follows (left to right): Ascomycota (Asco), Basidiobolomycota (Bolo), Basidiomycota (Basi), Mortierellomycota (Mort), Mucormycota (Mucor), Rozellomycota (Roz), Unassigned (NA). Fungal functional groups in subplots E and F are as follows (left to right): Pathotroph (Patho), Pathotroph-Saprotroph (Patho-Sapro), Pathotroph-Saprotroph-Symbiotroph (Patho-Sapro-Symbiotroph), Saprotroph (Sapro), Saprotroph-Symbiotroph (Sapro-Symbio), Symbiotroph (Symbio), Unassigned (NA).

7.4 Discussion

Soil samples were collected over a five-year period at the BIFoR FACE experiment to analyse the effects of eCO_2 treatment on soil fungal communities. This dataset is one of the most comprehensive datasets on fungi in any woodland FACE experiment, and provides an important contribution to the growing data within mature woodland “second generation” FACE experiments. The hypotheses for this experiment were: A) Fungal communities would be affected by eCO_2 treatment, and B) The effects of eCO_2 would continue as the duration of eCO_2 fumigation increased.

This study provided further evidence that soil fungal communities were not significantly different between eCO_2 and aCO_2 arrays at baseline (2017). This matches the pilot study done using these samples (Chapter 6). Although using the same 2017 and 2018 soil cores, this new study used different sub-samples, DNA extraction kits, and sequencing methodology, and showed very similar results. Both studies demonstrated that fungal community composition, richness, and relative abundances of phyla and functional groups were all very similar between aCO_2 and eCO_2 arrays at baseline. These results provide an important baseline for this study and future studies at the BIFoR FACE experiment, enabling more reliable interpretation of future datasets at the experiment, as well as comparisons with other woodlands and FACE experiments.

The fungal community responded very quickly to eCO_2, with differences in community composition being detected after one year of treatment and continuing across the duration of this experiment. Rapid responses to eCO_2 by mature trees have been detected at BIFoR FACE, as well as being a well-established response in other FACE experiments (Gardner et al., 2022; Ziegler et al., 2023). However, there is less evidence for the responses of non-photosynthetic organisms such as fungi. Previous FACE experiments have demonstrated positive, negative, and no response of fungal communities to eCO_2 (see Chapter 2 Table 2-i). These results from a second-generation FACE experiment contribute to the evidence on fungi, and may allow a better understanding of why previous FACE experiments have shown such varied responses of fungi to eCO_2.

The rapid response of fungal communities to eCO_2 at BIFoR FACE could be due to the high abundance of mycorrhizal fungi. Mycorrhizal fungi (particularly species in *Russulaceae*) dominated the soil. These fungi may respond more quickly to eCO_2 than fungi in other functional groups due to their direct association with tree roots, for example they may receive additional sugars due to the higher photosynthetic rates of the trees under eCO_2. Previous FACE experiments have demonstrated that other environmental constraints (e.g. nitrogen availability) can cause plant responses to plateau after the initial response, and it will therefore be important to continue these analyses at the BIFoR FACE experiment to see if the effects to trees, and fungi, continue over time (Norby and Zak, 2011).

The differences between aCO_2 and eCO_2 arrays were predominantly due to fungi with low abundances. For example, the *Russulaceae* family dominated in both treatment and control, and remained relatively stable in relative abundance across the experimental duration. Future analysis could focus further on these low abundance fungi, particularly groups such as saprotrophs, which may have a more delayed response to eCO_2 fumigation, and may be responding to accumulating changes in leaf litter and dead wood biochemistry.

Although fungal community composition differences responded very quickly to eCO_2 treatment, this was not demonstrated in other measures of the fungal communities such as richness and relative abundance. From 2017 to 2020 there was some variation in overall species richness, and the relative abundances of different fungal functional groups; however, there were no significant differences between eCO_2 and aCO_2 arrays. However, in 2021 there was an increase in richness in eCO_2 arrays, and decrease in the relative abundance of mycorrhizal fungi. These changes could be due to annual variation, however there may have been changes accumulating in the woodland due to the cumulative effects of four years of eCO_2 treatment. Although early work on fine root abundance was shown to increase under eCO_2 at BIFoR FACE (Ziegler et al., 2023), more recent work shows that in upper soil layers, root abundance may now be decreasing under eCO_2 (G. Handy, personal communication, 2023). This decrease in ECM fungal abundance may be correlated with the decrease in fine root abundance. Saprotroph relative abundance was higher in 2021, which may be due to higher rates of decomposition of dead plant and fungal biomass in the soil. Further work as the BIFoR FACE eCO_2 treatment continues, as well as using quantitative methods (for example qPCR) to quantify the abundance of fungi in the soil is important to understand these results further. Additionally, work investigating bacteria and soil invertebrates would be a valuable addition, and provide further context to the soil carbon cycling within the woodland.

7.5 Conclusions

This five-year study at the BIFoR FACE experiment demonstrated a significant effect of eCO_2 on fungal community composition. The results of this research are consistent with early pilot studies at BIFoR FACE, and show that the effects of eCO_2 on fungal communities continued across years one to four of the experiment.

Fungal richness and the relative abundances of fungal phyla and functional groups showed annual variation, but were not significantly different between control and treatment groups after one, two, and three years of fumigation. Changes in the system were detected after four years of fumigation, likely related to a drop in the relative abundance of mycorrhizal fungi under eCO_2 treatment.

This substantial study makes an important contribution to our understanding on the impacts of eCO_2 on woodland fungi. Future work continuing this dataset as eCO_2

fumigation continues is important to investigate these effects further, particularly relating to the fungal community differences, and the decreases in abundance of mycorrhizal fungi.

CHAPTER 8: INVESTIGATING THE IMPACTS OF SEASON AND ELEVATED CO_2 TREATMENT ON WOODLAND SOIL FUNGAL COMMUNTIES

8.1 Introduction

Soil fungal communities are an essential part of woodland ecosystems. As well as having inherent biodiversity value, they form a vital part of a number of key woodland processes. Despite their importance, our understanding of belowground fungal communities is lacking, and fungi have not been appropriately accounted for in global climate models (Hararuk et al., 2015; Ouimette et al., 2020). This is particularly surprising given the significance of fungi in carbon cycling, particularly in forest systems. For example, a recent study estimates that worldwide, 9.07 Gt CO_2e are allocated annually to ectomycorrhizal fungi, which are predominantly associated with trees (Hawkins et al., 2023). Given the key roles that fungi play in woodlands, in particular in carbon cycling, it is vital to understand how fungal communities may be affected by climate change, and what this means for our woodlands and the storage of carbon worldwide.

In our previous two studies (Chapter 6 and 7), we used the BIFoR FACE experiment to investigate the impacts of eCO_2 on soil fungal communities, using a DNA barcoding approach. These studies demonstrated that fungal community composition is affected by eCO_2 from an early stage (after one year of eCO_2 treatment), however effects on the relative abundance of fungal functional groups was a slower process, with decreases in mycorrhizal fungal abundance only beginning to become apparent after four years of eCO_2 treatment. These previous studies are important datasets for our understanding of the impacts of climate change on soil fungi. However, as with all studies, there were some limitations in the data collected, in particular that samples were only collected once per year. In this new study, we collected data to address these limitations, investigating the interactions between eCO_2 and season on soil fungal communities.

Studies of fungal sporocarps show clear evidence of seasonal patterns in fungal fruiting being affected by climate change, with the average fungal fruiting season in the UK both starting earlier and finishing later (Andrew et al., 2018c; Boddy et al., 2014; Gange et al., 2007; Kauserud et al., 2010). The strong seasonal patterns of fungal fruiting suggest there could be significant seasonal changes in below-ground fungal structures also. Previous studies have demonstrated that woodland soil fungal communities, particularly abundance and enzyme production can be significantly different temporally, however the effects of season on fungal community composition are less clear (Beidler et al., 2023; Gong et al., 2022; Jumpponen et al., 2010; Voříšková et al., 2014; Zhao et al., 2023; Žifčáková et al., 2016). However, unlike fungal fruiting studies, where there has been significant research investigating the interactions between season and climate change, there is not an equivalent body of research investigating the interactions between climate change and seasonality for soil fungal communities.

Despite the evidence demonstrating the potential for high levels of variation in soil fungal communities temporally, previous FACE experiments (including those done at BIFoR FACE) have rarely used multiple sampling points per year, occasionally only using sampling on a single date. This makes it impossible to know whether the samples collected are representative of fungal communities across a whole year.

To investigate the interactions between season and eCO_2 on soil fungi, we used DNA barcoding to study soil fungal communities across two years at the BIFoR FACE experiment. Our hypotheses were:

A) Fungal communities will be affected by eCO_2 treatment

B) Fungal communities will be different between seasons

8.2 Methods

8.2.1 BIFoR FACE

Soil samples were collected four times per year over a two-year period (2018 and 2019) at the BIFoR FACE facility. The BIFoR FACE facility design and methodology is described in detail in Chapter 2.3, with further detail on soils, tree species, and mycorrhizal types described in Chapter 6.2.1.

8.2.2 Soil sampling, processing, and storage

Four soil cores were collected from each of Arrays 1 – 6 on 27th March 2018, 01st June 2018, 25th September 2018, 05th November 2018, 16th April 2019, 26th June 2019, 10th September 2019, and 05th November 2019, totalling 192 samples. Sample collection dates corresponded to one sample per season: spring, summer, early-autumn, late-autumn.

Samples were collected using a sterile 50ml plastic screw-top centrifuge tube, pressed into the soil vertically to collect a sample directly into the centrifuge tube. Samples were homogenized in the tube using a sterile spatula before being stored at 4°C for up to 12 hours, with samples then being frozen at -70°C to -80°C for long term storage.

8.2.3 DNA extraction and sequencing

The 192 samples in this chapter were extracted and sequenced using fungal ITS1 primers exactly as described in 7.2.3 and 7.2.4. The samples from Chapter 7 and 8 were sequenced on the same sequencing run. The samples were also sequenced for bacterial communities using 16S primers, however the sequence quality and quantity were poor, and therefore this data was not included in these analyses.

8.2.4 Data pre-processing

Data pre-processing was completed using the method described in 7.2.5. After processing, there were a total of 22086977 reads, with a median of 10552 per sample (a range of 4288 to 29021 per sample).

8.2.5 Data processing, visualisation, and analysis

Data processing, visualisation, and analysis was completed using the method described in 7.2.6. For analysis investigating the impact of season on fungal communities (Section 8.3.1), data from aCO_2 arrays only was used.

8.3 Results

8.3.1 Seasonal variation in fungal communities

Across the 96 samples collected in the aCO_2 arrays for this season analysis, richness varied from 24 to 62 OTUs per sample, with a median of 45 (Figure 8-i A). Richness was significantly different between seasons ($p = 0.035$), with the lowest average richness in spring, and highest in early autumn. Despite the differences in richness, there was no significant difference in overall community composition between seasons, with no distinct seasonal clusters present in the PCA plot ($p = 1$, Figure 8-i B).

The relative abundance of fungal phyla and functional groups was broadly similar to previous experiments at BIFoR FACE (see Chapter 6 and 7), with samples dominated by species in *Basidiomycota* and *Ascomycota* (Figure 8-i C). The relative abundance of symbiotrophs (mycorrhizal fungi) was higher in spring and summer, making up around 58% of sequences, compared with 55% in autumn (Figure 8-i D). This decrease in relative abundance of symbiotrophs was replaced by small increases in abundances of several functional groups, particularly pathotroph-saprotrophs, saprotrophs, and OTUs unassigned to a functional group.

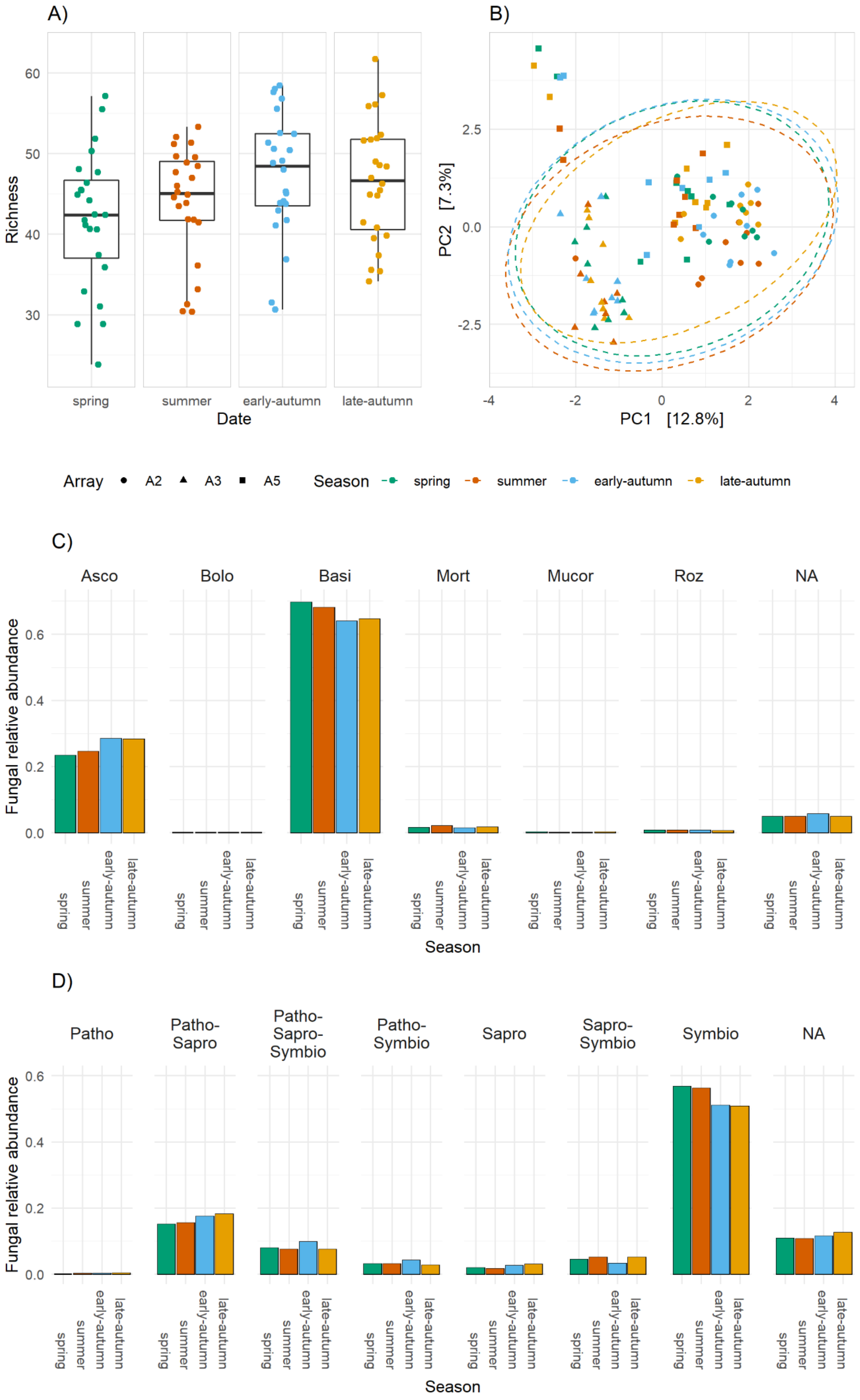

A)
Richness
60
50
40
30
spring
summer
early-autumn
late-autumn
Date
B)
PC2 [7.3%]
2.5
0.0
-2.5
-4
-2
0
2
4
PC1 [12.8%]
Array
● A2
▲ A3
■ A5
Season
spring
summer
early-autumn
late-autumn
C)
Asco
Bolo
Basi
Mort
Mucor
Roz
NA
Fungal relative abundance
0.6
0.4
0.2
0.0
spring
summer
early-autumn
late-autumn
Season
D)
Patho
Patho-Sapro
Patho-Sapro-Symbio
Patho-Symbio
Sapro
Sapro-Symbio
Symbio
NA
Fungal relative abundance
0.6
0.4
0.2
0.0
spring
summer
early-autumn
late-autumn
Season

Figure 8-i Fungal communities across four seasons. Subplots show: A) Mean fungal richness, B) PCA plot of fungal communities, C) Relative abundance of fungal phyla, D) Relative abundance of fungal functional groups.

Fungal phyla in subplot C are as follows (left to right): Ascomycota (Asco), Basidiobolomycota (Bolo), Basidiomycota (Basi), Mortierellomycota (Mort), Mucormycota (Mucor), Rozellomycota (Roz), Unassigned (NA). Fungal functional groups in subplot D are as follows (left to right): Pathotroph (Patho), Pathotroph-Saprotroph (Patho-Sapro), Pathotroph-Saprotroph-Symbiotroph (Patho-Sapro-Symbiotroph), Saprotroph (Sapro), Saprotroph-Symbiotroph (Sapro-Symbio), Symbiotroph (Symbio), Unassigned (NA).

