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Development of nitrogen cycling in recently deglaciated watersheds

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Abstract

Perturbation of natural environments through anthropogenic nitrogen (N) inputs and climate change can significantly alter the type of loading and cycling processes within soil systems. Few pristine environments remain in which to study natural controls on the development of soil N cycling over time and thus increase our understanding of the natural development of such mechanisms and determine the effects of anthropogenic change on these systems.

This study took place in Glacier Bay National Park and Preserve (GBNP), in southeast Alaska. Rapid de-glaciation within this region over the last 250 years has created watersheds of varying ages with minimal anthropogenic disturbance. This has presented a unique opportunity to study the evolution of microbial N cycling in pristine soil systems. Six river catchments were selected for study across a chronosequence of 200 years of primary succession.

Within each watershed a combination of both field and laboratory methods were used to evaluate the soil N status in addition to the net and potential microbial processes (mineralisation, nitrification and denitrification). Riparian soil and leaf samples were collected from near stream and floodplain areas. This approach allowed a cross stream comparison of microbial N processing for a range of soil ages to determine a time frame for riparian development.

Samples were also collected in the wider catchment area away from fluvial influences in order to investigate the effects of dominant vegetation types and slope steepness on the physical and microbial characteristics of the soil. These data were then coupled with percent vegetation type for each watershed which was generated by analysis of satellite imagery. This allowed the scaling up of soil variables to the watershed scale, and to quantify change over time through the use of historic satellite images. Surface and hyporheic water chemistry was also determined to link successional changes in watershed characteristics with riverine nutrient exports.

A key finding of this research was that vegetation type is the primary influence on nitrogen cycling processes and soil characteristics with successional change, allowing for the development of the soil layer with increased organic matter and N retention. This in turn benefits the development of the microbial community. With increasing age potential microbial activity increased in particular nitrification which outperformed denitrification by up to 40 times, however there was no resulting increase in soil NO_3^- concentration indicating a large heterotrophic microbial community in older soils.

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List of Abbreviations

A	Area
BBS	Burg Bay South
C	Carbon
DEA	Denitrification Enzyme Activity
DIN	Dissolved inorganic nitrogen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
EC	Electrical conductivity
EF	Enrichment factor
GBNP	Glacier Bay National Park
GIS	Geographic information system
GPS	Global positioning system
HDP	High density plastic
LWD	Large woody debris
N	Nitrogen
NFS	North Fingers Stream
OM	Organic Matter
P	Phosphorus
RPC	Rush point Creek
Q	Discharge
SFC	Stone Fly Creek
TDS	Total dissolved solids
TDN	Total dissolved nitrogen
TN	Total nitrogen
TIN	Total inorganic nitrogen
TON	Total organic nitrogen
TP	Total phosphorus
V	Velocity
WFPS	Water filled pore space
WPC	Wolf Point Creek

Contents

1: Introduction

1.1 Geomorphologic development

Understanding the form, historical context and behaviour of landscapes is crucial to understanding ecosystems on several spatial and temporal scales (Swanson *et al.*, 1988). Ecosystem development is influenced by the interactions between landforms, biota and geomorphic processes which influence the movement of material and energy within it.

Landform and geomorphic interactions are commonly so complex and entwined in the history of a site (contained in soils and other system components over time) that individual landform effects may be impossible to identify, particularly where anthropogenic disturbance is present (Swanson *et al*., 1988). A solution to this problem may be found through the study of pristine environments undergoing primary succession after a major disturbance event (e.g. volcanic eruption or glacial retreat).

Fluvial landforms within such environments (e.g. floodplains) are dynamic areas or ecotones where these processes interact, mediating fluxes of nutrients and organic matter between terrestrial, riverine and marine ecosystems. Riparian vegetation has been shown to play a pivotal role in the physical and biological development of floodplain areas, with trees and plants increasingly considered 'ecosystem engineers' of fluvial environments (Gurnell & Petts, 2006; Gurnell, 2013).

An understanding of nutrient cycling processes within forests is needed not only to explain forest ecosystem dynamics in space and time, but also for the wise husbandry of forest resources in a changing global environment (Waring, 1980). The study of recently deglaciated regions in environments with little anthropogenic influence, allows the opportunity to untangle the development of such complex interactions with minimal human interference.

1.1.1 Geomorphologic influences on habitat heterogeneity

Parent material and landforms of recently deglaciated surfaces strongly influence the development of river systems and the surrounding riparian zone. Glacially carved watersheds contain distinct remnants of their past in the form of moraines, lake systems, outwash sediment and fluvial topographies caused by catastrophic glacial floods (Naiman, 2005).

Riparian assemblages and their heterogeneity are constrained by the lithotopography (elevation, exposure, slope, groundwater dynamics and parent material) of these newly exposed surface landforms (Naiman, 2005; Swanson *et al.*, 1988; Waring, 1980).

Both parent material and topography also have significant micro and macro interactions with both the weather and climate of the local and wider watershed regions. Ultimately, this interplay between processes has a long lasting evolving effect on the form and structure of the riparian biotic community (Figure 1-1). Over time, continued biotic succession, geomorphic development and changing disturbance regimes interact and have an influence on each other, thus shaping the environment (Naiman, 2005; Waring, 1980).

The fluvial processes that result affect important physical (e.g. floodplain sediment sorting) and biological (e.g. disturbance regimes and species survival) characteristics and mechanisms within the floodplain area. Such processes help to shape the biological succession occurring and maintain both morphological and ecological habitat heterogeneity at a range of temporal and spatial scales (Naiman, 2005).

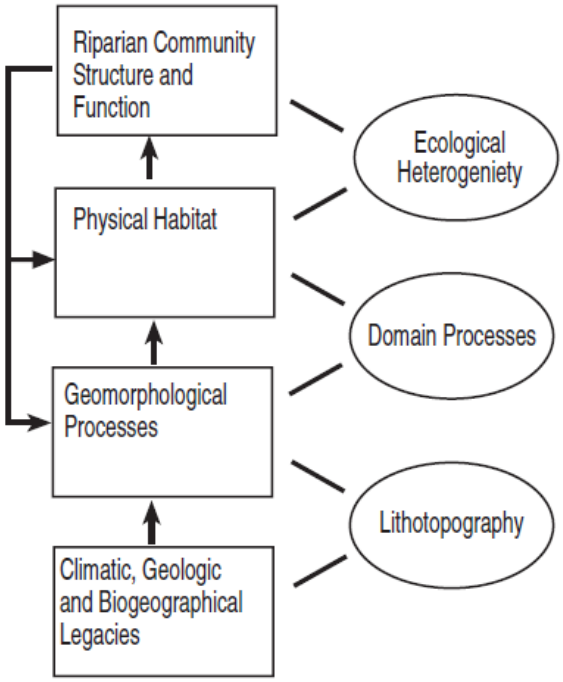

Figure 1-1: *Schematic representation of the relationships between drivers of ecological heterogeneity in riparian forests (Taken from Naiman, 2005).*

Changes to important driving forces of Earth ecosystems, such as global climate and atmospheric concentrations of carbon (C) and nitrogen (N) will have widespread, rapid effects on vegetation and microbial species composition, function and services. Understanding and predicting the future state of ecosystems caused by these changes is a key challenge for ecologists (Milner, 2007). However, much of the theory and understanding of ecological change is based on systems that are in a stable state. Furthermore, a large proportion of landscape development studies are based in areas of secondary succession, where a disturbance event (e.g. fire or flooding) results in the incomplete removal of the biotic community present.

A greater understanding of primary succession in rapidly changing environments is therefore necessary to bridge this gap in knowledge, and help predict how changing external factors will influence changing environments (Milner *et al.*, 2007). Such investigations provide the opportunity to study the development of natural processes and to help inform restoration programs and estimates for greenhouse gas emissions (Tockner *et al*, 2003).

1.2 Glacial recession

1.2.1 Global importance

Since the end of the little ice age (1850) most glaciers have been in decline globally, today glaciers cover ~ 3.1 % of Earth's surface, ~ 10.7 % of Earth's land area, and ~ 5 % of Alaska (USGS). Over the last century, mountain glaciers worldwide have, on average, been decreasing in mass and contributing to sea level rise (Dyurgerov and Meier, 1997; Arendt *et al*., 2002; Rignot *et al*., 2003; Paul *et al*., 2004; Dyurgerov and McCabe, 2006). Mid-latitude mountain ranges such as the Himalayas, Alps, Rocky Mountains, Cascade Range, and the southern Andes, as well as isolated tropical summits such as Mount Kilimanjaro in Africa, are showing some of the largest proportionate glacial losses (IPPC, 2001; Mizuno, 2014; Hobbs, 2011; Guelland, 2013).

Dyurgerov and Meier (2005) found that since 1970, of the 173 glaciers studied across the world, 83% were thinning at an average loss of 0.31 m yr^{-1}. According to the 2007 IPCC report, glaciers and icecaps have lost increasing amounts of mass since the middle of the last century. In the period between 1961 and 2004, glaciers and icecaps were losing 0.50 ± 0.18 mm yr^{-1} in sea level equivalent (SLE) mass. Between 1991 and 2004, however, the rate was actually above the average, at 0.77 ± 0.22 mm yr^{-1} SLE (IPCC, 2007). The numbers differ regionally, with the strongest mass losses per unit area in Patagonia, Alaska, and northwest USA and southwest Canada (IPCC, 2007).

Larsen (2007) used digital elevation models (DEMs) to track changes in glacial thickness, and found that in southeast Alaska and western Canada surface elevations of glaciers have lowered over 95% of the 14,580 km^2 of glacier-covered area analyzed, with some glaciers thinning as much as 640 m between 1948 to 2000 (Larsen, 2007). A similar study located in the European Alps found that Alpine glaciers lost 35% of their total area from 1850 until the 1970s, and almost 50% by 2000 (Zemp, 2006). Total glacier volume around 1850 is estimated at some 200 km^3 and was close to one-third of this value in 2006 (Zemp, 2006).

Glacial retreat will have damaging impacts on the supply of fresh water for irrigation and domestic use, mountain recreation, as well as on animals and plants that depend on

glacier-melt in the affected areas for habitat (Finn, 2013; Khamis, 2013; IPCC, 2007). The recent substantial retreat and acceleration of the rate of retreat since 1995 of a number of key outlet glaciers of the Greenland and West Antarctic ice sheets, may foreshadow a rise in sea level, having a potentially dramatic effect on coastal regions worldwide (IPCC, 2007).

1.2.2 **Succession following glacial retreat**

As discussed above glacial retreat is a worldwide problem with wide ranging environmental issues. However the existence of new ice-free areas in front of glaciers offers excellent opportunities to study the processes of primary colonization and succession in areas with little human influence.

Most research in glacial forefield primary succession to date is focused on Europe and North America where the majority of the field work has been carried out. The most influential research in the Northern Hemisphere has undoubtedly been that undertaken at Glacier Bay, Alaska (Crocker & Major 1955; Lawrence 1958; Chapin *et al*. 1994; Hobbie *et al*, 1998; Milner, 2007), also in similar comparable regions around the world, for example in North America and Canada (Jones & del Moral 2005; Knelman, 2012; Mori *et al*. 2008; Okitsu *et al*. 2004), Scandinavian glaciers (Robins and Mathews, 2009; Matthews & Whittaker 1987; Matthews 1992; Whittaker 1993), and in the Alps (Bardgett *et al,* 2007; Caccianiga *et al*. 2001; Garbarino *et al*. 2010; Guelland *et al,* 2013; Miniac *et al*, 2007; Raffl *et al*. 2006; Stöcklin & Bäumler 1996; Tscherko et al. 2003 & 2004). There are relatively fewer published studies based in Southern Hemisphere glacier forelands, however those available include sites in New Zealand (Burrows & Maunder 1975; Wardle 1980; Sommerville *et al*. 1982) and in some sub-Antarctic islands such as Heard Island (Scott 1990), South Georgia (Smith 1984) and at the Ampère Glacier, in Kerguelen Islands (Frenot, 1999).

The classic view of primary succession across these environments states that bare sediments are colonized by early successional plants and microbes that can survive in this nutrient poor environment, fixing atmospheric N, and progressively improving the surface sediment for themselves and later successional species, through the accumulation of OM, C and N (Chapin *et al*, 1994). Worldwide, most studies have

described vegetation changes on moraines of different ages and have described centuries of change (Okitsu *et al*. 2004; Jones & del Moral 2005; Raffl *et al*. 2006; Mori *et al*. 2008). Most studies have found that time, expressed as till age, is the main factor controlling species distributions (Whittaker 1987; Matthews 1992; Caccianiga *et al*. 2001). However, in addition to till age, substratum stability may strongly influence vegetation patterns around glaciers on high, tropical mountains (Mizuno 2014). Garbarino *et al*. (2010) found, by examining patterns of larch development, that the most influential factors determining vegetation stand density and age were proximity to the glacier terminus, seed sources, litter cover and elevation.

Microbial communities initiate the earliest stages of soil development and biogeochemical cycling (Nemergut *et al.*, 2007). Microbial functional diversity reaches stability within 50 yr of succession (Tscherko et al. 2003). In the foreland of the Coleman Glacier, USA, species richness and diversity were highest during early succession at small scales, and during late succession at larger scales (Jones & del Moral 2005). Until recently, it has been assumed that the colonisation of a glacier forefield starts with autotrophic organisms (Walker and del Moral 2003) that, upon death, decompose into soil organic matter (SOM), becoming bio-available to soil heterotrophs as a source of energy (Bardgett and Walker 2004).

However relatively recent research had questioned this theory in recently deglaciated areas. Tscherko *et al*. (2003) and Zumsteg *et al*. (2012) found a fast and diverse colonisation of heterotrophs after glacial retreat, before autotrophic colonisation. These heterotrophic bacteria are able to utilise ancient, recalcitrant C remnants as an energy source, only switching entirely too modern plant derived C after 50 years (Bardgett, 2007). Ancient C sources could be either parent rock material or aeolian inputs of organic C original accumulated in cryoconite holes on the ice sheet (Hawes 2008; Anesio *et al*. 2010).

In addition cyanobacteria could play an important role in keeping initial soil C levels from declining over time through inputs of C (Schmidt *et al.* 2008). Accumulated soil organic matter only becomes significant during later successional stages for the sustenance of soil heterotrophs. In these later stages soil C models assume that there is

a constant supply of litter and soil respiration, and hence under a stable climate, C inputs and outputs reach a ‘‘steady state’’ (Liski *et al*. 2005).

Studies indicate that rates of C accumulation in soils are greatest during the initial phase of soil formation (Schlesinger 1990). Soils could, therefore, act as a C sink during the first few decades of soil development. Consequently, an improved knowledge of the development of C cycling in early successional soils is essential for the quantification of soils as sources or sinks C.

Guelland *et al,* (2013) examined a chronosequence (7-128 years) along the Damma glacier forefield, Switzerland. Results showed a clear increase in soil CO_2 effluxes with increasing site age which was linked to soil C accumulation and development of vegetation cover (Guelland *et* al, 2013). The initial phase of C accumulation changed to one of high throughput with increasing age. The authors suggest that the relatively strong increase in soil C stocks compared to C fluxes is a characteristic feature of initial soil formation on freshly exposed rocks (Guelland *et* al, 2013). White *et al,* (2007) compared soil C samples over a glacial chronosqeunce in Svalbard, Norway. The sources contributing to C cycling and to the build-up of soil organic matter changed along this glacier formed chronosequences, with the appearance of more lignineous material in the soil C pool with increasing age (60 – 100 years) (White *et al*. 2007). These discoveries indicate that, although well studied, the mechanisms involved in primary succession still require research, particularly in regard to newly deglaciated environments.

Despite the importance of microbial colonisation for modifying and improving soil conditions in early successional environments, plant colonisation dramatically alters the composition and function of soil microbial communities (Knelman *et al.*, 2012). Plants are a driving factor for microbial communities, influencing important factors such as supplies of N and C from both root exudates and litter (Bardgett *et al*, 2005; Bardgett & Walker, 2004; Grayston *et al,* 1998). In addition, they have strong localised influences on important environmental variables such as soil moisture, temperature, shading, cation exchange capacity, nutrient retention and pH (Knelman *et al.*, 2012). Some studies have indicated that plants do not influence microbial soil communities of

very young alpine sites (0-43 years) (Tscherko, 2004) but these findings are likely to be due to the complex abiotic and climatic influences that can affect rates of succession.

In temperate climatic zones, significant plant effects on microbial community structure has been demonstrated in these early successional areas (>43 years) (Bardgett & Walker, 2004; Edwards, Bürgmann, Miniaci, & Zeyer, 2006). Here vegetated areas support greater bacterial and fungal communities in the rhizosphere (Grayston, 1998) and across a wider spatial scale in surrounding sediments, compared to bare un-vegetated sediments in the same glacial forefront areas (Knelman *et al*., 2012; Miniaci *et al*., 2007).

These studies indicate that plant-microbe interactions are a driving force for soil food web development during the early stages of primary succession across many geographical areas (Bardgett and Walker, 2004). The close relationship between vegetation type and soil is maintained as the systems develop over time. In mid-late successional forests, vegetation type influences the microbial community and function, through varying quality and quantity of leaf litter inputs, root exudates, rhizodeposition and physical alterations to the soil environment, all of which influence the storage, cycling and emission of C and N within these ecosystems (Knelman *et al*., 2012; Staelens *et al*., 2011).

Of particular importance to the N status and cycling of an environment is the quality and quantity of leaf litter inputs. Leaf litter with a low C quality, indicated by high lignin concentrations, constrains decomposition and heterotrophic N_2 fixation (Vitousek & Hobbie, 2000). In addition, differences in leaf litter decomposition rates may be attributed to variations in litter traits such as leaf toughness, N and lignin content, polyphenol concentration and C:N ratios (Hättenschwiler *et al*., 2005). This disconnection in resource limitation between producers and decomposers contributes to the maintenance of N limitation in terrestrial ecosystems (Vitousek & Hobbie, 2000).

Nutrient rich litter however, can enhance the decomposition of poor quality leaf litter (Chapman, 2006; Wardle, 1999). This suggests that a mixture of vegetation during mid successional phases may provide increased litter decay and a further increase in N

transformations, for which the decomposition of organic matter is the limiting step (Schimel & Bennett, 2004).

These studies illustrate that primary succession following glacial retreat follows similar developmental trajectories across a broad range of geographical areas. Therefore this study in GBNP, Alaska is comparable to many other high latitude/altitude regions worldwide.

1.2.3 **Geomorphic development in Glacier Bay, Alaska**

Glacier Bay National Park in Alaska (referred to hereafter as GBNP) provides an excellent environment in which to study primary succession on recently exposed sediments, undergoing rapid vegetation colonisation and succession. Over the past 250 years, this area has experienced a rapid, well documented glacial recession.

A number of studies have been conducted in the GBNP area revealing the similarity and patterns of successional development within the Bay area. In addition, these studies have also highlighted how well suited such environments are to study the interplay between abiotic, physical and biotic factors in controlling geomorphic and biotic succession over time from early to later successional sites (Milner *et al.*, 2007).

These environments are strongly influenced by physical factors during early succession, however these change in importance with time as biotic influences such as terrestrial vegetation and soil development become increasing important to biotic and geomorphic succession (Milner *et al.*, 2007).

Using the N cycle, and the quantification of its main processes as a tool it is possible to quantify N retention and the production capacity of riparian systems over time since de-glaciation. Recently deglaciated sites (0-5 years) are physically driven, formed of unconsolidated glacial till shaped into a mosaic of geomorphologic features by glacial ice melt water and rain. These early rivers are cold, turbid and unstable, migrating with ease through the landscape of unconsolidated sediments. These soils are low in N, C and Organic Matter (OM) and are subject to physical and chemical weathering that often results in high levels of soil leaching (Figure 1-2).

With increasing age (5-15 years) there is a corresponding increase in biotic controls within these environments. Bacterial, lichen and plant colonisation help to stabilize the sediment structure reducing sediment erosion and in addition fixing N and C from the atmosphere. This results in both soil layer development and organic matter retention. The classical view of early bacterial communities has shifted in recent years from one of autotrophic assemblages fixing N and C from the atmosphere to one of heterotrophic bacteria utilising ancient C contained within these post glacial sediment (Bardgett *et al., 2007)* (Figure 1-2).