8.3.2 Effects of eCO_2 on fungal communities

Similar to the results in previous DNA barcoding studies at BIFoR FACE (Chapter 6 and 7), there was a significant difference in fungal communities under eCO_2 treatment compared with aCO_2 controls ($p = 0.001$, Figure 8-ii B). Median richness was 46 OTUs per sample, ranging from a minimum of 23 to a maximum of 69. There were no significant differences in richness between eCO_2 and aCO_2 arrays ($p = 0.16$), however there were significant differences in richness between years ($p = 0.0004$). Richness was on average higher in 2019, but increased more in eCO_2 treatment arrays.

The relative abundance of mycorrhizal fungi (symbiotrophs) was over 50% in all treatment groups and years, although the abundance was higher in aCO_2 and in 2018. Species in *Russulaceae* made up the majority of the mycorrhizal fungi sequences (48% of total sequences), with other mycorrhizal fungi such as those in the *Laccaria* genus making up the remaining of sequences in this functional group. *Hydnandiaceae* and *Herpotrichiellaceae* were the next most abundant families in both eCO_2 and aCO_2, although their relative abundances were different between the treatment and control groups. Fungi in the *Hydnandiaceae* family (predominantly mycorrhizal fungi) made up 7% of sequences in aCO_2 and 4% in eCO_2, whereas *Herpotrichiellaceae* relative abundance was 12% and 18% aCO_2 and eCO_2 arrays respectively. No other fungal families had greater than 3% relative abundance in either eCO_2 or aCO_2, although these lower abundance families varied more significantly in abundance between treatment and control.

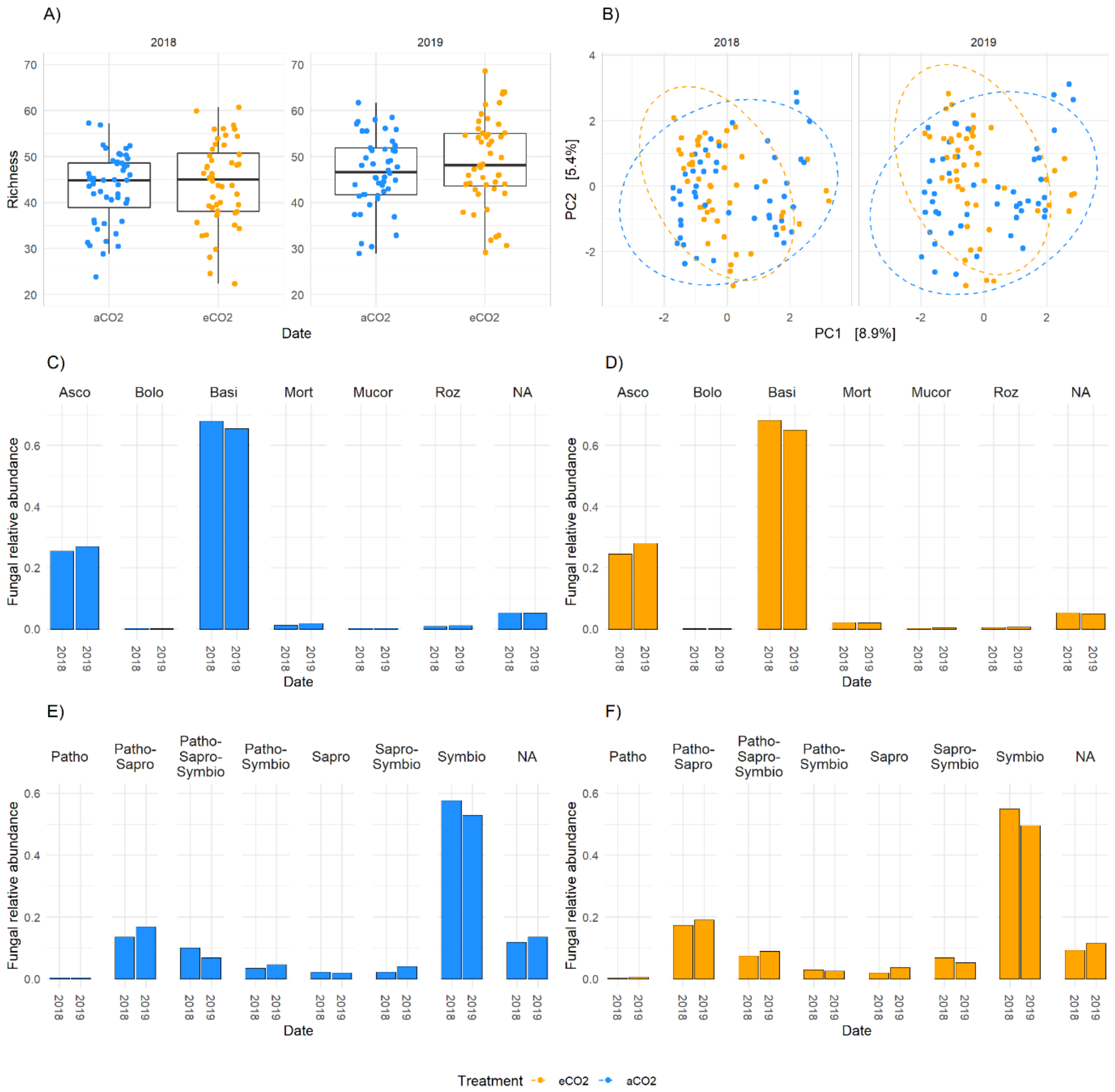


Figure 8-ii Fungal communities in eCO_2 treatment arrays compared with aCO_2 controls. Subplots show: A) Fungal richness, B) PCA plot of fungal communities, C) Relative abundance of fungal phyla in aCO_2 controls, D) Relative abundance of fungal phyla in eCO_2 treatment, E) Relative abundance of fungal functional groups in aCO_2 controls, F) Relative abundance of fungal functional groups in eCO_2 treatment.

Fungal phyla in subplots C and D are as follows (left to right): Ascomycota (Asco), Basidiobolomycota (Bolo), Basidiomycota (Basi), Mortierellomycota (Mort), Mucormycota (Mucor), Rozellomycota (Roz), Unassigned (NA). Fungal functional groups in subplots E and F are as follows (left to right): Pathotroph (Patho), Pathotroph-Saprotroph (Patho-Sapro), Pathotroph-Saprotroph-Symbiotroph (Patho-Sapro-Symbiotroph), Saprotroph (Sapro), Saprotroph-Symbiotroph (Sapro-Symbio), Symbiotroph (Symbio), Unassigned (NA).

8.4 Discussion

This is the third DNA barcoding study on soil fungal communities at BIFoR FACE, with this new data both providing further evidence on the effects of eCO_2 treatment on soil fungi, but also investigating the impacts of season on soil fungi. With this study we attempted to address some of the limitations in previous BIFoR FACE studies, in particular the seasonality of sample collection. For this new study, we tested the following hypotheses: A) Fungal communities will be affected by eCO_2 treatment and B) Fungal communities will be different between seasons.

Consistent with hypothesis A, fungal community composition was affected by eCO_2 treatment, with effects demonstrated both after one and two years of eCO_2 treatment. However, richness remained unaffected by eCO_2. The relative abundance of ECM fungi was slightly lower in eCO_2 arrays, both in 2018 and 2019. These results are consistent with results from the previous two fungal soil DNA barcoding studies at BIFoR FACE (Chapter 6 and 7). The consistency of the results across different sampling methods, DNA extraction methods, ITS primers, and sequencing methodologies is very strong evidence of the fungal community composition demonstrated. These results are particularly valuable in the context of the high levels of inconsistency in results shown in previous woodland FACE experiments (see Chapter 2.2).

Fungal community composition was extremely consistent across the seasons, however there were differences seen in the relative abundances of fungal functional groups. For example, the abundance of mycorrhizal fungi was higher in spring and summer than in autumn. These results are contrary to much of the literature, where several studies report seasonal variations in fungal community composition in woodlands (Beidler et al., 2023; Gong et al., 2022; Jumpponen et al., 2010; Zhao et al., 2023). However, several of these studies occur in forests with different climatic or seasonal patterns than experienced in temperate deciduous woodland, or the studies focussed solely on ECM fungi. A study using a temperate oak woodland site in Poland demonstrated very similar results to BIFoR FACE, with minimal effects of season on community composition, but changes in relative abundance and richness (Voříšková et al., 2014). This suggests that the results demonstrated at BIFoR FACE may be typical for a woodland of its type, however further study is warranted. It is highly likely that although the community composition remains consistent across the seasons, the enzyme activity and fungal biomass change across the year (Baldrian et al., 2013). To investigate this further, future work could incorporate qPCR, fungal biomass, or enzyme assays alongside DNA barcoding to enable comparison between fungal community composition, and the activity of the communities.

As with the previous two studies, no AM fungi were detected in this experiment, likely due to the ITS1 primers used. However due to the tree and ground flora species composition at BIFoR FACE (e.g. *Corylus avellana* (hazel), and *Acer pseudoplanatus* (sycamore), and *Hyacyinthoides non-scripta* (bluebell), it is highly likely that AM fungi are present at BIFoR FACE. Similarly to ECM fungi, oak woodland AM fungal communities have been shown to change seasonally (Helgason et al., 1999). Shifts in the ratios of ECM and AM fungi in soils have been demonstrated with climate change, and these shifts could have significant impacts on the wider woodland response to climatic changes. Combining ITS1 primer use with other primers more specific to AM community analysis is an important step for future studies to investigate the extent of AM fungal communities in the soil, and how any interactions between AM and ECM fungi could be affecting the wider fungal community and woodland response.

Overall, fungal communities, particularly community composition, were consistent across seasons, which suggests that previous studies at BIFoR FACE using only spring sampling points can be reasonably presumed to be representative of the fungal communities across the year. For future studies on fungal communities, taking annual samples could therefore be a reliable option, particularly if it encourages continuity in long ecological experiments such as BIFoR FACE. However, in the context of comparisons of fungal communities with enzymatic activity and other soil biochemistry analyses, seasonal soil fungal biodiversity sampling would provide more accuracy.

8.5 Conclusions

This is the third soil fungal DNA barcoding study at BIFoR FACE, providing further data to what is now a substantial body of research regarding the effects of eCO_2 on soil fungal communities at this site.

Contrary to our hypothesis, there was no seasonal variation in fungal community composition, however there were variations in richness and the relative abundance of different functional groups. Mycorrhizal fungi dominate the system (at the soil depth studied), and they seem to a be a driver of seasonal change in fungal abundance. To achieve a deeper understanding of the system, further work using quantitative analyses to give a more accurate measure of fungal abundance, as well as work investigating metabolic and enzyme activity in the soil would provide more information, particularly regarding carbon cycling and storage.

Soil fungal community results remained extremely consistent with previous studies, despite using a different sampling methodology and increased replicates. Fungal community composition was affected by eCO_2, both after one and two years of eCO_2 treatment, but richness remained unaffected. These results give further weight to the fungal community composition results in the early years of the BIFoR FACE experiment, providing essential baseline data and data on the early impacts of eCO_2 on fungi. These results are an important asset for future research at this site, and other FACE experiments worldwide.

CHAPTER 9: CONCLUSIONS AND FUTURE WORK

The studies reported in this thesis used a variety of methodologies to study woodland fungal communities under eCO_2. The work highlights the importance of studying fungal communities and the relevance this has to existing woodlands, climate forecasting, and climate mitigation efforts such as tree planting.

All of the studies were completed in the first four years of eCO_2 treatment at the BIFoR FACE facility. Previous FACE experiments have demonstrated a plateau in responses after the initial significant changes to woodland community functioning, due to nutrient limitations (Norby and Zak, 2011). This is a clear argument for continuing these fungal studies across the full duration of the BIFoR FACE experiment, which is planned to be a minimum of ten years. Few FACE experiments have fungal studies spanning more than 1 – 2 years, and so longer time series would be a particularly valuable way to continue on the work completed in this thesis. In addition, several of the studies in this thesis are the first of their type in any woodland FACE experiment worldwide. Running studies using the same methodology at the two other second generation FACE experiments worldwide (Amazon FACE and EucFACE) could enable valuable comparisons between woodland types.

The results demonstrated across the different experimental methodologies were varied, emphasizing the importance of using multiple methods to study fungal communities, and to choose methods appropriately depending on the hypotheses of the study. The bioaerosols studies (Chapters 3 and 4) demonstrated high levels of annual variation, with distinct differences in bioaerosol between eCO_2 and aCO_2 arrays between and within the two studies. Future work at the BIFoR FACE experiment to investigate how bioaerosol patterns change with continuing CO_2 treatment may help to resolve the questions arising from the differing eCO_2 responses in the two studies reported in this thesis. Running concurrent airborne spore measurements with fungal fruiting studies looking at the number and size of fungal fruit bodies would be a valuable addition, to enable intercomparisons of airborne spore concentrations with the number of fungi fruiting. Sampling across more seasons would allow further distinction of fungal and pollen seasons at BIFoR FACE, enabling investigation of the effects of eCO_2 on the sporulation season, as there has been some work demonstrating extensions to the airborne fungal spore season (in a similar way that fungal fruiting seasons have been shown to be extended) (Paudel et al., 2021). Finally, sampling and identification of aerosols (by microscopy, fluorescence in situ hybridization (FISH), or DNA sequencing) would be a clear next step (for example, using methodologies such as those in Aguayo et al., 2021, Prass et al., 2021, and Sadyś et al., 2016a). The ability to distinguish between types of biological particles would provide further evidence of the bioaerosol source at BIFoR FACE, and allow separation of bioaerosol signals from different fungal species throughout the season.

There were no reported changes to the number of fungal species fruiting, or sporocarp weight, under eCO_2 treatment. However, there were some early signs that fungal sporocarp weight may be changing under eCO_2. In addition to the benefits of continuing the surveys at BIFoR FACE for a longer period, including further DNA sequencing of sporocarps to improve identification, and utilising the expertise of other field mycologists could significantly increase the accuracy and diversity of fungal species recorded. Previous fungal sporocarp studies at forest FACE experiments showed an increase in sporocarp weight under eCO_2, so this aspect would be particularly interesting to continue with a longer time series as the eCO_2 fumigation at BIFoR FACE continues (Andrew and Lilleskov, 2009; Godbold et al., 2015). In addition, modifying future survey methodologies to incorporate additional analyses such as sporocarp numbers and wet and dry sporocarp weights would provide valuable data. Isotopic analysis of ^{13}C in fruit bodies would enable incorporation of current and future samples into the carbon cycling calculations for the BIFoR FACE site, and would enable comparison with several first-generation FACE experiments where similar analyses were completed (Chen et al., 2016; Hobbie et al., 2014; Keel et al., 2006).

The number of fungal species revealed in the fungal fruiting surveys was relatively low for the number of species expected in a woodland of this type, and when compared with the soil DNA barcoding work in Chapters 6 – 8. Although both survey types are likely to have missed considerable numbers of low-abundance fungal species across the time period measurement, work using species accumulation curves (for example, Unterseher et al., 2008) to indicate how well the surveys are capturing species richness at BIFoR FACE could be valuable additions to the analyses completed here as well as future studies.

Fungal fruiting surveys revealed more saprotrophic species fruiting than ectomycorrhizal species, compared with soil fungal communities which were dominated by ectomycorrhizae. Although species in *Russulaceae* were commonly found fruiting (particularly *Lactarius quietus*), more saprotrophic species were found fruiting across the surveys. As discussed above, incorporating the number of fruit bodies per species in the survey areas would be valuable addition, as for example there may be a high abundance ectomycorrhizal fruit bodies, but corresponding to a handful of species, compared with a lower abundance but higher species diversity of saprotrophs.

As noted across all three DNA barcoding studies, ectomycorrhizal fungi (in particular those in the *Russulaceae* family) dominated in both soil layers studied, and across both eCO_2 treatment and control, although ECM abundance showed signs of decreasing under eCO_2 in later years of the studies. Future work investigating the impacts of eCO_2 on ECM and saprotroph dynamics is warranted. Incorporating comparative analyses of fungal communities across a wider range of soil horizons with root imaging and sampling occurring at BIFoR FACE would enable a better understanding of the dynamics of ECM in the soil, particularly given their dominance (for example, using methodologies such as those used by Garcia et al., 2008, Godbold et al., 2015, or McCormack et al., 2017). Additionally, the sequencing completed in this experiment used primers specific to the ITS1 section of the genome, which poorly detects AM fungal communities (Blaalid et al., 2013; George et al., 2019a). Further barcoding studies using a variety of primers to better capture a wider range of mycorrhizal types at BIFoR FACE would be a valuable addition, particularly given the potential implications of mycorrhizal shifts on carbon storage belowground (Cheeke et al., 2017).

The results from these studies highlight the importance of using multiple methodologies to study the fungal kingdom, and the value of long-term ecological experiments. Although it is clear that further study is required, these results provide a valuable contribution to our understanding of the effects of climate change on forest fungi. The results from these studies could be incorporated into future research, at BIFoR FACE or further afield, and provide much-needed evidence for the importance of fungi in policy around woodland creation, protection of existing woodlands, and climate change.