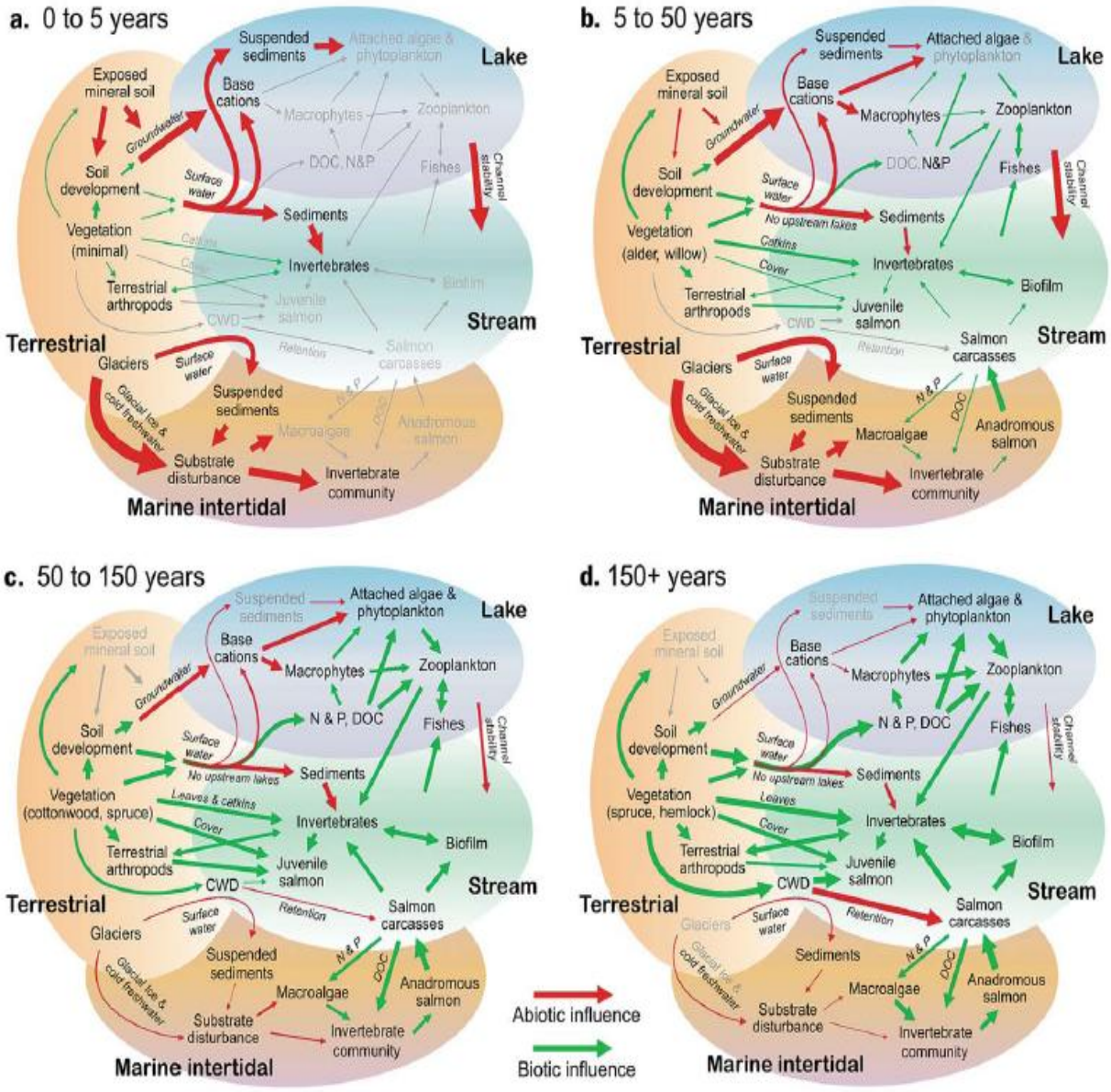

Figure 1-2: *Major linkages amongst and within a stream, lake, terrestrial, and marine intertidal environment at Glacier Bay at four time periods following deglaciation. Arrow thickness indicates the strength of the influence, which may be positive or negative. Red arrows represent abiotic processes; whilst green represent biotic processes. Grey components have little or no importance in the time period shown. Abbreviations: CWD, coarse woody debris; DOC, dissolved organic carbon; N, nitrogen; P, phosphorus (Milner, et al, 2007).*

Plant colonisation (5-50 years) stabilises the soil through leaf coverage and the development of root systems, increasing bank stability. During this period, bacterial community structure and function are also radically changed due to the increased input and retention of organic matter, particularly C and N (Bardgett & Walker, 2004; Tscherko, 2004, 2005; Wardle *et al*., 2004).

At the oldest sites (>150 years) within GBNP, the vegetation climax community is *Picea sitchensis*/*Tsuga heterophylla* forest. Vegetation development affects the form of terrestrial inputs to the river systems, directly through litter fall and woody debris and indirectly through the type of biota it supports and the terrestrial energy subsidy provided to the river system. The vegetation community of older streams provide large woody debris (LWD) that can change the geomorphology of the river such as island development, retention of nutrients and habitat complexity (Milner *et al.*, 2007).

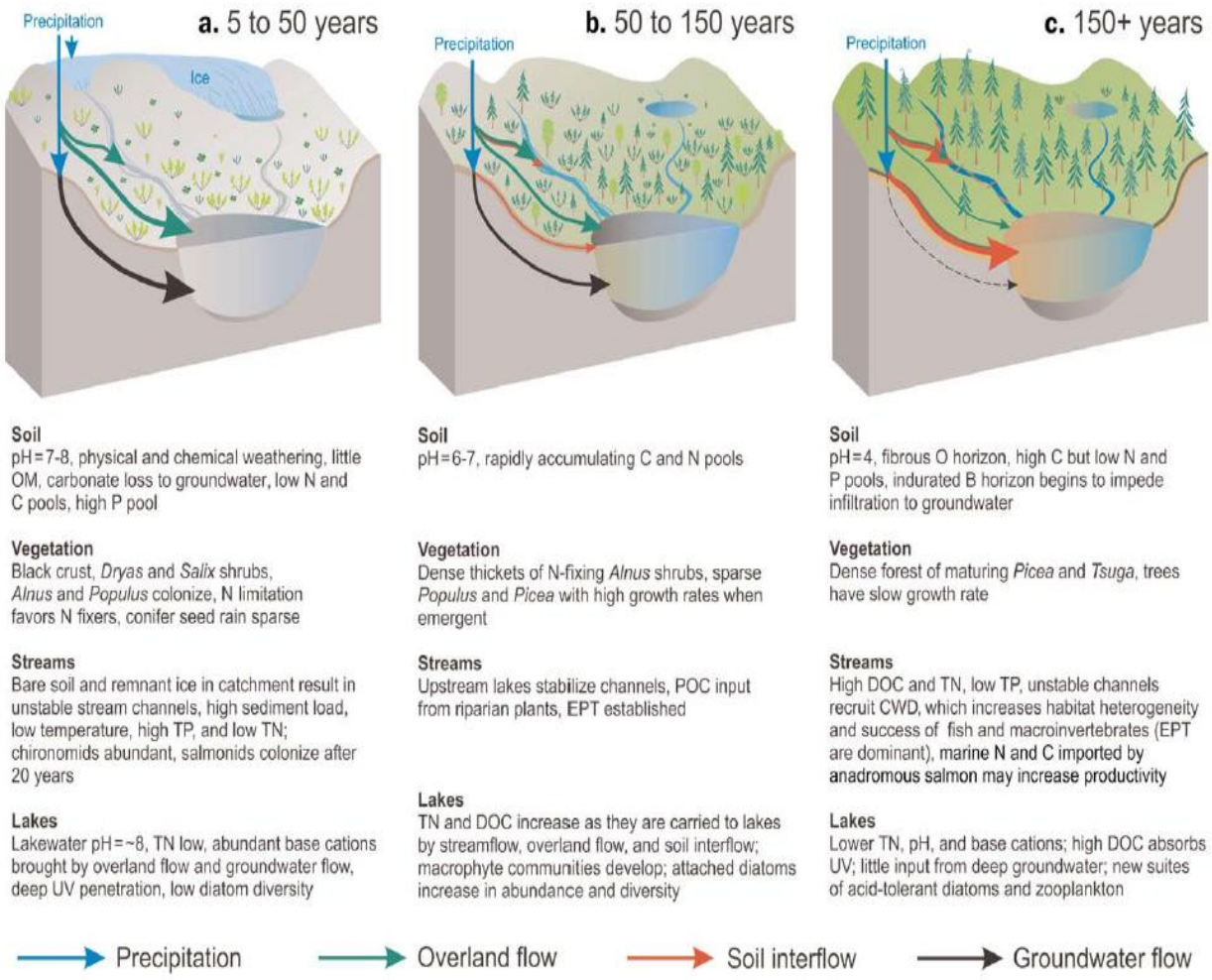

Figure 1-3: *Major features of terrestrial, stream and lake environments at Glacier Bay at three time periods following deglaciation. Arrow thickness represents relative contribution to stream and lake water from overland flow, soil interflow, and groundwater flow. The three panels represent different parts of the glacier bay landscape and do not constitute a sequence that has been followed at all sites (Milner, et al., 2007).*

One area yet to be investigated is the influence of these changing developmental processes over time in relation to the hyporheic zone, defined as the region of

sediments beneath and alongside a stream channel where shallow groundwater and surface water mix(Milner *et al.*, 2007; Triska, 2007). It is important to note that plant colonisation did not follow the same trajectory across the entire bay area (Fastie, 1995). The glacial terminus was bordered by a *T. heterophylla* and *P. sitchensis* forest therefore, the first areas to be exposed after glacial retreat in the lower bay were colonised by these species initially with no period of *Alnus sinuatta* dominance.

More recently, exposed surfaces further up the bay were exposed to a different seed rain composition, dominated by *A. sinuatta* and *Salix* spp. propagules, that leads to the development of *A. sinuatta* thickets at younger sites such as these. Thus, the proximity to plant propagules influences early colonist species composition (Milner *et al.*, 2007). This has had a lasting effect on the ecosystem with sites that had early dominance of *A. sinuatta* thicket vegetation (for first 100 years) showing greater productivity and N content than sites in the lower bay that had little or no *A. sinuatta* development prior to *P. sitchensis* and *T. heterophylla* dominance.

This increase in ecosystem productivity is demonstrated as a further increase in biotic control at older sites (50-150 years) that have, during a century of N fixation by *A. sinuatta*, accumulated soil N from near 0 to 2600 kg per hectare (ha), i.e. 30 % more than at older sites that experienced little *A. sinuatta* colonisation and early *P. sitchensis* dominance (Crocker & Major, 1955; Fastie, 1995; Milner *et al*., 2007). These shifts in vegetation type and soil development influence N, C and other chemical inputs to surface waters within these catchments (Figures 1-2 and 1-3).

1.3 Drivers of soil microbial development and function

1.3.1 Effect of slope

The gradient of slope steepness can affect soil physical and chemical characteristics, through interactions between water movement and soil particles (grain size distribution) and nutrient leaching (Swanson *et al.*, 1988). Steepness influences the gravitational movement of material effecting water retention time, influencing the length of contact time between nutrients and microorganisms.

The interactions of geomorphology, hydraulic residence times and substrate permeability create biogeochemically reactive interface zones (Grimm *et al*., 2003). Longer water retention results in greater removal of nutrients, even in areas with low microbial capacity (Grimm *et al*., 2003; McClain *et al*., 2003; Pinay *et al.*, 2003). Therefore it would be expected that shallow slope gradients will allow longer retention times, and conversely high sloping areas will have shorter retention times, resulting in differing microbial activity in these areas.

Also the water travel time can influence nutrient cycling, as demonstrated by Pinay & O'Keefe (2008) within the hyporheic zone of an Alaskan river. Increasing ground water travel time through sediments decreased water NO_3^- and increased N_2O emissions, as the longer the contact time between NO_3^- containing ground water and sediments increased the time that soil microbes had to denitrify the NO_3^- (Pinay & O'Keefe, 2008). Therefore this principle should apply to soil in areas of varying slope and water retention. Site slope should be reflected within the physical (sediment grain size), chemical (inorganic N pool), and microbial population (population size and activity) of the soil and within site vegetation.

Essentially hydraulic residence time and flow path are the important factors for reactions on water and soil chemistry, as length of contact is only important if flow paths bring together critical reactants (McClain *et al*., 2003).

1.3.2 **Vegetation- soil effects on microbial N transformations**

The interplay between plant-microbe succession has, in recent years been extensively researched across multiple environments. Plants play a driving role for the development of soil microbial communities of riparian forest and in wider catchment soils during primary succession (Bardgett & Walker, 2004; Chapin & Walker, 1994; Edwards *et al.*, 2006; Knelman *et al*., 2012; Tscherko *et al.*, 2005) and have a continuing influence in more mature systems (Grayston, 1998; Wardle, 1999).

The feedback between vegetation litter fall quality, specifically polymeric composition, polymer to N ratios and the form of microbial nutrient cycling processes play a major role in the regulation of nutrient availability and net primary production in terrestrial

ecosystems, as well as influencing pH and temperature (Chapman *et al*., 2006; Schweitzer *et al*., 2004; Scott & Binkley, 1997; Staelens *et al*., 2011). Many common garden studies have detailed the way in which plant species control the N status and microbial processes of soil, however these may not be representative of actual forest systems (Binkley & Valentine, 1991; Menyailo, Hungate, & Zech, 2002).

In forested areas the dominant tree species will provide the majority of nutrient and organic matter inputs to the soil, therefore regulating the microbial processes occurring within the soil layer (Scott & Binkley, 1997). This is demonstrated by two studies of sample plots under five different tree species in the Catskill Mountains, USA. These found that tree species influenced soil C:N ratios and net N mineralisation and nitrification rates (Lovet *et al*, 2004). Furthermore, these tree species were shown to be preferentially accessing supplies of soil NH_4^+ rather than NO_3^-, affecting the form of N available within the soil N pool for both plants and microbes (Templer, 2003).

Similarly Staelens *et al*., (2011) found in situ gross nitrogen transformations differ between temperate deciduous and coniferous forest soils. Coniferous forests were associated with less mineralisation, but greater nitrification than deciduous forested areas. However, on this occasion, this may be due to the increased N deposition associated with coniferous trees increased surface area, in an area with high atmospheric N.

In a similar study, Ambus *et al.,* (2006) attribute these differing N transformation rates between forest types to the characteristics of the leaf litter produced by the dominant tree species. NO production was found to be higher in association with nitrification activity in coniferous forest soil, favouring this transformation process due to its low soil moisture and thick well aerated soil layer. Conversely, deciduous forest soils had higher rates of N_2O production associated with denitrification due to the moist, compact nature of the soil layer (Ambus & Beier, 2006).

In addition to altering the physical structure of soil, vegetation also affects the N and C inputs. As illustrated by Zhang (2011), conifers supply more recalcitrant and less labile C than deciduous trees. This study also shows that the dominant transformation process

in coniferous areas is heterotrophic nitrification (Zhang, 2011), however this does change with N availability and soil N characteristics (Schimel & Bennett, 2004).

Having a more diverse mixture of vegetation types may result in a larger microbial community within the underlying soil as these provide a greater range of complementary reagents than those provided by a forest dominated by one species, which would decrease soil quality (Orwin *et al.*, 2010). Therefore mid successional forests which have higher species diversity, provide optimum soil inputs for microbial communities (Orwin *et al.*, 2010).

Over the next 100 years it is predicted that global temperatures will increase by 1.8 ± 4 °C, with arctic and alpine regions expected to be affected most (IPCC, 2007). High latitude ecosystems are likely to be more sensitive to this projected temperature increase as plant growth and microbial respiration are more sensitive to warming at low temperatures (Karner, 1998).

Experimental warming of alpine regions has found that plant production in addition to C, N source pools are strongly intensified by experimental warming (Na, 2011). It is therefore important to devise strategies to estimate the fluxes of important global warming gasses at the catchment scale. Due to the well documented influence of vegetation as a driver for soil microbial processes and gaseous emissions, it is reasonable to assume that the dominant vegetation type within a particular area will exert the largest control these processes (Ambus & Beier, 2006).

At the regional scale the most important factors when assessing N emissions are N deposition and the type of vegetation cover and soil (Ambus & Beier, 2006). The increased necessity to understand and estimate current and future C as well as closely linked N emissions from all forested environments, particularly post glacial areas at a catchment scale, requires a novel approach.

A useful tool for evaluating how changing vegetation compositions will influence microbial activity may therefore be the use of Landsat imagery coupled with field and laboratory based observations and experimentation. Utilising these data in conjunction, it is possible to estimate catchment scale processes based on field sample

measurements and using vegetation type and slope as an estimate of processes across the catchment. This method has already been used when modelling net primary productivity (Field, 1995) and C flux (Wang, 2006) at global scale, but has never, as far as we are aware, been used at the catchment scale, coupled to local and experimental measurements looking at soil N transformations and emissions.

1.4 Nitrogen cycling in soils

Nitrogen is a vital component of microbial, plant and animal life. As such, understanding the interactions and mechanisms involved in each stage is extremely important. Despite considerable research into the N transformation processes of nitrification and denitrification, new reaction pathways of the N cycle are being discovered, such as the anammox (Nannipieri, 2009; Schmid, 2005). The research included within this thesis and further continuing work is required to unravel the complexities of N cycling.

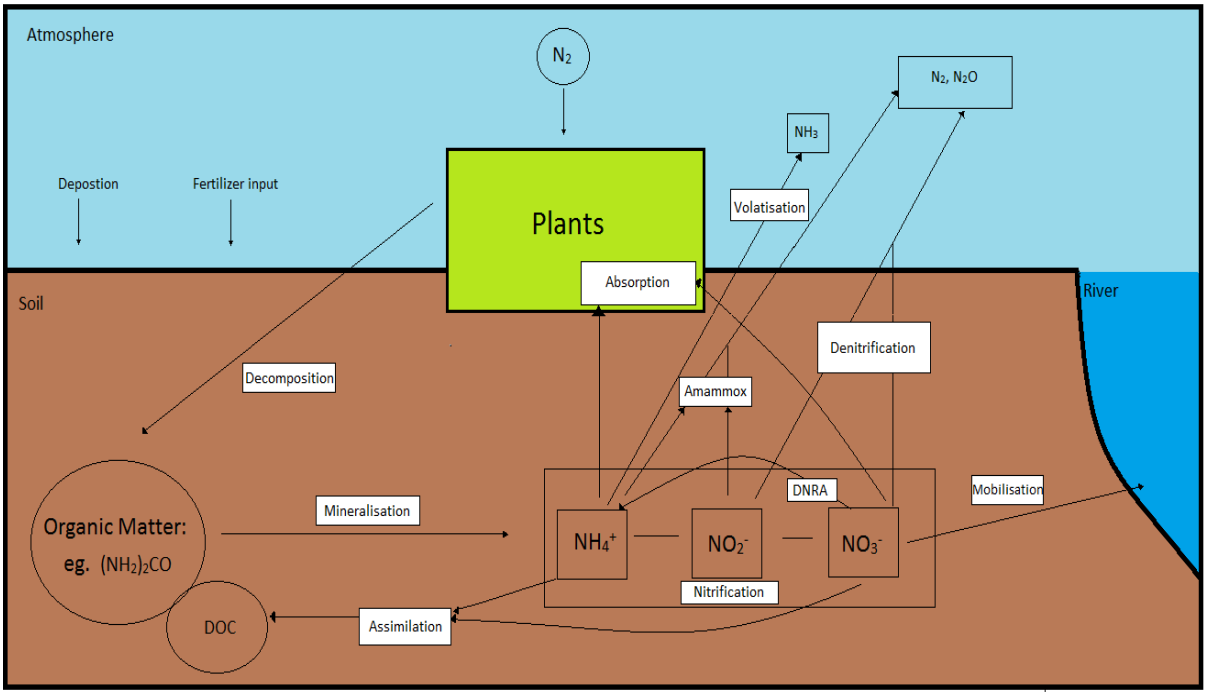

Figure 1-4: N transformation and movement in soil, atmosphere and surface water.

The largest N reservoir is the atmosphere (3.9 x 10^{15} tons) where N is stored as di-nitrogen gas (N_2) (Schlesinger, 1997). Since N_2 is largely inert, requiring substantial energy to split the N_2 into a reactive form, N is a common limiting nutrient for plant and microbial growth in many environments (Nadlehoffer and Fry, 1994).

N_2 can be split into a reactive biologically available state by abiotic forces such as lightning in the atmosphere followed by the deposition of the resulting nitrate (NO_3^-) or by biotic forces such as specialist N fixing bacteria or fungi containing nitrogenase enzymes directly oxidising N_2 to NO_3^-. These organisms can either be free living microorganisms or exist in a symbiotic partnership between them and a plant in a root nodule or in the surrounding rhizosphere. Once in the biosphere N is intensively cycled through the environment by a number of biotic and abiotic processes, and in a number of molecular forms.

Within the terrestrial environment three main N stores exist: 1) the biota (2.5 x 10^{10} tons); 2) organic matter (1.1 x 10^{11} tons) and 3) the Earth's crust (7.7 x 10^{14} tons) (Schlesinger, 1997). Soil N is mainly in an organic form (94%) with inorganic forms substantially less abundant (6%). The processes of mineralisation and nitrification transform these organic forms of N into inorganic forms (NO_3^-, NH_4^+, NO_2^-) making them available for plant and microbial uptake.

Table 1-1: *Nitrification and denitrification reaction pathways (Bock et al, 1986, Wrage, 2001).*

Reaction	Process	Bacterial taxa	Intermediary Reaction I.D	Intermediary reactions
A	Nitrification (*Aerobic)*	*Nitrosomonas, Nitrobacter.*	1i	$NH_3 + O_2 + 2H^+ \longrightarrow NH_2OH + H_2O$
			1ii	$NH_2OH + O_2 \longrightarrow NO_2^- + H_2O + H^+$
			1iii	$NO_2^- + H_2O \longrightarrow NO_3 + 2H^+$
			2	$NH_3 + O_2 + 2H^+ + H_2O \longrightarrow NO_3 + 9H^+$
B	Denitrification *(Anaerobic)*	*Pseudomonas, Bacillus, Thiobacillus, Propioni-bacterium.*	3i	$2HNO_3 + 4H^+ \longrightarrow 2HNO_2 + H_2O$
			3ii	$2HNO_2 + H^+ \longrightarrow 2NO + H_2O$
			3iii	$2NO + 2H^+ \longrightarrow N_2O + H_2O$
			3iv	$N_2O + 2H^+ \longrightarrow N_2 + H_2O$
			4	$NO_3 \longrightarrow N_2$

The classic paradigm of the N cycle states that the N mineralisation process is the rate limiting step of N transformations (Schimel & Bennett, 2004). However work over the past decade has revealed that depolymerisation of N containing polymers to simpler monomers is the limiting process (Schimel & Bennett, 2004). These N containing

monomers are then bio-available for uptake by plants, as well as mineralising microbes (Schimel & Bennett, 2004). Anthropogenic activities also provide further sources of N to the environment through fertilizer application (NO_3) for food supply and fossil fuels combustion for energy production causing nitrogen deposition.