LITERATURE CITED

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R.H., Kõljalg, U., 2021. UNITE general FASTA release for Fungi 2. https://doi.org/10.15156/BIO/1280089

Abrego, N., Halme, P., Purhonen, J., Ovaskainen, O., 2016a. Fruit body based inventories in wood-inhabiting fungi: Should we replicate in space or time? Fungal Ecology 20, 225–232. https://doi.org/10.1016/j.funeco.2016.01.007

Abrego, N., Oivanen, P., Viner, I., Nordén, J., Penttilä, R., Dahlberg, A., Heilmann-Clausen, J., Somervuo, P., Ovaskainen, O., Schigel, D., 2016b. Reintroduction of threatened fungal species via inoculation. Biological Conservation 203, 120–124. https://doi.org/10.1016/j.biocon.2016.09.014

Ágreda, T., Águeda, B., Fernández-Toirán, M., Vicente-Serrano, S.M., Òlano, J.M., 2016. Long-term monitoring reveals a highly structured interspecific variability in climatic control of sporocarp production. Agricultural and Forest Meteorology 223, 39–47. https://doi.org/10.1016/j.agrformet.2016.03.015

Aguayo, J., Husson, C., Chancerel, E., Fabreguettes, O., Chandelier, A., Fourrier-Jeandel, C., Dupuy, N., Dutech, C., Ioos, R., Robin, C., Thibaudon, M., Marçais, B., Desprez-Loustau, M.-L., 2021. Combining permanent aerobiological networks and molecular analyses for large-scale surveillance of forest fungal pathogens: A proof-of-concept. Plant Pathology 70, 181–194. https://doi.org/10.1111/ppa.13265

AmazonFACE, 2023. Amazon FACE - The Program [WWW Document]. AmazonFACE. URL https://www.google.com/maps/d/viewer?mid=1ulJZwz2qNapjUnFofSfZKZDrrtyNgK3k (accessed 1.13.23).

Amiro, B.D., 1990. Comparison of turbulence statistics within three boreal forest canopies. Boundary-Layer Meteorology 51, 99–121. https://doi.org/10.1007/BF00120463

an de Hulst, H.C., 1957. Light scattering by small particles. John Wiley and Sons, New York.

Andrew, C., Diez, J., James, T.Y., Kauserud, H., 2019. Fungarium specimens: a largely untapped source in global change biology and beyond. Philosophical Transactions of the Royal Society B: Biological Sciences 374, 20170392. https://doi.org/10.1098/rstb.2017.0392

Andrew, C., Heegaard, E., Gange, A.C., Senn-Irlet, B., Egli, S., Kirk, P.M., Büntgen, U., Kauserud, H., Boddy, L., 2018a. Congruency in fungal phenology patterns across dataset sources and scales. Fungal Ecology 32, 9–17. https://doi.org/10.1016/j.funeco.2017.11.009

Andrew, C., Heegaard, E., Halvorsen, R., Martinez-Peña, F., Egli, S., Kirk, P.M., Bässler, C., Büntgen, U., Aldea, J., Høiland, K., Boddy, L., Kauserud, H., 2016. Climate impacts on fungal community and trait dynamics. Fungal Ecology 22, 17–25. https://doi.org/10.1016/j.funeco.2016.03.005

Andrew, C., Heegaard, E., Høiland, K., Senn-Irlet, B., Kuyper, T.W., Krisai-Greilhuber, I., Kirk, P.M., Heilmann-Clausen, J., Gange, A.C., Egli, S., Bässler, C., Büntgen, U., Boddy, L., Kauserud, H., 2018b. Explaining European fungal fruiting phenology with climate variability. Ecology 99, 1306–1315. https://doi.org/10.1002/ecy.2237

Andrew, C., Lilleskov, E.A., 2009. Productivity and community structure of ectomycorrhizal fungal sporocarps under increased atmospheric CO2 and O3. Ecology Letters 12, 813–822. https://doi.org/10.1111/j.1461-0248.2009.01334.x

Anthony, M.A., Crowther, T.W., van der Linde, S., Suz, L.M., Bidartondo, M.I., Cox, F., Schaub, M., Rautio, P., Ferretti, M., Vesterdal, L., De Vos, B., Dettwiler, M., Eickenscheidt, N., Schmitz, A., Meesenburg, H., Andreae, H., Jacob, F., Dietrich, H.-P., Waldner, P., Gessler, A., Frey, B., Schramm, O., van den Bulk, P., Hensen, A., Averill, C., 2022. Forest tree growth is linked to mycorrhizal fungal composition and function across Europe. ISME J 1–10. https://doi.org/10.1038/s41396-021-01159-7

Asplund, J., Kauserud, H., Ohlson, M., Nybakken, L., 2019. Spruce and beech as local determinants of forest fungal community structure in litter, humus and mineral soil. FEMS Microbiology Ecology 95. https://doi.org/10.1093/femsec/fiy232

Averill, C., Dietze, M.C., Bhatnagar, J.M., 2018. Continental-scale nitrogen pollution is shifting forest mycorrhizal associations and soil carbon stocks. Global Change Biology 24, 4544–4553. https://doi.org/10.1111/gcb.14368

Aylor, D.E., 1978. Dispersal in Time and Space: Aerial Pathogens, in: Horsfall, J.G., Cowling, E.B. (Eds.), Plant Disease: An Advanced Treatise. Academic Press, New York, pp. 159–179. https://doi.org/10.1016/0048-3575(79)90101-9

Baird, A., Pope, F., 2022. ‘Can’t see the forest for the trees’: The importance of fungi in the context of UK tree planting. Food and Energy Security n/a, e371. https://doi.org/10.1002/fes3.371

Baird, A.B., Bannister, E.J., MacKenzie, A.R., Pope, F.D., 2021. Mass concentrations of autumn bioaerosol in a mature temperate woodland Free Air Carbon Dioxide Enrichment (FACE) experiment: investigating the role of meteorology and carbon dioxide levels. Biogeosciences Discussions 1–25. https://doi.org/10.5194/bg-2021-162

Baldocchi, D.D., Meyers, T.P., 1988. Turbulence structure in a deciduous forest. Boundary-Layer Meteorology 43, 345–364.

Baldrian, P., Šnajdr, J., Merhautová, V., Dobiášová, P., Cajthaml, T., Valášková, V., 2013. Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. Soil Biology and Biochemistry, Special Issue: Interactions of Soil Minerals with Organic Components and Microorganisms VII and Enzymes in the Environment IV 56, 60–68. https://doi.org/10.1016/j.soilbio.2012.01.020

Bannister, E., MacKenzie, A.R., Cai, X., 2021. Realistic forests and the modeling of forest-atmosphere exchange [WWW Document]. Earth and Space Science Open Archive. https://doi.org/10.1002/essoar.10506854.1

Bannister, E.J., Jesson, M., Harper, N.J., Hart, K.M., Curioni, G., Cai, X., MacKenzie, A.R., 2023. Residence times of air in a mature forest: observational evidence from a free-air CO_2 enrichment experiment. Atmospheric Chemistry and Physics 23, 2145–2165. https://doi.org/10.5194/acp-23-2145-2023

Beidler, K.V., Powers, J.S., Dupuy-Rada, J.M., Hulshof, C., Medvigy, D., Pizano, C., Salgado-Negret, B., Van Bloem, S.J., Vargas G, G., Waring, B.G., Kennedy, P.G., 2023. Seasonality regulates the structure and biogeochemical impact of ectomycorrhizal fungal communities across environmentally divergent neotropical dry forests. Journal of Ecology 111, 1598–1613. https://doi.org/10.1111/1365-2745.14112

Belcher, S.E., Harman, I.N., Finnigan, J.J., 2012. The Wind in the Willows: Flows in Forest Canopies in Complex Terrain. Annual Review of Fluid Mechanics 44, 479–504. https://doi.org/10.1146/annurev-fluid-120710-101036

Bennett, A.E., Classen, A.T., 2020. Climate change influences mycorrhizal fungal–plant interactions, but conclusions are limited by geographical study bias. Ecology 101, e02978. https://doi.org/10.1002/ecy.2978

Bento Lab, 2021. Bento Lab | The mobile genetics setup | Science Unbound [WWW Document]. Bento Lab. URL https://www.bento.bio/ (accessed 2.9.21).

Bergot, M., Cloppet, E., Pérarnaud, V., Déqué, M., Marçais, B., Desprez-Loustau, M.-L., 2004. Simulation of potential range expansion of oak disease caused by Phytophthora cinnamomi under climate change. Global Change Biology 10, 1539–1552. https://doi.org/10.1111/j.1365-2486.2004.00824.x

Bidartondo, M.I., Ellis, C., Kauserud, H., Kennedy, P.G., Lilleskov, E.A., Suz, L.M., Andrew, C., 2018. State of the World's Fungi 2018: Climate Change: Fungal responses and effects 12.

Biedermann, P.H.W., Vega, F.E., 2020. Ecology and Evolution of Insect–Fungus Mutualisms. Annual Review of Entomology 65, 431–455. https://doi.org/10.1146/annurev-ento-011019-024910

Blaalid, R., Kumar, S., Nilsson, R.H., Abarenkov, K., Kirk, P.M., Kauserud, H., 2013. ITS1 versus ITS2 as DNA metabarcodes for fungi. Molecular Ecology Resources 13, 218–224. https://doi.org/10.1111/1755-0998.12065

Boddy, L., 2021. Fungi and Trees Their Complex Relationships. Arboricultural Association.

Boddy, L., Büntgen, U., Egli, S., Gange, A.C., Heegaard, E., Kirk, P.M., Mohammad, A., Kauserud, H., 2014. Climate variation effects on fungal fruiting. Fungal Ecology, Fungi in a changing world: The role of fungi in ecosystem response to global change 10, 20–33. https://doi.org/10.1016/j.funeco.2013.10.006

Bödeker, I.T.M., Lindahl, B.D., Olson, Å., Clemmensen, K.E., 2016. Mycorrhizal and saprotrophic fungal guilds compete for the same organic substrates but affect decomposition differently. Functional Ecology 30, 1967–1978. https://doi.org/10.1111/1365-2435.12677

Brancalion, P.H.S., Holl, K.D., 2020. Guidance for successful tree planting initiatives. Journal of Applied Ecology 57, 2349–2361. https://doi.org/10.1111/1365-2664.13725

Brasier, C.M., Scott, J.K., 1994. European oak declines and global warming: a theoretical assessment with special reference to the activity of Phytophthora cinnamomi. EPPO Bulletin 24, 221–232. https://doi.org/10.1111/j.1365-2338.1994.tb01063.x

Brazee, N.J., Lindner, D.L., D'Amato, A.W., Fraver, S., Forrester, J.A., Mladenoff, D.J., 2014. Disturbance and diversity of wood-inhabiting fungi: effects of canopy gaps and downed woody debris. Biodivers Conserv 23, 2155–2172. https://doi.org/10.1007/s10531-014-0710-x

British Mycological Society, 2008. House of Lords - Science and Technology - Written Evidence [WWW Document]. URL https://publications.parliament.uk/pa/ld200708/ldselect/ldsctech/162/162we10.htm (accessed 3.30.21).

Broome, A., Ray, D., Mitchell, R., Harmer, R., 2019. Responding to ash dieback (Hymenoscyphus fraxineus) in the UK: woodland composition and replacement tree species. Forestry: An International Journal of Forest Research 92, 108–119. https://doi.org/10.1093/forestry/cpy040

Brundrett, M.C., Tedersoo, L., 2020. Resolving the mycorrhizal status of important northern hemisphere trees. Plant Soil 454, 3–34. https://doi.org/10.1007/s11104-020-04627-9

Buckley, P., Helliwell, D.R., Milne, S., Howell, R., 2017. Twenty-five years on – vegetation succession on a translocated ancient woodland soil at Biggins Wood, Kent, UK. Forestry: An International Journal of Forest Research 90, 561–572. https://doi.org/10.1093/forestry/cpx015

Büntgen, U., Kauserud, H., Egli, S., 2012. Linking climate variability to mushroom productivity and phenology. Frontiers in Ecology and the Environment 10, 14–19. https://doi.org/10.1890/110064

Burge, H.A., 2002. An update on pollen and fungal spore aerobiology. Journal of Allergy and Clinical Immunology 110, 544–552. https://doi.org/10.1067/mai.2002.128674

Buyck, B., 1999. Taxonomists are an endangered species in Europe. Nature 401, 321–321. https://doi.org/10.1038/43762

Caillaud, D., Cheriaux, M., Charpin, D., Chaabane, N., Thibaudon, M., 2018. Moisissures extérieures et santé respiratoire. Revue des Maladies Respiratoires 35, 188–196. https://doi.org/10.1016/j.rmr.2018.01.001

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869

Cannon, P.F., 1997. Strategies for rapid assessment of fungal diversity. Biodiversity and Conservation 6, 669–680. https://doi.org/10.1023/A:1018362002273

Carslaw, D.C., Ropkins, K., 2012. openair --- an R package for air quality data analysis. Environmental Modelling & Software. 27–28, 52–61.

Carteron, A., Beigas, M., Joly, S., Turner, B.L., Laliberté, E., 2020. Temperate Forests Dominated by Arbuscular or Ectomycorrhizal Fungi Are Characterized by Strong Shifts from Saprotrophic to Mycorrhizal Fungi with Increasing Soil Depth. Microb Ecol. https://doi.org/10.1007/s00248-020-01540-7

Castañeda-Gómez, L., Powell, J.R., Ellsworth, D.S., Pendall, E., Carrillo, Y., 2021. The influence of roots on mycorrhizal fungi, saprotrophic microbes and carbon dynamics in a low-phosphorus Eucalyptus forest under elevated CO2. Functional Ecology n/a. https://doi.org/10.1111/1365-2435.13832

Castañeda-Gómez, L., Walker, J.K.M., Powell, J.R., Ellsworth, D.S., Pendall, E., Carrillo, Y., 2020. Impacts of elevated carbon dioxide on carbon gains and losses from soil and associated microbes in a Eucalyptus woodland. Soil Biology and Biochemistry 143, 107734. https://doi.org/10.1016/j.soilbio.2020.107734

Chaudhary, V.B., Nolimal, S., Sosa-Hernández, M.A., Egan, C., Kastens, J., 2020. Trait-based aerial dispersal of arbuscular mycorrhizal fungi. New Phytologist n/a. https://doi.org/10.1111/nph.16667

Cheeke, T.E., Phillips, R.P., Brzostek, E.R., Rosling, A., Bever, J.D., Fransson, P., 2017. Dominant mycorrhizal association of trees alters carbon and nutrient cycling by selecting for microbial groups with distinct enzyme function. New Phytologist 214, 432–442. https://doi.org/10.1111/nph.14343

Chen, J., Hofmockel, K.S., Hobbie, E.A., 2016. Isotopic Analysis of Sporocarp Protein and Structural Material Improves Resolution of Fungal Carbon Sources. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.01994

Chung, H., Zak, D.R., Lilleskov, E.A., 2006. Fungal community composition and metabolism under elevated CO2 and O3. Oecologia 147, 143–154. https://doi.org/10.1007/s00442-005-0249-3

Cionco, R.M., 1978. Analysis of canopy index values for various canopy densities. Boundary-Layer Meteorology 15, 81–93. https://doi.org/10.1007/BF00165507

Cionco, R.M., 1965. A Mathematical Model for Air Flow in a Vegetative Canopy. Journal of Applied Meteorology. https://doi.org/10.1175/1520-0450(1965)004<0517:ammfaf>2.0.co;2

Clasen, L.A., Detheridge, A.P., Scullion, J., Griffith, G.W., 2020. Soil stabilisation for DNA metabarcoding of plants and fungi. Implications for sampling at remote locations or via third-parties. Metabarcoding and Metagenomics 4, e58365. https://doi.org/10.3897/mbmg.4.58365

Clubbe, C., Ainsworth, A.M., Bárrios, S., Bensusan, K., Brodie, J., Cannon, P., Chapman, T., Copeland, A.I., Corcoran, M., Dani Sanchez, M., David, J.C., Dines, T., Gardiner, L.M., Hamilton, M.A., Heller, T., Hollingsworth, P.M., Hutchinson, N., Llewelyn, T., Lowe Forrest, L., McGinn, K.J., Miles, S., O'Donnell, K., Woodfield-Pascoe, N., Rich, T.C.G., Rumsey, F., Sim, J., Smith, S.R., Spence, N., Stanworth, A., Stroh, P., Taylor, I., Trivedi, C., Twyford, A.D., Viruel, J., Walker, K., Wilbraham, J., Woodman, J., Fay, M.F., 2020. Current knowledge, status, and future for plant and fungal diversity in Great Britain and the UK Overseas Territories. PLANTS, PEOPLE, PLANET 2, 557–579. https://doi.org/10.1002/ppp3.10142

Compalati, E., Ridolo, E., Passalacqua, G., Braido, F., Villa, E., Canonica, G.W., 2010. The link between allergic rhinitis and asthma: the united airways disease. Expert Rev Clin Immunol 6, 413–423. https://doi.org/10.1586/eci.10.15