1.4.1 Nitrogen cycling processes

The microbial nitrogen cycle contains a number of redox processes that can control the availability and speciation of N in the environment;

Depolymerisation (decomposition) is the breakdown of N containing polymers such as amino acids, by microbial (including mycorrhizal) extracellular enzymes for energy. This results in the production and eventual release of N containing monomers, organic matter (Schimel & Bennett, 2004).

Mineralisation is performed by heterotrophic microorganisms that break down organic matter to NH_4^+. Organic matter containing N such as urea ($(NH_2)_2CO$) is decomposed for energy resulting in the production of NH_4^+ which can either be stored within the microbe or released into the environment depending on environmental pressures (Schimel and Bennett, 2004). In non-fertilized areas this can be a major factor controlling the availability of N for plants and microorganisms (Haynes, 1986).

Immobilisation and assimilation are processes by which mineralising microbes can retain and use N for cellular growth and repair. These mechanisms are particularly prevalent in areas with low N availability and therefore high C:N ratios, whereas in low C:N environments the bacteria are more likely to release the NH_4 into the soil (Nicolardot, 2001).

Nitrification is the oxidation of ammonium (NH_4^+) or ammonia (NH_3) produced by the decomposition of organic matter, to nitrate (NO_3^-) via the intermediary step of nitrite (NO_2^-). This process is performed by two separate micro-organisms (Figure 2-1). The first oxidises NH_4^+ to NO_2^- and is carried out by NH_3-oxidisers or primary nitrifiers, whereas the second step is performed by NO_2^-- oxidisers or secondary nitrifiers (Bock *et al*., 1986, Wrage, 2001). These two groups together are called Nitrobacteriaceae (Wrage,

Velthof, van Beusichem, & Oenema, 2001). These nitrogen transformations lead to the production of several intermediaries which can be lost from the ecosystem, depending on its age and development. Nitrous oxide gas (N_2O) produced during both the ammonia oxidation and nitrite oxidation steps is either emitted into the atmosphere, or utilised by N_2O reducing bacteria in the soil (Figure 1-5).

Nitrification can occur via autotrophic bacteria using NH_4^+ as an energy source for the fixation of carbon dioxide (CO_2), or by heterotrophic microbial nitrification that uses organic carbon (C) sources (Wrage *et al*., 2001).

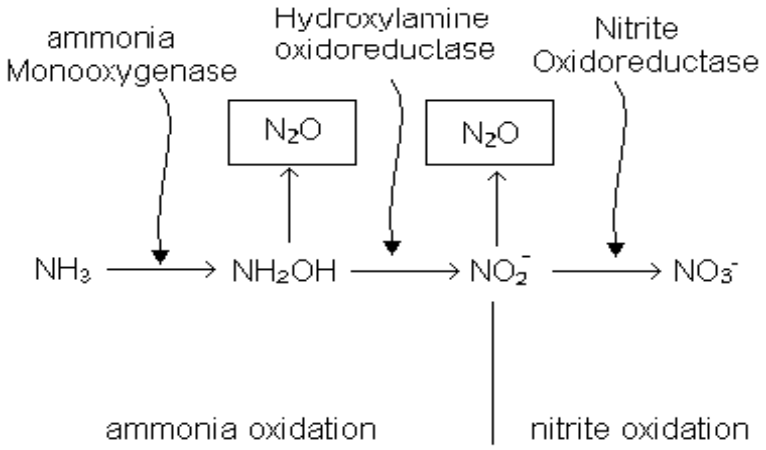

Figure 1-5: Outline of Nitrification pathways and enzymes. (Adapted from Wrage et al, 2001).

Denitrification is performed by particular groups of ubiquitous heterotrophic bacteria that utilise the step wise reduction of NO_3^- to produce gaseous N (N_2O and N_2) for energy production. These bacteria are facultative anaerobes using NO_3^- in place of O_2 in anaerobic environments as an electron acceptor (Wrage *et al*., 2001; McClain *et al*., 2003). During this process intermediary compounds are produced that can then be lost from the soil system, such as nitrous oxide (N_2O) and nitric oxide (NO) emitted into the atmosphere, also NO_2^- that can be reduced to ammonia via nitrate ammonification (Figure 1-5) (Wrage *et al*., 2001).

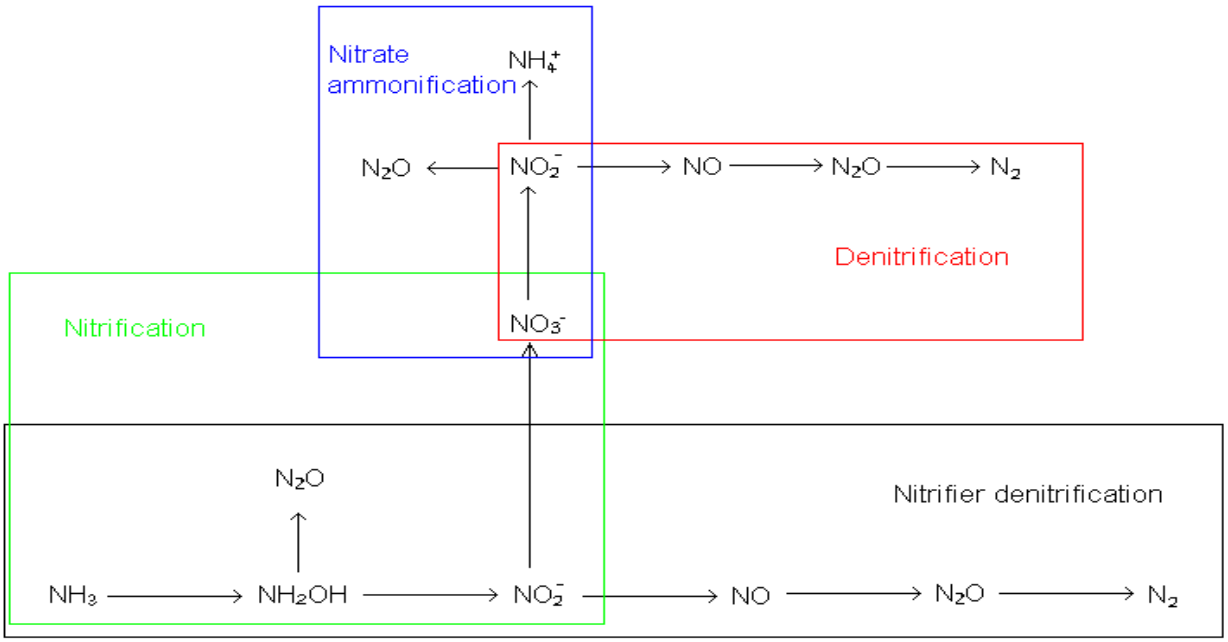

Figure 1-6: Microbial processes and sources of N_2O in soil. (Baggs, 2008).

Additional pathways exist for the transformation of NH_4^+ such as nitrifier-denitrification where the oxidation of NH_3 to NO_2^- is followed by its reduction to N_2O and N_2 by the same bacterium (Figure 1-6). This reaction pathway is produced by one group of bacteria, namely NH_3 oxidisers and varies from coupled nitrification-denitrification where each step is carried out by separate organisms (Wage et al, 2001).

In addition anaerobic ammonium oxidation (anammox) to produce NO_3^- has been shown in marine and terrestrial soils in small populations (Humbert, 2012). Dissimilarly nitrate reduction (DNRA) to NH_4^+ (Figure 1-4) can occur in some environments and has been demonstrated to outperform denitrification in obtaining NO_3^- in some cases (Rütting *et al*., 2008).

Leaching and loss of NO_3^- can occur in soil systems due to its highly mobile nature through water flow paths as dissolved organic nitrogen (DON) or through particulate sediment erosion losses of insoluble N after sediment binding (Chapin & Matson, 2002; Nannipieri & Eldor, 2009; Schimel & Bennett, 2004; Wu, Zhang, & Yu, 2012).

Volatilisation of N can occur in alkaline soils with a pH above 7.5. At this pH NH_4^+ can volatilise to $NH_3 + H^+$ and be lost to the atmosphere (Reddy and Patrick, 1984).

Non biological routes for N transformations can occur within certain environments, such as chemo-denitrification. This is the chemical decomposition of the intermediates from NH_4^+ oxidation to NO_2^- and of NO_2^- itself with organic (e.g. amines) or inorganic (e.g. Fe_2^+ and Cu_2^+) compounds to produce N_2O and N_2 (Wrage *et al*., 2001; Baggs, 2008). This reaction is thought to take place when NO_2^- accumulates in low pH soils and may be a significant source of N_2O. However, being closely linked to nitrification, it is difficult to determine the end products from one another to determine the source portioning in these environments (Baggs, 2008; Wrage *et al*., 2001).

1.4.2 **Controls on microbial cycling**

There are a number of physical variables that control microbial processes in riparian biogeochemical hot spots, leading to spatial variation of soil nutrients, organic matter (OM) and moisture content (Naiman, 2005). Denitrification rates, for example, have been reported to increase with increasing water content of soils of different textures and drainage particularly when it exceeds 60% water filled pore space (WFPS) (Groffman & Tiedje, 1991; Pinay, 2000). More recently, similar critical thresholds for denitrification activity have been observed of 53% to 56.8% water content among different types of peat (Agner & Schenk, 2005; Amha & Bohne, 2011). WFPS influences the availability and diffusion of oxygen to micro sites within the soil structure and will therefore significantly influence rates of denitrification and nitrification (Groffman & Tiedje, 1991).

There is clear evidence that sediment grain size has a significant impact on rates of microbial activity, with the highest for denitrification activity found in fine floodplain sediments (>65%) (Pinay *et al.*, 1993; Pinay, 2000). Fine sediments retain water and nutrients more efficiently than courser textured sediments causing in longer water residence times, a larger surface area and an increase in cation adsorption sites. Coarse textured soils however, tend to be prone to water loss and nitrate and organic matter (OM) leaching (Vitousek & Reiners, 2009).

Fine sediment characteristics increase the prevalence of anoxic conditions, favourable for certain microbial processes including denitrification (Naiman, 2005; Pinay, 2000). Such areas can exist in the saturated soils of swales and other depressions in wetland

areas not subject to scour and can become sinks for river borne OM and phosphate (P) which are frequently hotspots for denitrification (Naiman *et al.*, 2002). There is also a steep vertical gradient within riparian sediment for denitrification activity.

The upper soil layer (0-25cm) hosts the highest denitrifying population density; however these upper areas are not always water logged anaerobic environments (Burt *et al*., 2002; Clement *et al.*, 2003). The microbial community's existence is explained by the presence of denitrifying micro sites in soil particles, which are maintained by stable moisture content (Clement *et al*., 2003). Furthermore, water movement by capillary rise to the upper soil horizons also allows the maintenance of both aerobic and anaerobic environments in this organic horizon.

Mineralisation and nitrification will take place in aerobic soil layers or cracks, with denitrification occurring in water logged deeper layers or adjacent micro sites. Here, water not only creates the anoxic conditions but also transports reagents between sites allowing these processes to occur in close proximity increasing N transformation rates (Clement *et al*., 2003; Wrage *et al.*, 2001). This type of soil profile is an oxic-anoxic interface crossed continually with water flow, and is particularly conducive for N cycling.