Courty, P.-E., Franc, A., Pierrat, J.-C., Garbaye, J., 2008. Temporal Changes in the Ectomycorrhizal Community in Two Soil Horizons of a Temperate Oak Forest. Applied and Environmental Microbiology 74, 5792–5801. https://doi.org/10.1128/AEM.01592-08

Cox, L., Calderon, M.A., 2010. Subcutaneous specific immunotherapy for seasonal allergic rhinitis: a review of treatment practices in the US and Europe. Current Medical Research and Opinion 26, 2723–2733. https://doi.org/10.1185/03007995.2010.528647

Craig, M., Buckley, P., Howell, R., 2015. Responses of an ancient woodland field layer to soil translocation: methods and timing. Applied Vegetation Science 18, 579–590. https://doi.org/10.1111/avsc.12170

Crilley, L.R., Shaw, M., Pound, R., Kramer, L.J., Price, R., Young, S., Lewis, A.C., Pope, F.D., 2018. Evaluation of a low-cost optical particle counter (Alphasense OPC-N2) for ambient air monitoring. Atmos. Meas. Tech 11, 709–720. https://doi.org/10.5194/amt-11-709-2018

Crilley, L.R., Singh, A., Kramer, L.J., Shaw, M.D., Alam, M.S., Apte, J.S., Bloss, W.J., Hildebrandt Ruiz, L., Fu, P., Fu, W., Gani, S., Gatari, M., Ilyinskaya, E., Lewis, A.C., Ng'ang'a, D., Sun, Y., Whitty, R.C.W., Yue, S., Young, S., Pope, F.D., 2020. Effect of aerosol composition on the performance of low-cost optical particle counter correction factors. Atmospheric Measurement Techniques 13, 1181–1193. https://doi.org/10.5194/amt-13-1181-2020

Crous, K.Y., Ósvaldsson, A., Ellsworth, D.S., 2015. Is phosphorus limiting in a mature Eucalyptus woodland? Phosphorus fertilisation stimulates stem growth. Plant Soil 391, 293–305. https://doi.org/10.1007/s11104-015-2426-4

Dahlberg, A., Genney, D.R., Heilmann-Clausen, J., 2010. Developing a comprehensive strategy for fungal conservation in Europe: current status and future needs. Fungal Ecology, Rare and Endangered Fungi 3, 50–64. https://doi.org/10.1016/j.funeco.2009.10.004

Dales, R.E., Cakmak, S., Judek, S., Dann, T., Coates, F., Brook, J.R., Burnett, R.T., 2003. The Role of Fungal Spores in Thunderstorm Asthmaa. CHEST 123, 745–750. https://doi.org/10.1378/chest.123.3.745

D'Amato, G., Chong-Neto, H.J., Ortega, O.P.M., Vitale, C., Ansotegui, I., Rosario, N., Haahtela, T., Galan, C., Pawankar, R., Murrieta-Aguttes, M., Cecchi, L., Bergmann, C., Ridolo, E., Ramon, G., Diaz, S.G., D'Amato, M., Annesi-Maesano, I., 2020. The effects of climate change on respiratory allergy and asthma induced by pollen and mold allergens. Allergy 75, 2219–2228. https://doi.org/10.1111/all.14476

D'Amato, G., Holgate, S.T., Pawankar, R., Ledford, D.K., Cecchi, L., Al-Ahmad, M., Al-Enezi, F., Al-Muhsen, S., Ansotegui, I., Baena-Cagnani, C.E., Baker, D.J., Bayram, H., Bergmann, K.C., Boulet, L.-P., Buters, J.T.M., D'Amato, M., Dorsano, S., Douwes, J., Finlay, S.E., Garrasi, D., Gómez, M., Haahtela, T., Halwani, R., Hassani, Y., Mahboub, B., Marks, G., Michelozzi, P., Montagni, M., Nunes, C., Oh, J.J.-W., Popov, T.A., Portnoy, J., Ridolo, E., Rosário, N., Rottem, M., Sánchez-Borges, M., Sibanda, E., Sienra-Monge, J.J., Vitale, C., Annesi-Maesano, I., 2015. Meteorological conditions, climate change, new emerging factors, and asthma and related allergic disorders. A statement of the World Allergy Organization. World Allergy Organization Journal 8. https://doi.org/10.1186/s40413-015-0073-0

Darwin Tree of Life, 2020. All Things Fun-GAL [WWW Document]. URL https://www.darwintreeoflife.org/2020/07/20/all-things-fun-gal/ (accessed 2.9.21).

Davies, D.A.L., 1962. The Preservation of Larger Fungi by Freeze Drying. Transactions of the British Mycological Society 45, 424–428.

Davies, R.R., Denny, M.J., Newton, L.M., 1963. A Comparison Hetween the Summer and Autumn Air-Sporas at London and Liverpool. Allergy 18, 131–147. https://doi.org/10.1111/j.1398-9995.1963.tb03156.x

Dawes, M.A., Hagedorn, F., Handa, I.T., Streit, K., Ekblad, A., Rixen, C., Körner, C., Hättenschwiler, S., 2013. An alpine treeline in a carbon dioxide-rich world: synthesis of a nine-year free-air carbon dioxide enrichment study. Oecologia 171, 623–637. https://doi.org/10.1007/s00442-012-2576-5

Dawes, M.A., Hättenschwiler, S., Bebi, P., Hagedorn, F., Handa, I.T., Körner, C., Rixen, C., 2011. Species-specific tree growth responses to 9 years of CO2 enrichment at the alpine treeline. Journal of Ecology 99, 383–394. https://doi.org/10.1111/j.1365-2745.2010.01764.x

Department for Business, Energy & Industrial Strategy, 2020. The UK’s Nationally Determined Contribution under the Paris Agreement [WWW Document]. GOV.UK. URL https://www.gov.uk/government/publications/the-uks-nationally-determined-contribution-communication-to-the-unfccc (accessed 2.10.21).

Department for Environment, Food & Rural Affairs, 2021. UK Plant Health Risk Register [WWW Document]. URL https://secure.fera.defra.gov.uk/phiw/riskRegister/ (accessed 1.19.21).

Department for Environment, Food & Rural Affairs, Natural England, 2022. Habitats and species of principal importance in England [WWW Document]. GOV.UK. URL https://www.gov.uk/government/publications/habitats-and-species-of-principal-importance-in-england (accessed 1.8.23).

Di-Giovanni, F., Kevan, P.G., Nasr, M.E., 1995. The variability in settling velocities of some pollen and spores. Grana 34, 39–44. https://doi.org/10.1080/00173139509429031

Dr FunGi, 2019. Day 1.5 of the @LostFoundFungi community DNA 🧬 workshop and we’re grinding fungal specimens 🍄, mixing PCR ingredients, and running @theBentoLab. Please pray for our success! 🙏🏽🙏🏽 https://t.co/fZUszBtasN. @PowderyM. URL https://twitter.com/PowderyM/status/1155058834026745856 (accessed 2.9.21).

Dressaire, E., Yamada, L., Song, B., Roper, M., 2016. Mushrooms use convectively created airflows to disperse their spores. Proc Natl Acad Sci U S A 113, 2833–2838. https://doi.org/10.1073/pnas.1509612113

Dunbar, J., Gallegos-Graves, L.V., Steven, B., Mueller, R., Hesse, C., Zak, D.R., Kuske, C.R., 2014. Surface soil fungal and bacterial communities in aspen stands are resilient to eleven years of elevated CO2 and O3. Soil Biology and Biochemistry 76, 227–234. https://doi.org/10.1016/j.soilbio.2014.05.027

Ellis, C.J., 2016. Oceanic and temperate rainforest climates and their epiphyte indicators in Britain. Ecological Indicators, Navigating Urban Complexity: Advancing Understanding of Urban Social – Ecological Systems for Transformation and Resilience 70, 125–133. https://doi.org/10.1016/j.ecolind.2016.06.002

FACE Data Management System, 2021. Global List of FACE Experiments [WWW Document]. URL https://facedata.ornl.gov/global_face.html#BreuleuxPlus (accessed 6.1.21).

FAO Global Forest Resources Assessment, 2015.

Feng, X., Simpson, A.J., Schlesinger, W.H., Simpson, M.J., 2010. Altered microbial community structure and organic matter composition under elevated CO2 and N fertilization in the duke forest. Global Change Biology 16, 2104–2116. https://doi.org/10.1111/j.1365-2486.2009.02080.x

Fernandez, C.W., Kennedy, P.G., 2016. Revisiting the ‘Gadgil effect’: do interguild fungal interactions control carbon cycling in forest soils? New Phytologist 209, 1382–1394. https://doi.org/10.1111/nph.13648

Finnigan, J., 2000. Turbulence in Plant Canopies. Annual Review of Fluid Mechanics 32, 519–571.

Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L., Gurr, S.J., 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484, 186–194. https://doi.org/10.1038/nature10947

Forest Research, 2021. Dothistroma needle blight (Dothistroma septosporum) [WWW Document]. Forest Research. URL https://www.forestresearch.gov.uk/tools-and-resources/pest-and-disease-resources/dothistroma-needle-blight/ (accessed 2.11.21).

Forest Research, 2020. Forestry Statistics 2020 [WWW Document]. URL https://www.forestresearch.gov.uk/documents/7806/CompleteFS2020.pdf (accessed 1.25.21).

Frey, S.D., 2019. Mycorrhizal Fungi as Mediators of Soil Organic Matter Dynamics. Annu. Rev. Ecol. Evol. Syst. 50, 237–259. https://doi.org/10.1146/annurev-ecolsys-110617-062331

Friggens, N.L., Hester, A.J., Mitchell, R.J., Parker, T.C., Subke, J.-A., Wookey, P.A., 2020. Tree planting in organic soils does not result in net carbon sequestration on decadal timescales. Global Change Biology 26, 5178–5188. https://doi.org/10.1111/gcb.15229

Fröhlich-Nowoisky, J., Kampf, C.J., Weber, B., Huffman, J.A., Pöhlker, C., Andreae, M.O., Lang-Yona, N., Burrows, S.M., Gunthe, S.S., Elbert, W., Su, H., Hoor, P., Thines, E., Hoffmann, T., Després, V.R., Pöschl, U., 2016. Bioaerosols in the Earth system: Climate, health, and ecosystem interactions. Atmospheric Research 182, 346–376. https://doi.org/10.1016/j.atmosres.2016.07.018

Fröhlich-Nowoisky, J., Pickersgill, D.A., Despres, V.R., Poschl, U., 2009. High diversity of fungi in air particulate matter. Proceedings of the National Academy of Sciences 106, 12814–12819. https://doi.org/10.1073/pnas.0811003106

Frøslev, T.G., Kjøller, R., Bruun, H.H., Ejrnæs, R., Hansen, A.J., Læssøe, T., Heilmann-Clausen, J., 2019. Man against machine: Do fungal fruitbodies and eDNA give similar biodiversity assessments across broad environmental gradients? Biological Conservation 233, 201–212. https://doi.org/10.1016/j.biocon.2019.02.038

Furneaux, B., Song, Z., 2021. FUNGuildR: Look up Guild Information for Fungi.

Gabriel, M.F., Postigo, I., Tomaz, C.T., Martínez, J., 2016. Alternaria alternata allergens: Markers of exposure, phylogeny and risk of fungi-induced respiratory allergy. Environment International 89–90, 71–80. https://doi.org/10.1016/j.envint.2016.01.003

Gadgil, R.L., Gadgil, P.D., 1971. Mycorrhiza and Litter Decomposition. Nature 233, 133–133. https://doi.org/10.1038/233133a0

Galante, T.E., Horton, T.R., Swaney, D.P., 2011. 95% of basidiospores fall within 1 m of the cap: a field-and modeling-based study. Mycologia 103, 1175–1183. https://doi.org/10.3852/10-388

Gange, A.C., Allen, L.P., Nussbaumer, A., Gange, E.G., Andrew, C., Egli, S., Senn-Irlet, B., Boddy, L., 2019. Multiscale patterns of rarity in fungi, inferred from fruiting records. Global Ecology and Biogeography 28, 1106–1117. https://doi.org/10.1111/geb.12918

Gange, A.C., Gange, E.G., Mohammad, A.B., Boddy, L., 2011. Host shifts in fungi caused by climate change? Fungal Ecology, Conservation Underground: Fungi in a Changing World 4, 184–190. https://doi.org/10.1016/j.funeco.2010.09.004

Gange, A.C., Gange, E.G., Sparks, T.H., Boddy, L., 2007. Rapid and Recent Changes in Fungal Fruiting Patterns. Science 316, 71–71. https://doi.org/10.1126/science.1137489

Gange, A.C., Heegaard, E., Boddy, L., Andrew, C., Kirk, P., Halvorsen, R., Kuyper, T.W., Bässler, C., Diez, J., Heilman-Clausen, J., Høiland, K., Büntgen, U., Kauserud, H., 2018. Trait-dependent distributional shifts in fruiting of common British fungi. Ecography 41, 51–61. https://doi.org/10.1111/ecog.03233

Garcia, M.O., Ovasapyan, T., Greas, M., Treseder, K.K., 2008. Mycorrhizal dynamics under elevated CO2 and nitrogen fertilization in a warm temperate forest. Plant Soil 303, 301–310. https://doi.org/10.1007/s11104-007-9509-9

Gardner, A., Ellsworth, D.S., Crous, K.Y., Pritchard, J., MacKenzie, A.R., 2022. Is photosynthetic enhancement sustained through three years of elevated CO2 exposure in 175-year-old Quercus robur? Tree Physiology 42, 130–144. https://doi.org/10.1093/treephys/tpab090

George, P.B.L., Creer, S., Griffiths, R.I., Emmett, B.A., Robinson, D.A., Jones, D.L., 2019a. Primer and Database Choice Affect Fungal Functional but Not Biological Diversity Findings in a National Soil Survey. Frontiers in Environmental Science 7, 173. https://doi.org/10.3389/fenvs.2019.00173

George, P.B.L., Lallias, D., Creer, S., Seaton, F.M., Kenny, J.G., Eccles, R.M., Griffiths, R.I., Lebron, I., Emmett, B.A., Robinson, D.A., Jones, D.L., 2019b. Divergent national-scale trends of microbial and animal biodiversity revealed across diverse temperate soil ecosystems. Nat Commun 10, 1107. https://doi.org/10.1038/s41467-019-09031-1

Gielen, B., Calfapietra, C., Lukac, M., Wittig, V.E., De Angelis, P., Janssens, I.A., Moscatelli, M.C., Grego, S., Cotrufo, M.F., Godbold, D.L., Hoosbeek, M.R., Long, S.P., Miglietta, F., Polle, A., Bernacchi, C.J., Davey, P.A., Ceulemans, R., Scarascia-Mugnozza, G.E., 2005. Net carbon storage in a poplar plantation (POPFACE) after three years of free-air CO2 enrichment. Tree Physiology 25, 1399–1408. https://doi.org/10.1093/treephys/25.11.1399

Gilbert, G.S., Reynolds, D.R., 2005. Nocturnal Fungi: Airborne Spores in the Canopy and Understory of a Tropical Rain Forest. Biotropica 37, 462–464.