To summarise, riparian areas can act as 'hotspots' for nitrification and denitrification with microbial and vegetative communities playing pivotal roles in the nitrogen buffering capacity of these areas and the movement of material and nutrients between the terrestrial and aquatic environments. However, what has not been made clear to date is the time frame for the development of such interfaces in areas with little anthropogenic influence, such as in recently deglaciated environments. Therefore, by careful interpretation of these processes over the chronosequence present in Glacier Bay National Park their development over time can be determined.

1.4.3 **Influence of riparian areas on water chemistry**

Headwater streams have the largest influence on overall river water chemistry throughout the entire river system (Burt *et al*., 2010; Wipfli, 2007). In these small 1st and 2nd order streams the surrounding catchment provides the greater flow of inputs

such as dissolved organic matter (DOM) and nutrients (N, P and C) to the riparian zone and stream systems than vice versa (Figure 1-6). Riparian areas are the most effective at removing nutrients from incoming ground water in small headwater streams.

Microbial processes occurring at biogeochemical interfaces within the wider watershed and riparian zone will have a substantial influence on riverine inputs (Burt *et al*., 2010; McClain *et al.*, 2003). Because of this close link water chemistry (N and C isotopic ratio and concentrations) can be used as an indication of the microbial processes occurring in the surrounding watershed. Investigating how these indicators change over a chronosequence of river watersheds will shed light on to how microbial processes develop over time.

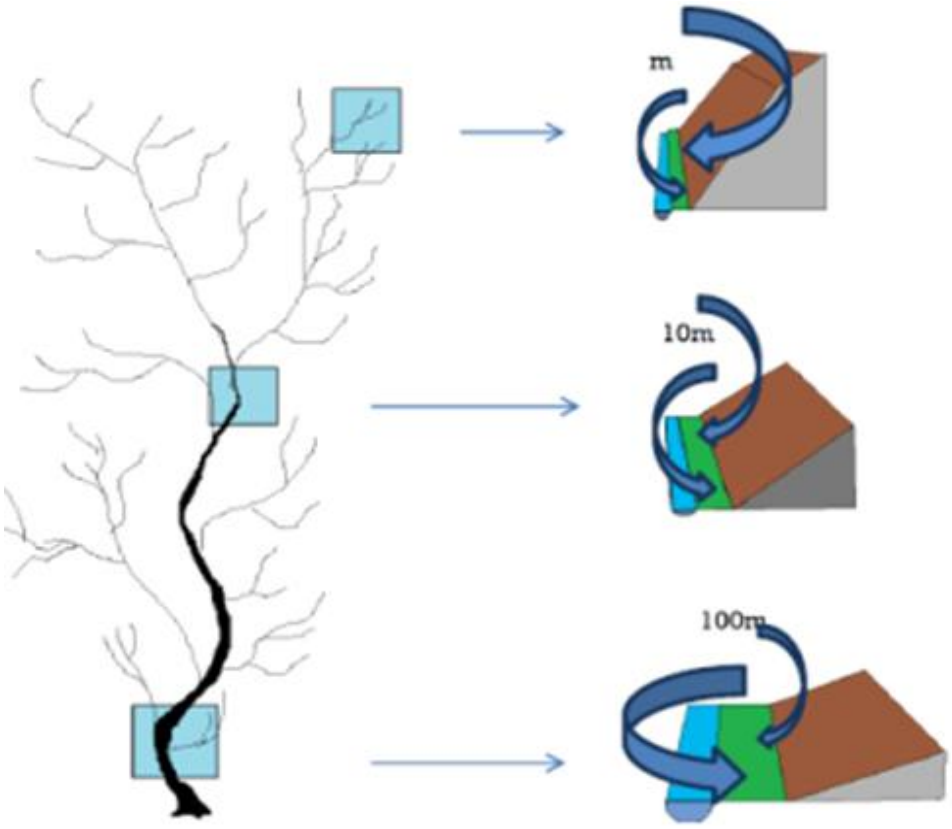

Figure 1-7: *Exchanges of matter and energy down the river continuum. Tabacchi et al. 1998.*

Riverine outflows provide subsidies of nutrients and DOM to coastal marine environments. Due to global warming these subsidies are increasing from sub-arctic watersheds as the stores of these substances become sources, released by receding ice sheets and permafrost (Hood, 2008). GBNP provides an opportunity to quantify the amount of nutrients released from recently deglaciated environments and determine

how this varies over time (0 - 207 years). These data will give an insight into how other subarctic regions will develop in a warming global environment.

1.5 Thesis aims and objectives

This thesis aims to demonstrate the development of soil physico-chemical properties and microbial cycling in riparian and wider catchment soils in relation to geomorphology, vegetation and age. Through the assessment of the important driving factors behind such processes it aims to scale these up to the watershed using vegetation cover data provided by satellite imagery. The influences that this development has on surface and hyporheic water chemistry will also be investigated.

Specifically the objectives of this thesis are to:

- Quantify the change over time of riparian sediment properties, such as physical characteristics, nutrient retention and microbial population size and activity.
- Determine the time frame for riparian sediment development from abiotic to biotic using space for time comparison.
- Quantify watershed soil nutrient content (N and C) and microbial community size and activity across GBNP chronosequence.
- Determine the driving factors behind any differences at these sites (e.g. age, slope and forest cover).
- Use satellite imagery data to scale up these processes for entire watersheds for present (2009) and past environments (22 years) and calculate the rate of change for these watersheds over this time frame.
- Assess the change in surface water chemistry of six streams of different ages in GBNP.
- Determine the effect of watershed and sub-catchment vegetation cover on surface and hyporheic water chemistry.

1.6 Thesis structure

Chapter 1: Introduction. This chapter provides a brief contextual background on the importance of landform, biotic and geomorphic processes on primary succession in a post glacial environments and GBNP. This is then followed by a summary of the important microbial cycling processes that are the focus of this thesis.

Chapter 2: Methodology. This provides a detailed overview of sample sites and field work design as well as field and laboratory methods used for the estimation of microbial N cycling and soil properties.

Chapter 3: Soil and microbial development in the riparian zone of watersheds of different ages. This chapter details the investigation of nitrogen cycling rates and storage in riparian zones over a chronosequence of recently deglaciated river catchments. A combination of field observations and laboratory experiments were used to assess these characteristics across the separate ecotones.

Laboratory net and potential assays were used to assess the functional structure of the microbial communities at these sites. These assays provide a powerful tool to quantify and compare the functional structure of microbial communities between sites. These data are then compared across the chronosequence utilizing space for time substitution, providing valuable data on N cycling development in these systems.

Chapter 4: Catchment scale processes, the influence of slope, vegetation and age. This chapter assesses the use of Landsat imagery to scale up soil nutrient retention and microbial processes to the catchment scale. Sample sites were selected away from riverine influences within sample stream catchments amid dominant vegetation types and across different topographies. This provides an indication of average microbial transformation rates and soil N and C contents for a given age, vegetation type and slope, and couples these data with GIS data provided by Klaar *et al.* (in revision).

Using these data hydro-system N saturation, N and C storage, microbial transformation rates and functional microbial community structure is scaled up to the catchment scale. This enables an evaluation of the process fluxes in the current year (using 2009 imagery). In addition, it allows a within stream comparison between vegetation covers

of the various catchments (independent of age) and also a cross stream comparison of processes within a particular vegetation cover through time.

Applying this method to the previous 22 years of remote sensing data it allows an interesting opportunity to evaluate the rate of change of these processes over this time period and make comparisons between current data and any overlapping development ages from the back calculated data within the current chronosequence.

Chapter 5: Water chemistry of Glacier Bay. The final data chapter looks at the effect that watershed vegetation cover and age has on surface and hyporheic water chemistry. Surface water samples were taken during the period of highest biological activity (May-September) from each study stream at the mouth and its tributaries and selected sub catchments and analysed for nitrogen (nitrate, ammonium) and DOC.

Chapter 6: Conclusions. This chapter provides a summary of the main conclusions of the data chapters in relation to the aims of this thesis along with suggestions for future research.

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2: Methodology

2.1 Project Area

This study was conducted in Glacier Bay National Park and Preserve (GBNP), southeast Alaska (58°10'- 59°15'N; 135°15'- 138°10'W). GBNP is located approximately 105 kilometres west of the state capital of Juneau. The National Park covers an area of approximately 11,030 km^2, most of which is dominated by a 'Y' shaped tidal fjord over 100km in length and 20km in width (Figure 2-1). A Neoglacial ice sheet once covered the entire area surrounding GBNP, reaching its maximum near the mouth of the current fjord around 1700 AD.

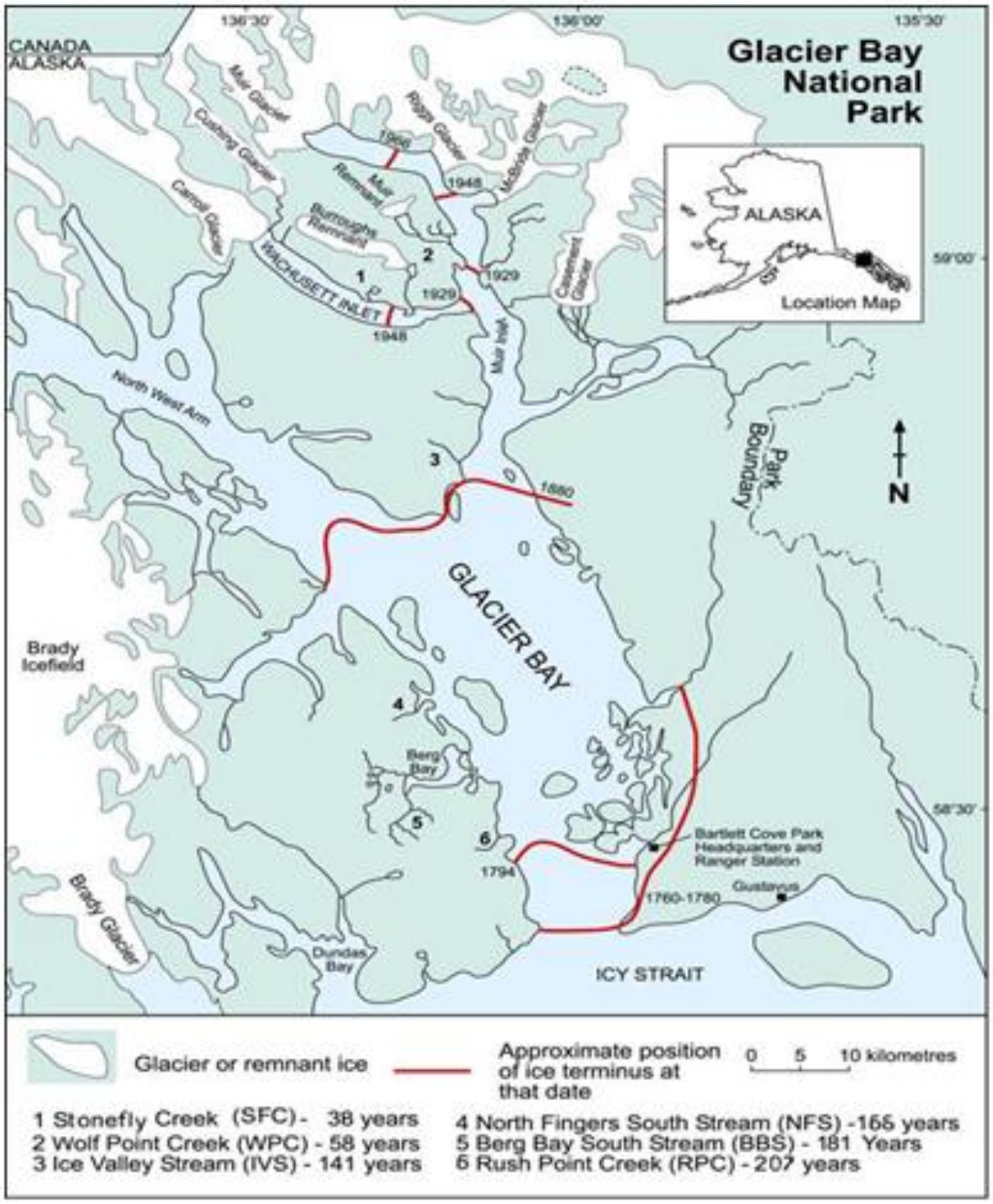

Figure 2-1: *Map of Glacier Bay National Park, Alaska that includes the location of study streams (Milner, 2007).*

Sampling activity was concentrated along 6 streams of various ages within GBNP ranging from the oldest Rush Point Creek (207 years) to the youngest Stone Fly Creek (38 years) (Figure 2-1).

The rapid glacial recession of a neoglacial ice sheet within this area has created an opportunity to monitor stream development and associated biological and physical interactions over time. Detailed historical, geological and dendrochronological data allows the glacial recession within Glacier Bay to be accurately dated whilst the extreme

nature of the disturbance to the area following the glacial recession provides the opportunity to study primary habitat development (Chapin *et al*., 1994; Crocker and Major, 1955; Reiners *et al.,* 1971).

Individual stream age is determined by the distance of a stream from the retreating glacier termini, thus temporal changes in stream biogeochemical interactions can be studied on the basis of spatial differences (Milner *et al.*, 2000). Through the use of space for time substitution the project area spans a temporal scale of 200 years across a spatial scale of 11,000 km^2. All of the six chosen streams are of a similar size (ca. 10 km^2) with geology of glacial moraine, till and outwash deposits (Table 2-1).

The area has a maritime climate that is heavily influenced by ocean currents, resulting in cool summers (10 to 20°C) and relatively mild winters (-2 to5°C) with annual average precipitation of 1778mm, experiencing the warmest and driest months during the sampling period chosen (May-September). The lower bay, where the fjord joins to Icy Strait, is dominated by temperate rain forest; in contrast, the upper bay remains in the early stages of vegetation colonisation and vegetative successional development due to comparatively recent ice recession. These early stages of vegetative development are dominated by *Alnus sinuatta* (Sitka alder) and *Dryas drummondii*, leading into stands of *Populus trichocarpa* (black cottonwood) before the later succession stages of *P. sitchensis* (Sitka spruce) and eventually *Tsuga heterophylla* (Western Hemlock) forests (*as discussed in Chapter 1 section 1.1.2)* (Milner, 2008).

The similarity between climate and catchment size of each study stream allows for a cross stream comparison, that represents a range of stream ages and development present within Glacier Bay.

Table *2-1*: *Summary of the physical characteristics of the 6 study streams. (SFC- Stone Fly Creek, WPC- Wolf Point Creek; IVS- Ice Valley Stream; NFS- North Fingers Stream; BBS- Berg Bay Stream; RPC- Rush Point Creek; QS- Quaternary deposits; Tg- tertiary intrusive (biotite granodiorite); Kg- Cretaceous intrusive (biotite-hornblende granodiorite and tonalite); Sc- Silurian-Devonian sediments and carbonates (Rendu Formation); Ss- Silurian sediments (Tidal Formation)). (Adapted from Roberson and Milner, (2006) and Hill et al, (2009)).*

Study stream		Reach gradient	Stream length	Drainage area	Average discharge	Stream order	Dom. subs.*	Geology
Name	Age	(%)	(km)	(km^2)	(m^3/s)			
SFC	38			10		2	Boulder	Qs[b], Kg
WPC	65	1.14	5.6	29.8	2.29	2	Boulder	Qs, Kg[b]
IVS	141	0.98	8.3	19.4	3.02	2	Cobble	Qs[b], Ss
NFS	166	1.14	8.0	16.8	5.65	2	Boulder	Qs, Sc+Ss[b], Kg
BBS	181	0.8	7.2	33.1	4.95	3	Gravel	Qs, Ss+Ss[b], Tg
RPC	206	0.88	6.6	23.3	7.51	2	Cobble	Qs, Sc+Ss, Tg[b]

[b] *Dominant geology*

Please see Appendix A for GPS locations of each sample site.

* Dominant substrate

2.1.1 **Aerial photographs of sampling sites**

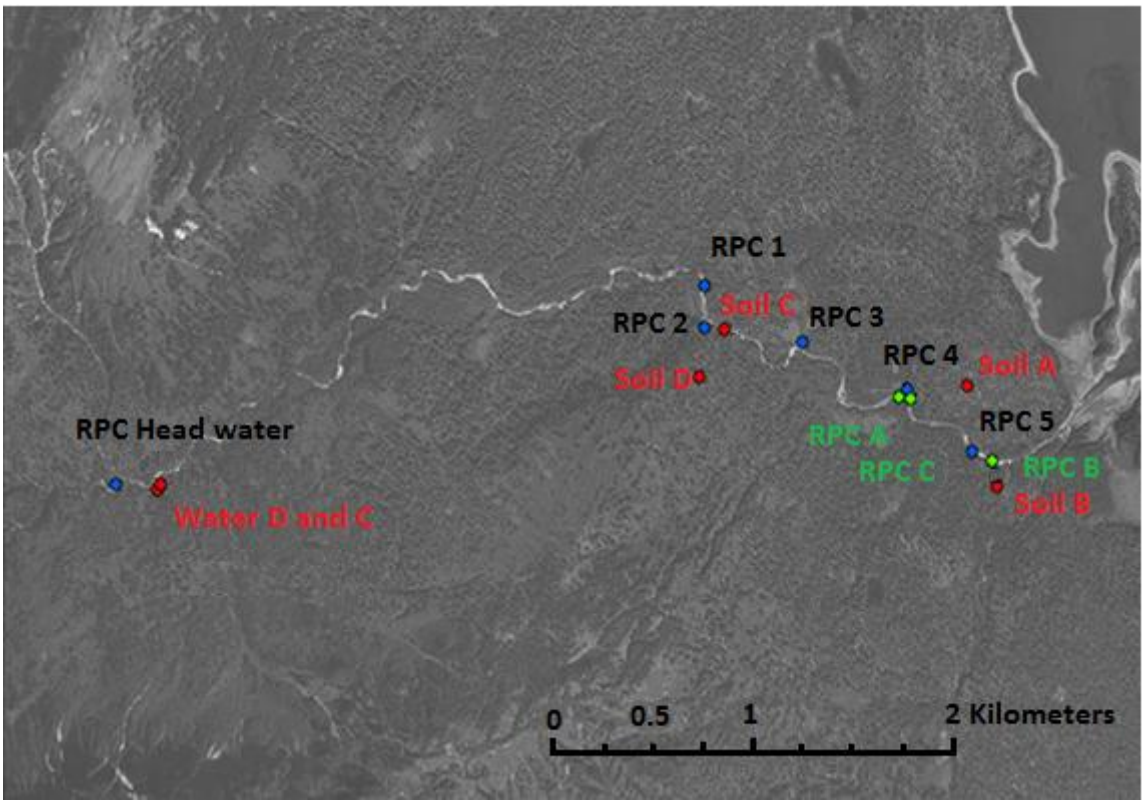

Figure 2-2: *Aerial photo of Rush Point Creek (RPC). Dots represent sampling sites; blue: water; green: riparian; red: wider catchment soil and water.*

Figure 2-3: *RPC Left: Soil sites C and right A, stream bank, floodplain and terrace locations.*

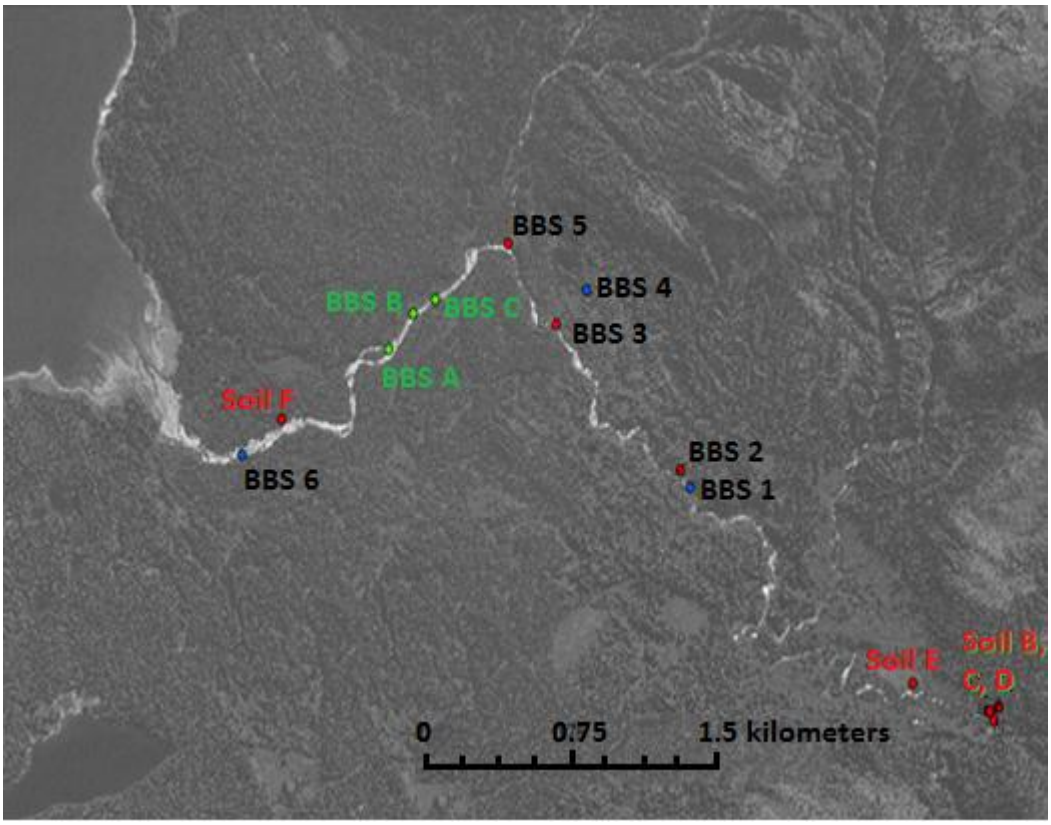

Figure 2-4: *Aerial photograph of Burg Bay Stream BBS. Dots represent sampling sites; blue: water; green: riparian; red: wider catchment soil and water.*