Gimeno, T.E., Crous, K.Y., Cooke, J., O'Grady, A.P., Ósvaldsson, A., Medlyn, B.E., Ellsworth, D.S., 2016. Conserved stomatal behaviour under elevated CO2 and varying water availability in a mature woodland. Functional Ecology 30, 700–709. https://doi.org/10.1111/1365-2435.12532

Gloor, G.B., Macklaim, J.M., Pawlowsky-Glahn, V., Egozcue, J.J., 2017. Microbiome Datasets Are Compositional: And This Is Not Optional. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.02224

Godbold, D.L., Hoosbeek, M.R., Lukac, M., Cotrufo, M.F., Janssens, I.A., Ceulemans, R., Polle, A., Velthorst, E.J., Scarascia-Mugnozza, G., De Angelis, P., Miglietta, F., Peressotti, A., 2006. Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. Plant and Soil 281, 15–24. https://doi.org/10.1007/s11104-005-3701-6

Godbold, D.L., Vašutová, M., Wilkinson, A., Edwards-Jonášová, M., Bambrick, M., Smith, A.R., Pavelka, M., Cudlin, P., 2015. Elevated Atmospheric CO2 Affects

Ectomycorrhizal Species Abundance and Increases Sporocarp Production under Field Conditions. Forests 6, 1256–1273. https://doi.org/10.3390/f6041256

Gong, S., Feng, B., Jian, S.-P., Wang, G.S., Ge, Z.-W., Yang, Z.L., 2022. Elevation Matters More than Season in Shaping the Heterogeneity of Soil and Root Associated Ectomycorrhizal Fungal Community. https://doi.org/10.1128/spectrum.01950-21

Gosselin, M.I., Rathnayake, C.M., Crawford, I., Pöhlker, C., Fröhlich-Nowoisky, J., Schmer, B., Després, V.R., Engling, G., Gallagher, M., Stone, E., Pöschl, U., Huffman, J.A., 2016. Fluorescent bioaerosol particle, molecular tracer, and fungal spore concentrations during dry and rainy periods in a semi-arid forest. Atmos. Chem. Phys. 16, 15165–15184. https://doi.org/10.5194/acp-16-15165-2016

Griffiths, P.T., Borlace, J.-S., Gallimore, P.J., Kalberer, M., Herzog, M., Pope, F.D., 2012. Hygroscopic growth and cloud activation of pollen: a laboratory and modelling study. Atmospheric Science Letters 13, 289–295. https://doi.org/10.1002/asl.397

Grinn-Gofroń, A., Nowosad, J., Bosiacka, B., Camacho, I., Pashley, C., Belmonte, J., De Linares, C., Ianovici, N., Manzano, J.M.M., Sadyś, M., Skjøth, C., Rodinkova, V., Tormo-Molina, R., Vokou, D., Fernández-Rodríguez, S., Damialis, A., 2019. Airborne Alternaria and Cladosporium fungal spores in Europe: Forecasting possibilities and relationships with meteorological parameters. Science of The Total Environment 653, 938–946. https://doi.org/10.1016/j.scitotenv.2018.10.419

Grundström, M., Adams-Groom, B., Pashley, C.H., Dahl, Å., Rasmussen, K., de Weger, L.A., Thibaudon, M., Fernández-Rodríguez, S., Silva-Palacios, I., Skjøth, C.A., 2019. Oak pollen seasonality and severity across Europe and modelling the season start using a generalized phenological model. Science of The Total Environment 663, 527–536. https://doi.org/10.1016/j.scitotenv.2019.01.212

Gunina, A., Smith, A.R., Godbold, D.L., Jones, D.L., Kuzyakov, Y., 2017. Response of soil microbial community to afforestation with pure and mixed species. Plant Soil 412, 357–368. https://doi.org/10.1007/s11104-016-3073-0

Hagan, D., 2017. py-opc, available at https://github.com/dhhagan/py-opc [WWW Document]. URL https://github.com/dhhagan/py-opc (accessed 1.27.21).

Halbwachs, H., Bässler, C., 2015. Gone with the wind – a review on basidiospores of lamellate agarics. Mycosphere 6, 78–112. https://doi.org/10.5943/mycosphere/6/1/10

Halme, P., Holec, J., Heilmann-Clausen, J., 2017. The history and future of fungi as biodiversity surrogates in forests. Fungal Ecology, Fungi in Natural Forests 27, 193–201. https://doi.org/10.1016/j.funeco.2016.10.005

Halme, P., Kotiaho, J.S., 2012. The importance of timing and number of surveys in fungal biodiversity research. Biodivers Conserv 21, 205–219. https://doi.org/10.1007/s10531-011-0176-z

Hararuk, O., Smith, M.J., Luo, Y., 2015. Microbial models with data-driven parameters predict stronger soil carbon responses to climate change. Glob Change Biol 21, 2439–2453. https://doi.org/10.1111/gcb.12827

Harley, K.G., Macher, J.M., Lipsett, M., Duramad, P., Holland, N.T., Prager, S.S., Ferber, J., Bradman, A., Eskenazi, B., Tager, I.B., 2009. Fungi and pollen exposure in the first months of life and risk of early childhood wheezing. Thorax 64, 353–358. https://doi.org/10.1136/thx.2007.090241

Harris, N.L., Gibbs, D.A., Baccini, A., Birdsey, R.A., de Bruin, S., Farina, M., Fatoyinbo, L., Hansen, M.C., Herold, M., Houghton, R.A., Potapov, P.V., Suarez, D.R., Roman-Cuesta, R.M., Saatchi, S.S., Slay, C.M., Turubanova, S.A., Tyukavina, A., 2021. Global maps of twenty-first century forest carbon fluxes. Nature Climate Change 1–7. https://doi.org/10.1038/s41558-020-00976-6

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., MacKenzie, A.R., 2019. Characteristics of Free Air Carbon Dioxide Enrichment of a Northern Temperate Mature Forest. Global Change Biology 0. https://doi.org/10.1111/gcb.14786

Haskins, R.H., 1960. Freeze-Drying of Macrofungi for Display. Mycologia 52, 161–164. https://doi.org/10.2307/3756262

Hawkins, H.-J., Cargill, R.I.M., Nuland, M.E.V., Hagen, S.C., Field, K.J., Sheldrake, M., Soudzilovskaia, N.A., Kiers, E.T., 2023. Mycorrhizal mycelium as a global carbon pool. Current Biology 33, R560–R573. https://doi.org/10.1016/j.cub.2023.02.027

Heald, C.L., Spracklen, D.V., 2009. Atmospheric budget of primary biological aerosol particles from fungal spores. Geophysical Research Letters 36. https://doi.org/10.1029/2009GL037493

Healy, D.A., O’Connor, D.J., Sodeau, J.R., 2012. Measurement of the particle counting efficiency of the “Waveband Integrated Bioaerosol Sensor” model number 4 (WIBS-4). Journal of Aerosol Science 47, 94–99. https://doi.org/10.1016/j.jaerosci.2012.01.003

Heilmann-Clausen, J., Barron, E.S., Boddy, L., Dahlberg, A., Griffith, G.W., Nordén, J., Ovaskainen, O., Perini, C., Senn-Irlet, B., Halme, P., 2015. A fungal perspective on conservation biology: Fungi and Conservation Biology. Conservation Biology 29, 61–68. https://doi.org/10.1111/cobi.12388

Helgason, T., Fitter, A.H., Young, J.P.W., 1999. Molecular diversity of arbuscular mycorrhizal fungi colonising Hyacinthoides non-scripta (bluebell) in a seminatural woodland. Molecular Ecology 8, 659–666. https://doi.org/10.1046/j.1365-294x.1999.00604.x

Hemkemeyer, M., Christensen, B.T., Tebbe, C.C., Hartmann, M., 2019. Taxon-specific fungal preference for distinct soil particle size fractions. European Journal of Soil Biology 94, 103103. https://doi.org/10.1016/j.ejsobi.2019.103103

Hicke, J.A., Allen, C.D., Desai, A.R., Dietze, M.C., Hall, R.J., (Ted) Hogg, E.H., Kashian, D.M., Moore, D., Raffa, K.F., Sturrock, R.N., Vogelmann, J., 2012. Effects of biotic disturbances on forest carbon cycling in the United States and Canada. Global Change Biology 18, 7–34. https://doi.org/10.1111/j.1365-2486.2011.02543.x

Hill, L., Jones, G., Atkinson, N., Hector, A., Hemery, G., Brown, N., 2019. The £15 billion cost of ash dieback in Britain. Current Biology 29, R315–R316. https://doi.org/10.1016/j.cub.2019.03.033

Hobbie, E.A., 2006. Carbon Allocation to Ectomycorrhizal Fungi Correlates with Belowground Allocation in Culture Studies. Ecology 87, 563–569. https://doi.org/10.1890/05-0755

Hobbie, E.A., Hofmockel, K.S., van Diepen, L.T.A., Lilleskov, E.A., Ouimette, A.P., Finzi, A.C., 2014. Fungal carbon sources in a pine forest: evidence from a 13C-labeled global change experiment. Fungal Ecology, Fungi in a changing world: The role of fungi in ecosystem response to global change 10, 91–100. https://doi.org/10.1016/j.funeco.2013.11.001

Hollins, P.D., Kettlewell, P.S., Atkinson, M.D., Stephenson, D.B., Corden, J.M., Millington, W.M., Mullins, J., 2004. Relationships between airborne fungal spore concentration of Cladosporium and the summer climate at two sites in Britain. Int J Biometeorol 48, 137–141. https://doi.org/10.1007/s00484-003-0188-9

Hollis, J., Jones, B., 2021. Soil Profile Pit at BIFoR-FACE, Norbury Junction, Staffordshire. https://doi.org/10.25500/edata.bham.00000706

Horton, T.R., 2017. Spore Dispersal in Ectomycorrhizal Fungi at Fine and Regional Scales, in: Tedersoo, L. (Ed.), Biogeography of Mycorrhizal Symbiosis, Ecological Studies. Springer International Publishing, Cham, pp. 61–78. https://doi.org/10.1007/978-3-319-56363-3_3

Hugill, P., Lucas, A., 2021. The Resupinates of Hampshire, 4th ed. The Tree Council.

Humphrey, J.W., 2005. Benefits to biodiversity from developing old-growth conditions in British upland spruce plantations: a review and recommendations. Forestry: An International Journal of Forest Research 78, 33–53. https://doi.org/10.1093/forestry/cpi004

Humphrey, J.W., Newton, AC., Peace, A.J., Holden, E., 2000. The importance of conifer plantations in northern Britain as a habitat for native fungi. Biological Conservation 96, 241–252. https://doi.org/10.1016/S0006-3207(00)00077-X

Ing, B., 2020. The Myxomycetes of Britain and Ireland : An Identification Handbook, 2nd ed. The Richmond Publishing Co. Ltd, Great Britain.

Jarvis, S.G., Woodward, S., Taylor, A.F.S., 2015. Strong altitudinal partitioning in the distributions of ectomycorrhizal fungi along a short (300 m) elevation gradient. New Phytologist 206, 1145–1155. https://doi.org/10.1111/nph.13315

Jepson, P.R., Arakelyan, I., 2017. Developing publicly acceptable tree health policy: public perceptions of tree-breeding solutions to ash dieback among interested publics in the UK. Forest Policy and Economics 80, 167–177. https://doi.org/10.1016/j.forpol.2017.03.002

JNCC, 2019. UK Post-2010 Biodiversity Framework [WWW Document]. URL https://jncc.gov.uk/our-work/uk-bap/ (accessed 1.8.23).

Jones, A.M., Harrison, R.M., 2004. The effects of meteorological factors on atmospheric bioaerosol concentrations—a review. Science of The Total Environment 326, 151–180. https://doi.org/10.1016/j.scitotenv.2003.11.021

Jönsson, M.T., Ruete, A., Kellner, O., Gunnarsson, U., Snäll, T., 2017. Will forest conservation areas protect functionally important diversity of fungi and lichens over time? Biodivers Conserv 26, 2547–2567. https://doi.org/10.1007/s10531-015-1035-0

Joshi, N., Fass, J., 2011. Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files.

Jumpponen, A., Jones, K.L., David Mattox, J., Yaege, C., 2010. Massively parallel 454-sequencing of fungal communities in Quercus spp. ectomycorrhizas indicates seasonal dynamics in urban and rural sites. Molecular Ecology 19, 41–53. https://doi.org/10.1111/j.1365-294X.2009.04483.x

Kaimal, J.C., Finnigan, J.J., 1994. Atmospheric boundary layer flows—their structure and measurement. Oxford University Press, New York. https://doi.org/10.16085/j.issn.1000-6613.2012.02.016

Karnosky, D.F., Mankovska, B., Percy, K., Dickson, R.E., Podila, G.K., Sober, J., Noormets, A., Hendrey, G., Coleman, M.D., Kubiske, M., Pregitzer, K.S., Isebrands, J.G., 1999. Effects of Tropospheric O3 on Trembling Aspen and Interaction with Co2: Results from an O3-Gradient and a Face Experiment. Water, Air, & Soil Pollution 116, 311–322. https://doi.org/10.1023/A:1005276824459

Kauserud, H., Heegaard, E., Buntgen, U., Halvorsen, R., Egli, S., Senn-Irlet, B., Krisai-Greilhuber, I., Damon, W., Sparks, T., Norden, J., Hoiland, K., Kirk, P., Semenov, M., Boddy, L., Stenseth, N.C., 2012. Warming-induced shift in European mushroom fruiting phenology. Proceedings of the National Academy of Sciences 109, 14488–14493. https://doi.org/10.1073/pnas.1200789109

Kauserud, H., Heegaard, E., Semenov, M.A., Boddy, L., Halvorsen, R., Stige, L.Chr., Sparks, T.H., Gange, A.C., Stenseth, N.Chr., 2010. Climate change and spring-fruiting fungi. Proceedings of the Royal Society B: Biological Sciences 277, 1169–1177. https://doi.org/10.1098/rspb.2009.1537

Keel, S.G., Siegwolf, R.T.W., Körner, C., 2006. Canopy CO2 enrichment permits tracing the fate of recently assimilated carbon in a mature deciduous forest. New Phytologist 172, 319–329. https://doi.org/10.1111/j.1469-8137.2006.01831.x

Khokon, A.M., Schneider, D., Daniel, R., Polle, A., 2021. Soil Layers Matter: Vertical Stratification of Root-Associated Fungal Assemblages in Temperate Forests Reveals Differences in Habitat Colonization. Microorganisms 9, 2131. https://doi.org/10.3390/microorganisms9102131

Kibby, G., 2021. Mushrooms and Toadstools of Britain & Europe, 1st ed. Geoffrey Kibby, Great Britain.

Kibby, G., 2020a. Mushrooms and Toadstools of Britain & Europe, 3rd ed. Geoffrey Kibby, Great Britain.

Kibby, G., 2020b. Mushrooms and Toadstools of Britain & Europe, 1st ed. Geoffrey Kibby, Great Britain.

Kicklighter, D.W., Melillo, J.M., Monier, E., Sokolov, A.P., Zhuang, Q., 2019. Future nitrogen availability and its effect on carbon sequestration in Northern Eurasia. Nat Commun 10, 3024. https://doi.org/10.1038/s41467-019-10944-0

Kivlin, S.N., Winston, G.C., Goulden, M.L., Treseder, K.K., 2014. Environmental filtering affects soil fungal community composition more than dispersal limitation at regional scales. Fungal Ecology, Fungal ecology beyond boundaries: from communities to the globe 12, 14–25. https://doi.org/10.1016/j.funeco.2014.04.004

Klironomos, J.N., Rillig, M.C., Allen, M.F., Zak, D.R., Pregitzer, K.S., Kubiske, M.E., 1997. Increased levels of airborne fungal spores in response to *Populus tremuloides* grown under elevated atmospheric CO $_2$. Can. J. Bot. 75, 1670–1673. https://doi.org/10.1139/b97-880

Komonen, A., Sundström, L.M., Wall, A., Halme, P., 2016. Afforested fields benefit nutrient-demanding fungi. Restoration Ecology 24, 53–60. https://doi.org/10.1111/rec.12282

Kourmouli, A., 2022. Soil respiration and nutrient dynamics at a mature temperate woodland - the BIFoR FACE experiment (d_ph). University of Birmingham.

Kriebel, D., Tickner, J., Epstein, P., Lemons, J., Levins, R., Loechler, E.L., Quinn, M., Rudel, R., Schettler, T., Stoto, M., 2001. The precautionary principle in environmental science. Environ Health Perspect 109, 871–876.

Kutszegi, G., Siller, I., Dima, B., Merényi, Z., Varga, T., Takács, K., Turcsányi, G., Bidló, A., Ódor, P., 2020. Revealing hidden drivers of macrofungal species richness by analyzing fungal guilds in temperate forests, West Hungary. COMMUNITY ECOLOGY. https://doi.org/10.1007/s42974-020-00031-6

Kutszegi, G., Siller, I., Dima, B., Takács, K., Merényi, Z., Varga, T., Turcsányi, G., Bidló, A., Ódor, P., 2015. Drivers of macrofungal species composition in temperate forests, West Hungary: functional groups compared. Fungal Ecology 17, 69–83. https://doi.org/10.1016/j.funeco.2015.05.009

La Porta, N., Capretti, P., Thomsen, I.M., Kasanen, R., Hietala, A.M., Von Weissenberg, K., 2008. Forest pathogens with higher damage potential due to climate change in Europe. Canadian Journal of Plant Pathology 30, 177–195. https://doi.org/10.1080/07060661.2008.10540534

Læssøe, T., Petersen, J.H., 2019. Fungi of Temperate Europe. Princeton University Press.

Lagomarsino, A., Knapp, B.A., Moscatelli, M.C., De Angelis, P., Grego, S., Insam, H., 2007. Structural and functional diversity of soil microbes is affected by elevated

[CO2] and N addition in a poplar plantation. J Soils Sediments 7, 399–405. https://doi.org/10.1065/jss2007.04.223

Lahti, L., Shetty, S., 2012. microbiome R package.

Lee, J., Lee, S., Young, J.P.W., 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiology Ecology 65, 339–349. https://doi.org/10.1111/j.1574-6941.2008.00531.x

Lekberg, Y., Vasar, M., Bullington, L.S., Sepp, S.-K., Antunes, P.M., Bunn, R., Larkin, B.G., Öpik, M., 2018. More bang for the buck? Can arbuscular mycorrhizal fungal communities be characterized adequately alongside other fungi using general fungal primers? New Phytologist 220, 971–976. https://doi.org/10.1111/nph.15035

Leski, T., Rudawska, M., Kujawska, M., Stasińska, M., Janowski, D., Karliński, L., Wilgan, R., 2019. Both forest reserves and managed forests help maintain ectomycorrhizal fungal diversity. Biological Conservation 238, 108206. https://doi.org/10.1016/j.biocon.2019.108206

Lewis, S.L., Wheeler, C.E., Mitchard, E.T.A., Koch, A., 2019. Restoring natural forests is the best way to remove atmospheric carbon. Nature 568, 25–28. https://doi.org/10.1038/d41586-019-01026-8

Li, D.-W., Kendrick, B., 1995. A year-round study on functional relationships of airborne fungi with meteorological factors. Int J Biometeorol 39, 74–80. https://doi.org/10.1007/BF01212584

Lilleskov, E.A., Bruns, T.D., 2005. Spore dispersal of a resupinate ectomycorrhizal fungus, Tomentella sublilacina, via soil food webs. Mycologia 97, 762–769. https://doi.org/10.1080/15572536.2006.11832767

Lonsdale, D., Pautasso, M., Holdenrieder, O., 2008. Wood-decaying fungi in the forest: conservation needs and management options. Eur J Forest Res 127, 1–22. https://doi.org/10.1007/s10342-007-0182-6

López-Mondéjar, R., Brabcová, V., Štursová, M., Davidová, A., Jansa, J., Cajthaml, T., Baldrian, P., 2018. Decomposer food web in a deciduous forest shows high share of generalist microorganisms and importance of microbial biomass recycling. ISME J 12, 1768–1778. https://doi.org/10.1038/s41396-018-0084-2

Lukac, M., Calfapietra, C., Godbold, D.L., 2003. Production, turnover and mycorrhizal colonization of root systems of three Populus species grown under elevated CO2 (POPFACE). Global Change Biology 9, 838–848. https://doi.org/10.1046/j.1365-2486.2003.00582.x

MacKenzie, A.R., Krause, S., Hart, K., Thomas, R., Blaen, P., Hamilton, R., Curioni, G., Quick, S., Kourmouli, A., Hannah, D., Warner, S.C., Brekenfeld, N., Ullah, S., Press, M., 2020. BIFoR FACE: Water-soil-vegetation-atmosphere research in a temperate deciduous forest catchment, including under elevated CO2 (preprint). Preprints. https://doi.org/10.22541/au.160157598.86879557

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10–12. https://doi.org/10.14806/ej.17.1.200

Marzano, M., Woodcock, P., Quine, C.P., 2019. Dealing with dieback: forest manager attitudes towards developing resistant ash trees in the United Kingdom. Forestry: An International Journal of Forest Research 92, 554–567. https://doi.org/10.1093/forestry/cpz022

McCormack, M.L., Fernandez, C.W., Brooks, H., Pritchard, S.G., 2017. Production dynamics of Cenococcum geophilum ectomycorrhizas in response to long-term elevated CO2 and N fertilization. Fungal Ecology 26, 11–19. https://doi.org/10.1016/j.funeco.2016.11.001

McElrone, A.J., Reid, C.D., Hoye, K.A., Hart, E., Jackson, R.B., 2005. Elevated CO2 reduces disease incidence and severity of a red maple fungal pathogen via changes in host physiology and leaf chemistry. Global Change Biology 11, 1828–1836. https://doi.org/10.1111/j.1365-2486.2005.001015.x

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE 8, e61217. https://doi.org/10.1371/journal.pone.0061217

Meier, I.C., Pritchard, S.G., Brzostek, E.R., McCormack, M.L., Phillips, R.P., 2014. The rhizosphere and hyphosphere differ in their impacts on carbon and nitrogen cycling in forests exposed to elevated CO2. New Phytologist 205, 1164–1174. https://doi.org/10.1111/nph.13122

Met Office, 2019. UK Climate Projections: Headline Findings [WWW Document]. URL https://www.metoffice.gov.uk/binaries/content/assets/metofficegovuk/pdf/research/ukcp/ukcp-headline-findings-v2.pdf (accessed 2.10.21).

Midlands Asthma and Allergy Research Association, 2021. Pollen and Spore Report [WWW Document]. MAARA. URL https://www.maara.org/2013-08-19-06-21-06/pollen-spore-report (accessed 3.22.21).

Miglietta, F., Peressotti, A., Vaccari, F.P., Zaldei, A., DeAngelis, P., Scarascia-Mugnozza, G., 2001. Free-air CO2 enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. New Phytologist 150, 465–476. https://doi.org/10.1046/j.1469-8137.2001.00115.x

Mitchell, R.J., Beaton, J.K., Bellamy, P.E., Broome, A., Chetcuti, J., Eaton, S., Ellis, C.J., Gimona, A., Harmer, R., Hester, A.J., Hewison, R.L., Hodgetts, N.G., Iason, G.R., Kerr, G., Littlewood, N.A., Newey, S., Potts, J.M., Pozsgai, G., Ray, D., Sim, D.A., Stockan, J.A., Taylor, A.F.S., Woodward, S., 2014. Ash dieback in the UK: A review of the ecological and conservation implications and potential management options. Biological Conservation 175, 95–109. https://doi.org/10.1016/j.biocon.2014.04.019

Mitchell, R.J., Bellamy, P.E., Ellis, C.J., Hewison, R.L., Hodgetts, N.G., Iason, G.R., Littlewood, N.A., Newey, S., Stockan, J.A., Taylor, A.F.S., 2019. Oak-associated biodiversity in the UK (OakEcol). NERC Environmental Information Data Centre. https://doi.org/10.5285/22b3d41e-7c35-4c51-9e55-0f47bb845202

Mitchell, R.J., Hewison, R.L., Hester, A.J., Broome, A., Kirby, K.J., 2016a. Potential impacts of the loss of Fraxinus excelsior (Oleaceae) due to ash dieback on woodland vegetation in Great Britain. New Journal of Botany 6, 2–15. https://doi.org/10.1080/20423489.2016.1171454

Mitchell, R.J., Pakeman, R.J., Broome, A., Beaton, J.K., Bellamy, P.E., Brooker, R.W., Ellis, C.J., Hester, A.J., Hodgetts, N.G., Iason, G.R., Littlewood, N.A., Pozsgai, G., Ramsay, S., Riach, D., Stockan, J.A., Taylor, A.F.S., Woodward, S., 2016b. How to Replicate the Functions and Biodiversity of a Threatened Tree Species? The Case of Fraxinus excelsior in Britain. Ecosystems 19, 573–586. https://doi.org/10.1007/s10021-015-9953-y

Miyamoto, Y., Terashima, Y., Nara, K., 2018. Temperature niche position and breadth of ectomycorrhizal fungi: Reduced diversity under warming predicted by a nested community structure. Global Change Biology 24, 5724–5737. https://doi.org/10.1111/gcb.14446

Money, N.P., 2016. Chapter 3 - Spore Production, Discharge, and Dispersal, in: Watkinson, S.C., Boddy, L., Money, N.P. (Eds.), The Fungi (Third Edition). Academic Press, Boston, pp. 67–97. https://doi.org/10.1016/B978-0-12-382034-1.00003-7

Moore-Landecker, E., 2011. Fungal Spores, in: eLS. American Cancer Society. https://doi.org/10.1002/9780470015902.a0000378.pub2

Müller, J., Hothorn, T., Pretzsch, H., 2007. Long-term effects of logging intensity on structures, birds, saproxylic beetles and wood-inhabiting fungi in stands of

European beech Fagus sylvatica L. Forest Ecology and Management 242, 297–305. https://doi.org/10.1016/j.foreco.2007.01.046

Mundra, S., Kjønaas, O.J., Morgado, L.N., Krabberød, A.K., Ransedokken, Y., Kauserud, H., 2021. Soil depth matters: shift in composition and inter-kingdom co-occurrence patterns of microorganisms in forest soils. FEMS Microbiology Ecology 97, fiab022. https://doi.org/10.1093/femsec/fiab022

Nathan, R., Horn, H.S., Chave, J., Levin, S.A., 2002. Mechanistic models for tree seed dispersal by wind in dense forests and open landscapes., in: Levey, D.J., Silva, W.R., Galetti, M. (Eds.), Seed Dispersal and Frugivory: Ecology, Evolution and Conservation. Third International Symposium-Workshop on Frugivores and Seed Dispersal, São Pedro, Brazil, 6-11 August 2000. CABI, Wallingford, pp. 69–82. https://doi.org/10.1079/9780851995250.0069

National Pollen and Aerobiology Research Unit, 2012. . Pollen Calendars by area - University Of Worcester. URL https://www.worcester.ac.uk/about/academic-schools/school-of-science-and-the-environment/science-and-the-environment-research/national-pollen-and-aerobiology-research-unit/pollen-calendar.aspx (accessed 7.16.20).

Newton, A.C., Holden, E., Davy, L.M., Ward, S.D., Fleming, L.V., Watling, R., 2002. Status and distribution of stipitate hydnoid fungi in Scottish coniferous forests. Biological Conservation 107, 181–192. https://doi.org/10.1016/S0006-3207(02)00060-5

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Nolan, V., Reader, T., Gilbert, F., Atkinson, N., 2020. The Ancient Tree Inventory: a summary of the results of a 15 year citizen science project recording ancient, veteran and notable trees across the UK. Biodivers Conserv 29, 3103–3129. https://doi.org/10.1007/s10531-020-02033-2

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., Zaehle, S., 2016. Model-data synthesis for the next generation of forest free-air CO2 enrichment (FACE) experiments. New Phytologist 209, 17–28. https://doi.org/10.1111/nph.13593

Norby, R.J., Wullschleger, S.D., Hanson, P.J., Gunderson, C.A., Tschaplinski, T.J., Jastrow, J.D., 2006. CO2 Enrichment of a Deciduous Forest: The Oak Ridge FACE Experiment, in: Nösberger, J., Long, S.P., Norby, Richard J., Stitt, M., Hendrey, G.R., Blum, H. (Eds.), Managed Ecosystems and CO2: Case Studies, Processes, and Perspectives, Ecological Studies. Springer, Berlin, Heidelberg, pp. 231–251. https://doi.org/10.1007/3-540-31237-4_13

Norby, R.J., Zak, D.R., 2011. Ecological Lessons from Free-Air CO2 Enrichment (FACE) Experiments. Annual Review of Ecology, Evolution, and Systematics 42, 181–203. https://doi.org/10.1146/annurev-ecolsys-102209-144647

Nordén, J., Abrego, N., Boddy, L., Bässler, C., Dahlberg, A., Halme, P., Hällfors, M., Maurice, S., Menkis, A., Miettinen, O., Mäkipää, R., Ovaskainen, O., Penttilä, R., Saine, S., Snäll, T., Junninen, K., 2020. Ten principles for conservation translocations of threatened wood-inhabiting fungi. Fungal Ecology 44, 100919. https://doi.org/10.1016/j.funeco.2020.100919

Norros, V., Penttilä, R., Suominen, M., Ovaskainen, O., 2012. Dispersal may limit the occurrence of specialist wood decay fungi already at small spatial scales. Oikos 121, 961–974. https://doi.org/10.1111/j.1600-0706.2012.20052.x

Norros, V., Rannik, Ü., Hussein, T., Petäjä, T., Vesala, T., Ovaskainen, O., 2014. Do small spores disperse further than large spores? Ecology 95, 1612–1621. https://doi.org/10.1890/13-0877.1

Observatree, Forest Research, 2018. Observatree - An early warning system for tree health and tree disease [WWW Document]. Observatree - the official project website. URL https://www.observatree.org.uk/ (accessed 2.11.21).

O'Connor, D.J., Sadyś, M., Skjøth, C.A., Healy, D.A., Kennedy, R., Sodeau, J.R., 2014. Atmospheric concentrations of Alternaria, Cladosporium, Ganoderma and Didymella spores monitored in Cork (Ireland) and Worcester (England) during the summer of 2010. Aerobiologia 30, 397–411. https://doi.org/10.1007/s10453-014-9337-3

O'Hanlon, R., Harrington, T.J., 2012a. Macrofungal diversity and ecology in four Irish forest types. Fungal Ecology 5, 499–508. https://doi.org/10.1016/j.funeco.2011.12.008

O'Hanlon, R., Harrington, T.J., 2012b. Similar taxonomic richness but different communities of ectomycorrhizas in native forests and non-native plantation forests. Mycorrhiza 22, 371–382. https://doi.org/10.1007/s00572-011-0412-0

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2020. vegan: Community Ecology Package.

Oneto, D.L., Golan, J., Mazzino, A., Pringle, A., Seminara, A., 2020. Timing of fungal spore release dictates survival during atmospheric transport. PNAS 117, 5134–5143. https://doi.org/10.1073/pnas.1913752117

Ouimette, A.P., Ollinger, S.V., Lepine, L.C., Stephens, R.B., Rowe, R.J., Vadeboncoeur, M.A., Tumber-Davila, S.J., Hobbie, E.A., 2020. Accounting for Carbon Flux to Mycorrhizal Fungi May Resolve Discrepancies in Forest Carbon Budgets. Ecosystems 23, 715–729. https://doi.org/10.1007/s10021-019-00440-3

Page, C.N., 1997. The Ferns of Britain and Ireland, 2nd ed. CUP.

Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, A.D., Piao, S., Rautiainen, A., Sitch, S., Hayes, D., 2011. A Large and Persistent Carbon Sink in the World's Forests. Science 333, 988–993. https://doi.org/10.1126/science.1201609

Parihar, M., Rakshit, A., Meena, V.S., Gupta, V.K., Rana, K., Choudhary, M., Tiwari, G., Mishra, P.K., Pattanayak, A., Bisht, J.K., Jatav, S.S., Khati, P., Jatav, H.S., 2020. The potential of arbuscular mycorrhizal fungi in C cycling: a review. Arch Microbiol 202, 1581–1596. https://doi.org/10.1007/s00203-020-01915-x

Parrent, J.L., Morris, W.F., Vilgalys, R., 2006. Co2-Enrichment and Nutrient Availability Alter Ectomycorrhizal Fungal Communities. Ecology 87, 2278–2287. https://doi.org/10.1890/0012-9658(2006)87[2278:CANAAE]2.0.CO;2

Parrent, J.L., Vilgalys, R., 2007a. Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO2 and nitrogen fertilization. New Phytologist 176, 164–174. https://doi.org/10.1111/j.1469-8137.2007.02155.x

Parrent, J.L., Vilgalys, R., 2007b. Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO2 and nitrogen fertilization. New Phytologist 176, 164–174. https://doi.org/10.1111/j.1469-8137.2007.02155.x

Pasanen, H., Junninen, K., Kouki, J., 2014. Restoring dead wood in forests diversifies wood-decaying fungal assemblages but does not quickly benefit red-listed species. Forest Ecology and Management 312, 92–100. https://doi.org/10.1016/j.foreco.2013.10.018

Paudel, B., Chu, T., Chen, M., Sampath, V., Prunicki, M., Nadeau, K.C., 2021. Increased duration of pollen and mold exposure are linked to climate change. Sci Rep 11, 12816. https://doi.org/10.1038/s41598-021-92178-z

Pepin, S., Körner, C., 2002. Web-FACE: a new canopy free-air CO2 enrichment system for tall trees in mature forests. Oecologia 133, 1–9. https://doi.org/10.1007/s00442-002-1008-3

Pérez, I., Anadón, J.D., Díaz, M., Nicola, G.G., Tella, J.L., Giménez, A., 2012. What is wrong with current translocations? A review and a decision-making proposal. Frontiers in Ecology and the Environment 10, 494–501. https://doi.org/10.1890/110175

Petters, M.D., Kreidenweis, S.M., 2007. A single parameter representation of hygroscopic growth and cloud condensation nucleus activity. Atmospheric Chemistry and Physics 7, 1961–1971. https://doi.org/10.5194/acp-7-1961-2007

Pope, F.D., 2010. Pollen grains are efficient cloud condensation nuclei. Environ. Res. Lett. 5, 044015. https://doi.org/10.1088/1748-9326/5/4/044015

Pöschl, U., 2005. Atmospheric Aerosols: Composition, Transformation, Climate and Health Effects. Angewandte Chemie International Edition 44, 7520–7540. https://doi.org/10.1002/anie.200501122

Potter, C., Harwood, T., Knight, J., Tomlinson, I., 2011. Learning from history, predicting the future: the UK Dutch elm disease outbreak in relation to contemporary tree disease threats. Philosophical Transactions of the Royal Society B: Biological Sciences 366, 1966–1974. https://doi.org/10.1098/rstb.2010.0395

Prada-Salcedo, L.D., Goldmann, K., Heintz-Buschart, A., Reitz, T., Wambsganss, J., Bauhus, J., Buscot, F., 2021. Fungal guilds and soil functionality respond to tree community traits rather than to tree diversity in European forests. Molecular Ecology 30, 572–591. https://doi.org/10.1111/mec.15749

Prass, M., Andreae, M.O., de Araùjo, A.C., Artaxo, P., Ditas, F., Elbert, W., Förster, J.-D., Franco, M.A., Hrabe de Angelis, I., Kesselmeier, J., Klimach, T., Kremper, L.A., Thines, E., Walter, D., Weber, J., Weber, B., Fuchs, B.M., Pöschl, U., Pöhlker, C., 2021. Bioaerosols in the Amazon rain forest: temporal variations and vertical profiles of Eukarya, Bacteria, and Archaea. Biogeosciences 18, 4873–4887. https://doi.org/10.5194/bg-18-4873-2021

Pritchard, S.G., Taylor, B.N., Cooper, E.R., Beidler, K.V., Strand, A.E., McCormack, M.L., Zhang, S., 2014. Long-term dynamics of mycorrhizal root tips in a loblolly pine forest grown with free-air CO2 enrichment and soil N fertilization for 6 years. Global Change Biology 20, 1313–1326. https://doi.org/10.1111/gcb.12409

Pulimood, T.B., Corden, J.M., Bryden, C., Sharples, L., Nasser, S.M., 2007. Epidemic asthma and the role of the fungal mold Alternaria alternata. Journal of Allergy and Clinical Immunology 120, 610–617. https://doi.org/10.1016/j.jaci.2007.04.045

Purahong, W., Wubet, T., Krüger, D., Buscot, F., 2018. Molecular evidence strongly supports deadwood-inhabiting fungi exhibiting unexpected tree species preferences in temperate forests. ISME J 12, 289–295. https://doi.org/10.1038/ismej.2017.177

Quine, C.P., Humphrey, J.W., 2010. Plantations of exotic tree species in Britain: irrelevant for biodiversity or novel habitat for native species? Biodivers Conserv 19, 1503–1512. https://doi.org/10.1007/s10531-009-9771-7

R Core Team, 2020. R: A Language and Environment for Statistical Computing.

Raupach, M.R., Finnigan, J.J., Brunet, Y., 1996. Coherent Eddies and Turbulence in Vegetation Canopies: The Mixing-Layer Analogy. Boundary-Layer Meteorology

25th Anniversary Volume, 1970–1995 351–382. https://doi.org/10.1007/978-94-017-0944-6_15

Reinmuth-Selzle, K., Kampf, C.J., Lucas, K., Lang-Yona, N., Fröhlich-Nowoisky, J., Shiraiwa, M., Lakey, P.S.J., Lai, S., Liu, F., Kunert, A.T., Ziegler, K., Shen, F., Sgarbanti, R., Weber, B., Bellinghausen, I., Saloga, J., Weller, M.G., Duschl, A., Schuppan, D., Pöschl, U., 2017. Air Pollution and Climate Change Effects on Allergies in the Anthropocene: Abundance, Interaction, and Modification of Allergens and Adjuvants. Environ Sci Technol 51, 4119–4141. https://doi.org/10.1021/acs.est.6b04908

Reponen, T., Willeke, K., Ulevicius, V., Reponen, A., Grinshpun, S.A., 1996. Effect of relative humidity on the aerodynamic diameter and respiratory deposition of fungal spores. Atmospheric Environment 30, 3967–3974. https://doi.org/10.1016/1352-2310(96)00128-8

Rinne-Garmston, K.T., Peltoniemi, K., Chen, J., Peltoniemi, M., Fritze, H., Mäkipää, R., 2019. Carbon flux from decomposing wood and its dependency on temperature, wood N2 fixation rate, moisture and fungal composition in a Norway spruce forest. Global Change Biology 25, 1852–1867. https://doi.org/10.1111/gcb.14594

Roberts, A.J., Crowley, L.M., Sadler, J.P., Nguyen, T.T.T., Gardner, A.M., Hayward, S.A.L., Metcalfe, D.B., 2022. Effects of Elevated Atmospheric CO2 Concentration on Insect Herbivory and Nutrient Fluxes in a Mature Temperate Forest. Forests 13, 998. https://doi.org/10.3390/f13070998

Rodrigues, J., Caruthers, C., Azmeh, R., Dykewicz, M.S., Slavin, R.G., Knutsen, A.P., 2016. The spectrum of allergic fungal diseases of the upper and lower airways. Expert Review of Clinical Immunology 12, 531–550. https://doi.org/10.1586/1744666X.2016.1142874

Rodriguez-Ramos, J.C., Cale, J.A., Cahill, J.F., Simard, S.W., Karst, J., Erbilgin, N., 2021. Changes in soil fungal community composition depend on functional group and forest disturbance type. New Phytologist 229, 1105–1117. https://doi.org/10.1111/nph.16749

Royal Botanic Gardens, Kew, 2021. The Fungarium [WWW Document]. URL https://www.kew.org/science/collections-and-resources/collections/fungarium (accessed 2.9.21).

Royal Botanic Gardens, Kew, 2019. The Lost and Found Fungi Project [WWW Document]. URL http://fungi.myspecies.info/content/lost-and-found-fungi-project (accessed 6.25.20).

Runnel, K., Tamm, H., Lõhmus, A., 2015. Surveying wood-inhabiting fungi: Most molecularly detected polypore species form fruit-bodies within short distances. Fungal Ecology 18, 93–99. https://doi.org/10.1016/j.funeco.2015.08.008

Rygiewicz, P.T., Andersen, C.P., 1994. Mycorrhizae alter quality and quantity of carbon allocated below ground. Nature 369, 58–60. https://doi.org/10.1038/369058a0

Sacco, A.D., Hardwick, K.A., Blakesley, D., Brancalion, P.H.S., Breman, E., Rebola, L.C., Chomba, S., Dixon, K., Elliott, S., Ruyonga, G., Shaw, K., Smith, P., Smith, R.J., Antonelli, A., 2021. Ten golden rules for reforestation to optimize carbon sequestration, biodiversity recovery and livelihood benefits. Global Change Biology n/a, 1–21. https://doi.org/10.1111/gcb.15498

Sadyś, M., Adams-Groom, B., Herbert, R.J., Kennedy, R., 2016a. Comparisons of fungal spore distributions using air sampling at Worcester, England (2006–2010). Aerobiologia 32, 619–634. https://doi.org/10.1007/s10453-016-9436-4

Sadyś, M., Kennedy, R., West, J.S., 2016b. Potential impact of climate change on fungal distributions: analysis of 2 years of contrasting weather in the UK. Aerobiologia 32, 127–137. https://doi.org/10.1007/s10453-015-9402-6

Sadyś, M., Skjøth, C.A., Kennedy, R., 2014. Back-trajectories show export of airborne fungal spores (Ganoderma sp.) from forests to agricultural and urban areas in England. Atmospheric Environment 84, 88–99. https://doi.org/10.1016/j.atmosenv.2013.11.015

Santini, A., Ghelardini, L., Pace, C.D., Desprez-Loustau, M.L., Capretti, P., Chandelier, A., Cech, T., Chira, D., Diamandis, S., Gaitniekis, T., Hantula, J., Holdenrieder, O., Jankovsky, L., Jung, T., Jurc, D., Kirisits, T., Kunca, A., Lygis, V., Malecka, M., Marcais, B., Schmitz, S., Schumacher, J., Solheim, H., Solla, A., Szabò, I., Tsopelas, P., Vannini, A., Vettraino, A.M., Webber, J., Woodward, S., Stenlid, J., 2013. Biogeographical patterns and determinants of invasion by forest pathogens in Europe. New Phytologist 197, 238–250. https://doi.org/10.1111/j.1469-8137.2012.04364.x

Sapsford, S.J., Paap, T., Hardy, G.E.St.J., Burgess, T.I., 2017. The 'chicken or the egg': which comes first, forest tree decline or loss of mycorrhizae? Plant Ecol 218, 1093–1106. https://doi.org/10.1007/s11258-017-0754-6

Sato, H., Morimoto, S., Hattori, T., 2012. A Thirty-Year Survey Reveals That Ecosystem Function of Fungi Predicts Phenology of Mushroom Fruiting. PLOS ONE 7, e49777. https://doi.org/10.1371/journal.pone.0049777

Schlesinger, W.H., Bernhardt, E.S., DeLucia, E.H., Ellsworth, D.S., Finzi, A.C., Hendrey, G.R., Hofmockel, K.S., Lichter, J., Matamala, R., Moore, D., Oren, R., Pippen, J.S., Thomas, R.B., 2006. The Duke Forest FACE Experiment: CO2 Enrichment of a Loblolly Pine Forest, in: Nösberger, J., Long, S.P., Norby, R.J., Stitt, M., Hendrey, George R., Blum, H. (Eds.), Managed Ecosystems and CO2: Case Studies, Processes, and Perspectives, Ecological Studies. Springer, Berlin, Heidelberg, pp. 197–212. https://doi.org/10.1007/3-540-31237-4_11

Schulte-Uebbing, L., de Vries, W., 2018. Global-scale impacts of nitrogen deposition on tree carbon sequestration in tropical, temperate, and boreal forests: A meta-analysis. Glob Change Biol 24, e416–e431. https://doi.org/10.1111/gcb.13862

Sesartic, A., Dallafior, T.N., 2011. Global fungal spore emissions, review and synthesis of literature data. Biogeosciences 8, 1181–1192. https://doi.org/10.5194/bg-8-1181-2011

Sgouridis, F., Reay, M., Cotchim, S., Ma, J., Radu, A., Ullah, S., 2023. Stimulation of soil gross nitrogen transformations and nitrous oxide emission under Free air CO2 enrichment in a mature temperate oak forest at BIFoR-FACE. Soil Biology and Biochemistry 184, 109072. https://doi.org/10.1016/j.soilbio.2023.109072

Skjøth, C.A., Damialis, A., Belmonte, J., De Linares, C., Fernández-Rodríguez, S., Grinn-Gofroń, A., Jędryczka, M., Kasprzyk, I., Magyar, D., Myszkowska, D., Oliver, G., Páldy, A., Pashley, C.H., Rasmussen, K., Satchwell, J., Thibaudon, M., Tormo-Molina, R., Vokou, D., Ziemianin, M., Werner, M., 2016. Alternaria spores in the air across Europe: abundance, seasonality and relationships with climate, meteorology and local environment. Aerobiologia 32, 3–22. https://doi.org/10.1007/s10453-016-9426-6

Smith, A.R., Lukac, M., Hood, R., Healey, J.R., Miglietta, F., Godbold, D.L., 2013. Elevated CO2 enrichment induces a differential biomass response in a mixed species temperate forest plantation. New Phytologist 198, 156–168. https://doi.org/10.1111/nph.12136

Šnajdr, J., Valášková, V., Merhautová, V., Herinková, J., Cajthaml, T., Baldrian, P., 2008. Spatial variability of enzyme activities and microbial biomass in the upper layers of Quercus petraea forest soil. Soil Biology and Biochemistry, Special Section: Enzymes in the Environment 40, 2068–2075. https://doi.org/10.1016/j.soilbio.2008.01.015

Sousan, S., Koehler, K., Hallett, L., Peters, T.M., 2016. Evaluation of the Alphasense optical particle counter (OPC-N2) and the Grimm portable aerosol spectrometer

(PAS-1.108). Aerosol Science and Technology 50, 1352–1365. https://doi.org/10.1080/02786826.2016.1232859

Spake, R., van der Linde, S., Newton, A.C., Suz, L.M., Bidartondo, M.I., Doncaster, C.P., 2016. Similar biodiversity of ectomycorrhizal fungi in set-aside plantations and ancient old-growth broadleaved forests. Biological Conservation 194, 71–79. https://doi.org/10.1016/j.biocon.2015.12.003

Steidinger, B.S., Bhatnagar, J.M., Vilgalys, R., Taylor, J.W., Qin, C., Zhu, K., Bruns, T.D., Peay, K.G., 2020. Ectomycorrhizal fungal diversity predicted to substantially decline due to climate changes in North American Pinaceae forests. Journal of Biogeography 47, 772–782. https://doi.org/10.1111/jbi.13802

Stephens, R.B., Rowe, R.J., 2020. The underappreciated role of rodent generalists in fungal spore dispersal networks. Ecology 101, e02972. https://doi.org/10.1002/ecy.2972

Su, H.B., Shaw, R.H., Pawu, K.T., Moeng, C.H., Sullivan, P.P., 1998. Turbulent statistics of neutrally stratified flow within and above a sparse forest from large-eddy simulation and field observations. Boundary-Layer Meteorology 88, 363–397. https://doi.org/10.1023/A:1001108411184

Suz, L.M., Barsoum, N., Benham, S., Dietrich, H.-P., Fetzer, K.D., Fischer, R., García, P., Gehrman, J., Kristöfel, F., Manninger, M., Neagu, S., Nicolas, M., Oldenburger, J., Raspe, S., Sánchez, G., Schröck, H.W., Schubert, A., Verheyen, K., Verstraeten, A., Bidartondo, M.I., 2014. Environmental drivers of ectomycorrhizal communities in Europe’s temperate oak forests. Molecular Ecology 23, 5628–5644. https://doi.org/10.1111/mec.12947

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K., Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., De Kesel, A., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. Science 346, 1256688. https://doi.org/10.1126/science.1256688

Tedersoo, L., Bahram, M., Zinger, L., Nilsson, R.H., Kennedy, P.G., Yang, T., Anslan, S., Mikryukov, V., 2022. Best practices in metabarcoding of fungi: From experimental design to results. Molecular Ecology 31, 2769–2795. https://doi.org/10.1111/mec.16460

Teklehaimanot, Z., Jones, M., Sinclair, F.L., 2002. Tree and livestock productivity in relation to tree planting configuration in a silvopastoral system in North Wales, UK. Agroforestry Systems 56, 47–55. https://doi.org/10.1023/A:1021131026092

Tesmer, J., Schnittler, M., 2007. Sedimentation velocity of myxomycete spores. Mycological Progress 6, 229–234. https://doi.org/10.1007/s11557-007-0539-8

The British Mycological Society, 2015. Conservation Policy [WWW Document]. URL https://www.britmycolsoc.org.uk/mycology/conservation/policy (accessed 1.31.21).

The Climate Change Committee, 2020. Sixth Carbon Budget [WWW Document]. URL https://www.theccc.org.uk/publication/sixth-carbon-budget/ (accessed 1.18.21).

The Woodland Trust, 2021. Ash Dieback (Hymenoscyphus fraxineus) [WWW Document]. Woodland Trust. URL https://www.woodlandtrust.org.uk/trees-woods-and-wildlife/tree-pests-and-diseases/key-tree-pests-and-diseases/ash-dieback/ (accessed 1.19.21).

Thomas, P., Büntgen, U., 2017. First harvest of Périgord black truffle in the UK as a result of climate change. Climate Research 74, 67–70. https://doi.org/10.3354/cr01494

Tláskal, V., Brabcová, V., Větrovský, T., Jomura, M., López-Mondéjar, R., Monteiro, L.M.O., Saraiva, J.P., Human, Z.R., Cajthaml, T., Rocha, U.N. da, Baldrian, P., 2021. Complementary Roles of Wood-Inhabiting Fungi and Bacteria Facilitate Deadwood Decomposition. mSystems 6. https://doi.org/10.1128/mSystems.01078-20

Tomao, A., Antonio Bonet, J., Castaño, C., de-Miguel, S., 2020. How does forest management affect fungal diversity and community composition? Current knowledge and future perspectives for the conservation of forest fungi. Forest Ecology and Management 457, 117678. https://doi.org/10.1016/j.foreco.2019.117678

Tóth, B.B., Barta, Z., 2010. Ecological studies of ectomycorrhizal fungi: an analysis of survey methods. Fungal Diversity 45, 3–19. https://doi.org/10.1007/s13225-010-0052-2

Treseder, K.K., Allen, M.F., 2000. Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO2 and nitrogen deposition. New Phytologist 147, 189–200.

Truong, C., Mujic, A.B., Healy, R., Kuhar, F., Furci, G., Torres, D., Niskanen, T., Sandoval-Leiva, P.A., Fernández, N., Escobar, J.M., Moretto, A., Palfner, G., Pfister, D., Nouhra, E., Swenie, R., Sánchez-García, M., Matheny, P.B., Smith, M.E., 2017. How to know the fungi: combining field inventories and DNA-barcoding to document fungal diversity. New Phytologist 214, 913–919. https://doi.org/10.1111/nph.14509

University of Worcester, 2021. Pollen Forecasting [WWW Document]. URL https://www.worc.ac.uk/about/academic-schools/school-of-science-and-the-environment/science-and-the-environment-research/national-pollen-and-aerobiology-research-unit/home.aspx (accessed 2.9.21).

Unterseher, M., Schnittler, M., Dormann, C., Sickert, A., 2008. Application of species richness estimators for the assessment of fungal diversity. FEMS Microbiology Letters 282, 205–213. https://doi.org/10.1111/j.1574-6968.2008.01128.x

Urbanová, M., Šnajdr, J., Baldrian, P., 2015. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. Soil Biology and Biochemistry 84, 53–64. https://doi.org/10.1016/j.soilbio.2015.02.011

van der Linde, S., Holden, E., Parkin, P.I., Alexander, I.J., Anderson, I.C., 2012. Now you see it, now you don’t: The challenge of detecting, monitoring and conserving ectomycorrhizal fungi. Fungal Ecology 5, 633–640. https://doi.org/10.1016/j.funeco.2012.04.002

van der Linde, S., Suz, L.M., Orme, C.D.L., Cox, F., Andreae, H., Asi, E., Atkinson, B., Benham, S., Carroll, C., Cools, N., De Vos, B., Dietrich, H.-P., Eichhorn, J., Gehrmann, J., Grebenc, T., Gweon, H.S., Hansen, K., Jacob, F., Kristöfel, F., Lech, P., Manninger, M., Martin, J., Meesenburg, H., Merilä, P., Nicolas, M., Pavlenda, P., Rautio, P., Schaub, M., Schröck, H.-W., Seidling, W., Šrámek, V., Thimonier, A., Thomsen, I.M., Titeux, H., Vanguelova, E., Verstraeten, A., Vesterdal, L., Waldner, P., Wijk, S., Zhang, Y., Žlindra, D., Bidartondo, M.I., 2018. Environment and host as large-scale controls of ectomycorrhizal fungi. Nature 558, 243–248. https://doi.org/10.1038/s41586-018-0189-9

Varenius, K., Kårén, O., Lindahl, B., Dahlberg, A., 2016. Long-term effects of tree harvesting on ectomycorrhizal fungal communities in boreal Scots pine forests. Forest Ecology and Management, Special section: Drought and US Forests:

Impacts and Potential Management Responses 380, 41–49. https://doi.org/10.1016/j.foreco.2016.08.006

Veldman, J.W., Overbeck, G.E., Negreiros, D., Mahy, G., Le Stradic, S., Fernandes, G.W., Durigan, G., Buisson, E., Putz, F.E., Bond, W.J., 2015. Where Tree Planting and Forest Expansion are Bad for Biodiversity and Ecosystem Services. BioScience 65, 1011–1018. https://doi.org/10.1093/biosci/biv118

Verheyen, K., Vanhellemont, M., Auge, H., Baeten, L., Baraloto, C., Barsoum, N., Bilodeau-Gauthier, S., Bruelheide, H., Castagneyrol, B., Godbold, D., Haase, J., Hector, A., Jactel, H., Koricheva, J., Loreau, M., Mereu, S., Messier, C., Muys, B., Nolet, P., Paquette, A., Parker, J., Perring, M., Ponette, Q., Potvin, C., Reich, P., Smith, A., Weih, M., Scherer-Lorenzen, M., 2016. Contributions of a global network of tree diversity experiments to sustainable forest plantations. Ambio 45, 29–41. https://doi.org/10.1007/s13280-015-0685-1

Větrovský, T., Kohout, P., Kopecký, M., Machac, A., Man, M., Bahnmann, B.D., Brabcová, V., Choi, J., Meszárošová, L., Human, Z.R., Lepinay, C., Lladó, S., López-Mondéjar, R., Martinović, T., Mašínová, T., Morais, D., Navrátilová, D., Odriozola, I., Štursová, M., Švec, K., Tláskal, V., Urbanová, M., Wan, J., Žifčáková, L., Howe, A., Ladau, J., Peay, K.G., Storch, D., Wild, J., Baldrian, P., 2019. A meta-analysis of global fungal distribution reveals climate-driven patterns. Nature Communications 10, 5142. https://doi.org/10.1038/s41467-019-13164-8

Větrovský, T., Morais, D., Kohout, P., Lepinay, C., Algora, C., Awokunle Hollá, S., Bahnmann, B.D., Bílohnědá, K., Brabcová, V., D'Alò, F., Human, Z.R., Jomura, M., Kolařík, M., Kvasničková, J., Lladó, S., López-Mondéjar, R., Martinović, T., Mašínová, T., Meszárošová, L., Michalčíková, L., Michalová, T., Mundra, S., Navrátilová, D., Odriozola, I., Piché-Choquette, S., Štursová, M., Švec, K., Tláskal, V., Urbanová, M., Vlk, L., Voříšková, J., Žifčáková, L., Baldrian, P., 2020. GlobalFungi, a global database of fungal occurrences from high-throughput-sequencing metabarcoding studies. Scientific Data 7, 228. https://doi.org/10.1038/s41597-020-0567-7

Voříšková, J., Brabcová, V., Cajthaml, T., Baldrian, P., 2014. Seasonal dynamics of fungal communities in a temperate oak forest soil. New Phytologist 201, 269–278. https://doi.org/10.1111/nph.12481

Weber, C.F., Balasch, M.M., Gossage, Z., Porras-Alfaro, A., Kuske, C.R., 2012. Soil Fungal Cellobiohydrolase I Gene (cbhI) Composition and Expression in a Loblolly Pine Plantation under Conditions of Elevated Atmospheric CO2 and Nitrogen Fertilization. Applied and Environmental Microbiology 78, 3950–3957. https://doi.org/10.1128/AEM.08018-11

Weber, C.F., Vilgalys, R., Kuske, C.R., 2013. Changes in fungal community composition in response to elevated atmospheric CO2 and nitrogen fertilization varies with soil horizon. Frontiers in Microbiology 4, 1–14. https://doi.org/10.3389/fmicb.2013.00078

Welsh, K.G., Holden, K.A., Wardlaw, A.J., Satchwell, J., Monteiro, W., Pashley, C.H., Gaillard, E.A., 2020. Fungal sensitization and positive fungal culture from sputum in children with asthma are associated with reduced lung function and acute asthma attacks respectively. Clinical & Experimental Allergy 00, 1–11. https://doi.org/10.1111/cea.13799

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis.

Wilson, E.O., 2017. Biodiversity research requires more boots on the ground. Nature Ecology & Evolution 1, 1590–1591. https://doi.org/10.1038/s41559-017-0360-y

Wolf, J., Johnson, N.C., Rowland, D.L., Reich, P.B., 2003. Elevated CO2 and plant species richness impact arbuscular mycorrhizal fungal spore communities. New Phytologist 157, 579–588.

Wolf, J., O’Neill, N.R., Rogers, C.A., Muilenberg, M.L., Ziska, L.H., 2010. Elevated atmospheric carbon dioxide concentrations amplify Alternaria alternate sporulation and total antigen production. Environmental Health Perspectives 118, 1223–1228. https://doi.org/10.1289/ehp.0901867

Woods, A.J., Martín-García, J., Bulman, L., Vasconcelos, M.W., Boberg, J., Porta, N.L., Peredo, H., Vergara, G., Ahumada, R., Brown, A., Diez, J.J., 2016. Dothistroma needle blight, weather and possible climatic triggers for the disease’s recent emergence. Forest Pathology 46, 443–452. https://doi.org/10.1111/efp.12248

Wright, R.J., 2020. Influences of climate change on childhood asthma and allergy risk. The Lancet Child & Adolescent Health 4, 859–860. https://doi.org/10.1016/S2352-4642(20)30315-1

Zhao, W., Wang, D.-D., Huang, K.-C., Liu, S., Reyila, M., Sun, Y.-F., Li, J.-N., Cui, B.-K., 2023. Seasonal variation in the soil fungal community structure of Larix gmelinii forests in Northeast China. Frontiers in Microbiology 14.

Zhou, Z., Wang, C., Luo, Y., 2020. Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. Nature Communications 11, 3072. https://doi.org/10.1038/s41467-020-16881-7

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

Žifčáková, L., Větrovský, T., Howe, A., Baldrian, P., 2016. Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Environ Microbiol 18, 288–301. https://doi.org/10.1111/1462-2920.13026

APPENDICES

11.1 BIFoR fungal and myxomycete species list from field IDs

Common name	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
	Amoebozoa	*Myxogastria*	*Physarales*	*Didymiaceae*	*Didymium*	
	Amoebozoa	*Myxogastria*	*Physarales*	*Didymiaceae*	*Mucilago*	*crustacea*
	Amoebozoa	*Myxogastria*	*Physarales*	*Physaraceae*	*Fuligo*	*septica*
	Amoebozoa	*Myxogastria*	*Stemonitidales*	*Stemonitidaceae*	*Stemonitis*	*fusca*
Oak blackhead	*Ascomycota*	*Ascomycetes*	*Xylariales*	*Xylariaceae*	*Diatrype*	*quercina*
Oak powdery mildew	*Ascomycota*	*Leotiomycetes*	*Erysiphales*	*Erysiphaceae*	*Erysiphe*	*alphitoides*
Brown cup	*Ascomycota*	*Leotiomycetes*	*Helotiales*	*Rutstroemiaceae*	*Rutstroemia*	*firma*
Purple jellydisc	*Ascomycota*	*Leotiomycetes*	*Leotiales*	*Gelatinodiscaceae*	*Ascocoryne*	*sarcoides*
Lemon disco	*Ascomycota*	*Leotiomycetes*	*Leotiales*	*Helotiaceae*	*Bisporella*	*citrina*
Scarlet elf cup	*Ascomycota*	*Pezizomycetes*	*Pezizales*	*Sarcoscyphaceae*	*Sarcoscypha*	*austriaca*
Coral spot fungus	*Ascomycota*	*Sordariomycetes*	*Hypocreales*	*Nectriaceae*	*Nectria*	*cinnabarina*
Hazel woodwart	*Ascomycota*	*Sordariomycetes*	*Xylariales*	*Hypoxylaceae*	*Hypoxylon*	*fuscum*
	Ascomycota	*Sordariomycetes*	*Xylariales*	*Hypoxylaceae*	*Hypoxylon*	
	Ascomycota	*Sordariomycetes*	*Xylariales*	*Xylariaceae*	*Biscogniauxia*	
	Ascomycota	*Sordariomycetes*	*Xylariales*	*Xylariaceae*	*Jackrogersella*	*multiformis*
Candlesnuff fungus	*Ascomycota*	*Sordariomycetes*	*Xylariales*	*Xylariaceae*	*Xylaria*	*hypoxylon*
Dead man's fingers	*Ascomycota*	*Sordariomycetes*	*Xylariales*	*Xylariaceae*	*Xylaria*	*polymorpha*
	Ascomycota	*Sordariomycetes*	*Xylariales*	*Xylariaceae*	*Xylaria*	
Rosy bonnet	*Basidiomycota*	*Agaricomycetees*	*Agaricales*	*Mycenaceae*	*Mycena*	*rosea*
Honey fungus	*Basidiomycota*	*Agaricomycetees*	*Agaricales*	*Physalacriaceae*	*Armillaria*	
Shaggy parasol	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Agaricaceae*	*Chlorophyllum*	*rhacodes*

	Basidiomycota	*Agaricomycetes*	*Agaricales*	*Agaricaceae*	*Lepiota*	
Puffball	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Agaricaceae*	*Lycoperdon*	
	Basidiomycota	*Agaricomycetes*	*Agaricales*	*Agaricaceae*		
Blusher	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Amanitaceae*	*Amanita*	*rubescens*
	Basidiomycota	*Agaricomycetes*	*Agaricales*	*Amanitaceae*	*Amanita*	
	Basidiomycota	*Agaricomycetes*	*Agaricales*	*Bolbitiaceae*	*Pholiotina*	
	Basidiomycota	*Agaricomycetes*	*Agaricales*	*Hydnangiaceae*	*Laccaria*	*amethystina*
Amethyst deceiver	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Hydnangiaceae*	*Laccaria*	*laccata*
Oysterling	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Inocybaceae*	*Crepidotus*	
Spindleshank	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Marasmiaceae*	*Gymnopus*	*fusipes*
Buttercap	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Marasmiaceae*	*Rhodocollybia*	*butyracea*
	Basidiomycota	*Agaricomycetes*	*Agaricales*	*Marasmiaceae*	*Xeromphalina*	
	Basidiomycota	*Agaricomycetes*	*Agaricales*	*Marasmiaceae*		
	Basidiomycota	*Agaricomycetes*	*Agaricales*	*Mycenaceae*	*Hemimycena*	
Clustered bonnet	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Mycenaceae*	*Mycena*	*inclinata*
	Basidiomycota	*Agaricomycetes*	*Agaricales*	*Mycenaceae*	*Mycena*	
Burgundydrop Bonnet	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Mycenaceae*	*Mycena*	*haematopus*
	Basidiomycota	*Agaricomycetes*	*Agaricales*	*Mycenaceae*		
Deer shield	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Pluteaceae*	*Pluteus*	*cervinus*
	Basidiomycota	*Agaricomycetes*	*Agaricales*	*Pluteaceae*	*Pluteus*	
	Basidiomycota	*Agaricomycetes*	*Agaricales*	*Psathrellaceae*	*Psathyrella*	
Hare's foot inkcap	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Psathyrellaceae*	*Coprinopsis*	*lagopus*

Common stump brittlestem	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Psathyrellaceae*	*Psathyrella*	*piluliformis*
Sulphur tuft	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Strophariaceae*	*Hypholoma*	*fasciculare*
Common funnel	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Tricholomataceae*	*Clitocybe*	*gibba*
Common funnel	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Tricholomataceae*	*Clitocybe*	*nuda*
Pipe club	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Typhulaceae*	*Typhula*	*fistulosa*
	Basidiomycota	*Agaricomycetes*	*Agaricales*			
Wood ear	*Basidiomycota*	*Agaricomycetes*	*Auriculariales*	*Auriculariaceae*	*Auricularia*	*auricula*
	Basidiomycota	*Agaricomycetes*	*Boletales*	*Boletaceae*		
Earthball	*Basidiomycota*	*Agaricomycetes*	*Boletales*	*Sclerodermataceae*	*Scleroderma*	
	Basidiomycota	*Agaricomycetes*	*Boletales*			
Chanterelle	*Basidiomycota*	*Agaricomycetes*	*Cantharellales*	Cantharellales	*Cantharellus*	
Common stinkhorn	*Basidiomycota*	*Agaricomycetes*	*Phallales*	*Phallaceae*	*Phallus*	*impudicus*
	Basidiomycota	*Agaricomycetes*	*Phallales*	*Phallaceae*	*Phallus*	
Birch bracket	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Fomitopsidaceae*	*Fomitopsis*	*betulina*
Oak curtain crust	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Hymenochaetaceae*	*Hymenochaete*	*rubiginosa*
Shaggy bracket	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Hymenochaetaceae*	*Inonotus*	*hispidus*
Chicken of the woods	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Polyporaceae*	*Laetiporus*	*sulphureus*
Dryads saddle	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Polyporaceae*	*Polyporus*	*squamosus*
	Basidiomycota	*Agaricomycetes*	*Polyporales*	*Steccherinaceae*	*Steccherinum*	*ochraceum*
Hairy curtain crust	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Stereaceae*	*Stereum*	*hirsutum*
Bleeding broadleaf crust	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Stereaceae*	*Stereum*	*rugosum*
	Basidiomycota	*Agaricomycetes*	*Polyporales*	*Stereaceae*	*Stereum*	

	Basidiomycota	*Agaricomycetes*	*Polyporales*			
	Basidiomycota	*Agaricomycetes*	*Russulales*	*Peniophoraceae*	*Peniophora*	*quercina*
Oakbug milkcap	*Basidiomycota*	*Agaricomycetes*	*Russulales*	*Russulaceae*	*Lactarius*	*quietus*
Ochre brittlegill	*Basidiomycota*	*Agaricomycetes*	*Russulales*	*Russulaceae*	*Russula*	*ochroleuca*
Brittlegill	*Basidiomycota*	*Agaricomycetes*	*Russulales*	*Russulaceae*	*Russula*	
	Basidiomycota	*Agaricomycetes*	*Russulales*	*Russulaceae*		
Cinnamon porecrust	*Basidiomycota*	*Basidiomycetes*	*Hymenochaetales*	*Hymenochaetaceae*	*Fuscoporia*	*ferrea*
Common jellyspot	*Basidiomycota*	*Dacrymcetes*	*Dacrymycetales*	*Dacrymycetaceae*	*Dacrymyces*	*stillatus*
Yellow stagshorn	*Basidiomycota*	*Dacrymycetes*	*Dacrymycetales*	*Dacrymycetaceae*	*Calocera*	*viscosa*
	Basidiomycota	*Pucciniomycetes*	*Pucciniales*	*Phragmidiaceae*	*Kuehneola*	*uredinis*
	Basidiomycota	*Tremellomycetes*	*Auriculariales*	*Auriculariaceae*	*Exidia*	*glandulosa*
Witches butter	*Basidiomycota*	*Tremellomycetes*	*Auriculariales*	*Auriculariaceae*	*Exidia*	*nigricans*
	Basidiomycota	*Tremellomycetes*	*Auriculariales*	*Auriculariaceae*	*Exidia*	*nucleata*
	Basidiomycota	*Tremellomycetes*	*Auriculariales*	*Auriculariaceae*	*Exidia*	*thuretiana*
	Basidiomycota	*Tremellomycetes*	*Auriculariales*	*Auriculariaceae*	*Exidia*	
	Basidiomycota	*Tremellomycetes*	*Tremellales*	*Tremellaceae*	*Tremella*	
Wolf's milk	*Mycetozoa*	*Myxogastria*	*Liceales*	*Tubiferaceae*	*Lycogala*	*epidendrum*
Bonnet mould	*Zygomycota*	*Incertae sedis*	*Mucorales*	*Phycomycetaceae*	*Spinellus*	*fusiger*