Figure 2-5: *BBS left: Floodplain (A. sinuatta) and forest (P. sitchensis). Right: river with near stream, floodplain and forested areas, large woody debris (LWD) in stream channel.*

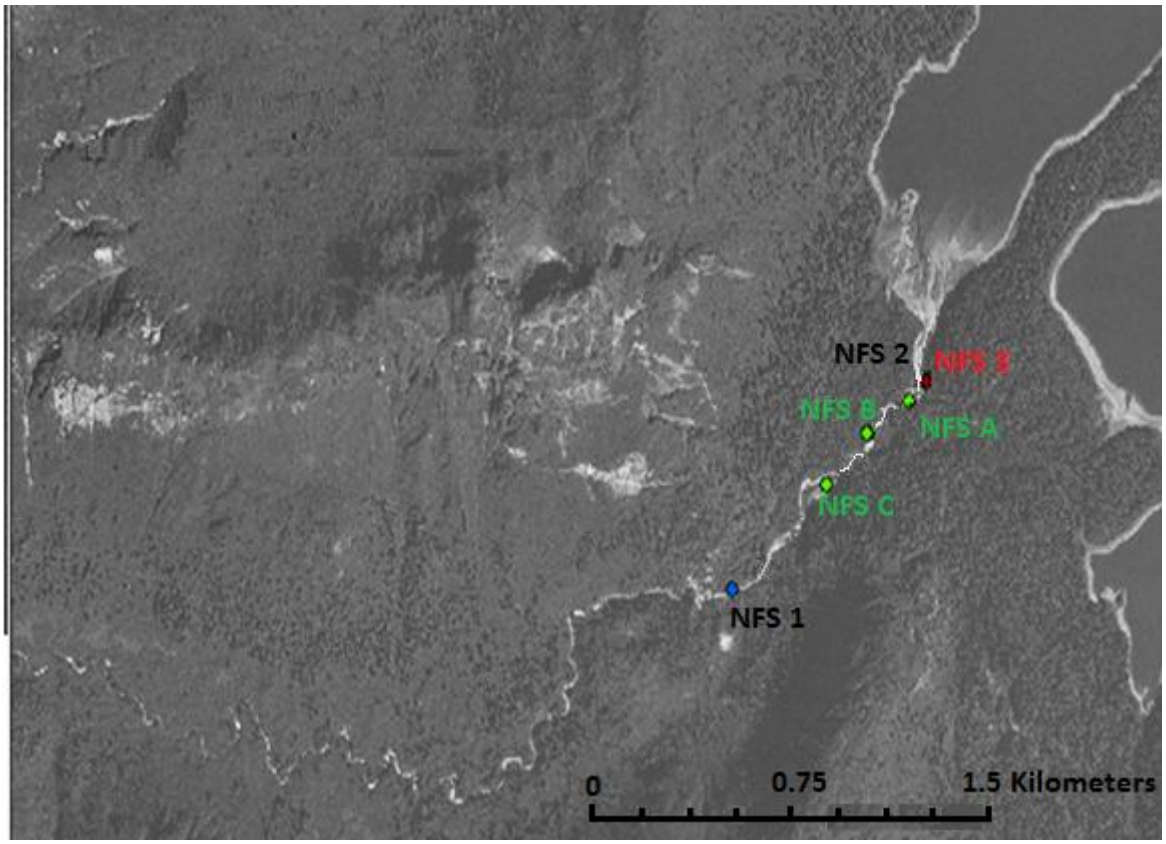

Figure 2-6: *Aerial photograph of North Fingers Stream (NFS). Dots represent sampling sites; blue: water; green: riparian; red: wider catchment soil and water.*

Figure 2-7: *NFS left: Soil site A; foreground river bank and floodplain, background P. sitchensis forest. Right: Lower river.*

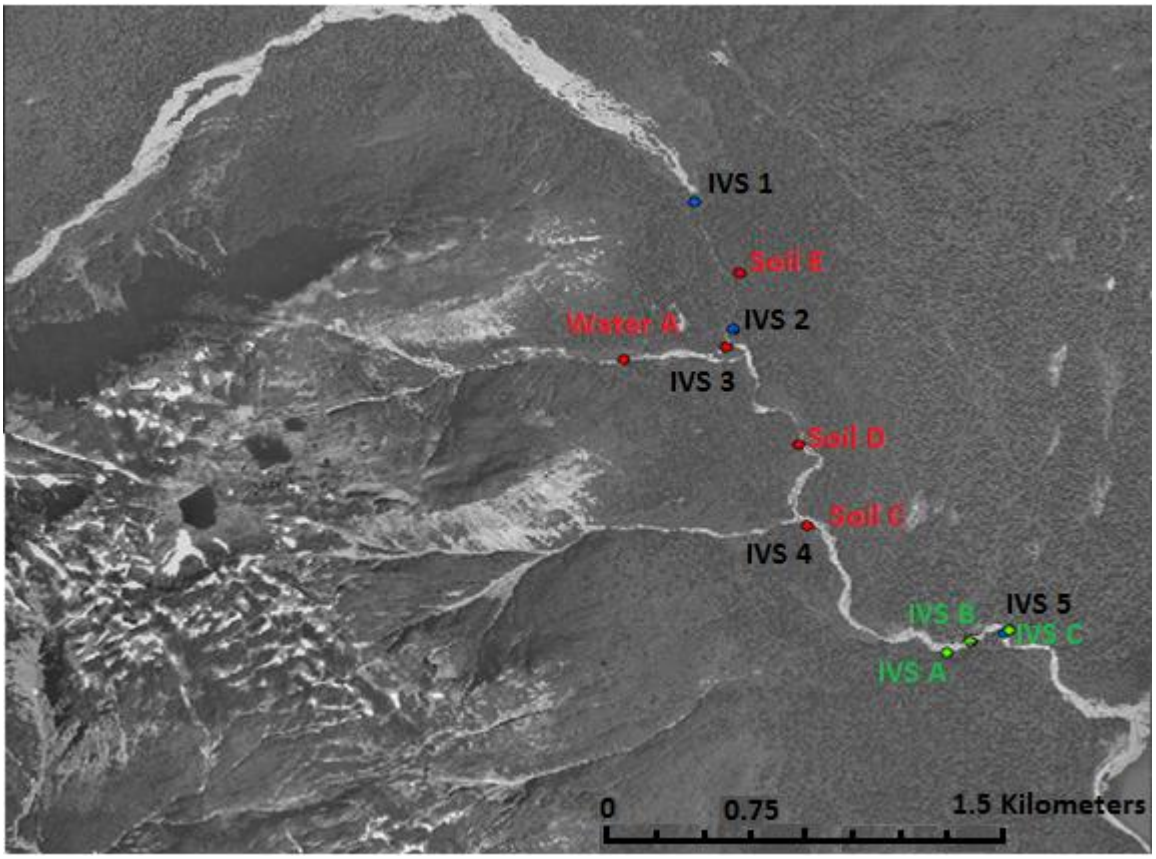

Figure 2-8: *Aerial photograph of Ice Valley Stream (IVS). Dots represent sampling sites; blue: water; green: riparian; red: wider catchment soil and water.*

Figure 2-9: *Left: IVS lower soil and water sampling site, P. sitchensis dominant forest in background. Right: Tributary stream (stream a) and riparian vegetation, source waterfall in background.*

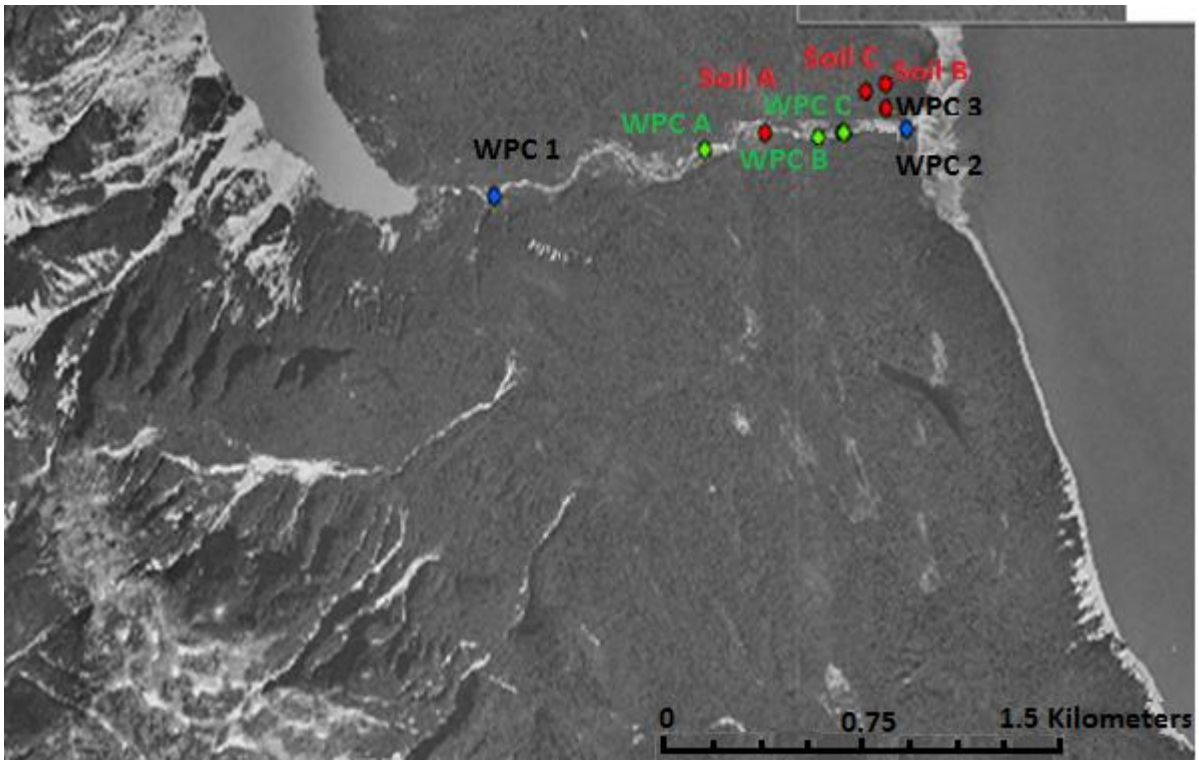

Figure 2-10*: Aerial photograph of Wolf Point Creek (WPC). Dots represent sampling sites; blue: water; green: riparian; red: wider catchment soil and water.*

Figure 2-11*: Confluence of stream and tributary, near stream and floodplain emergent vegetation, background P. trichocarpa and P. sitchensis forest.*

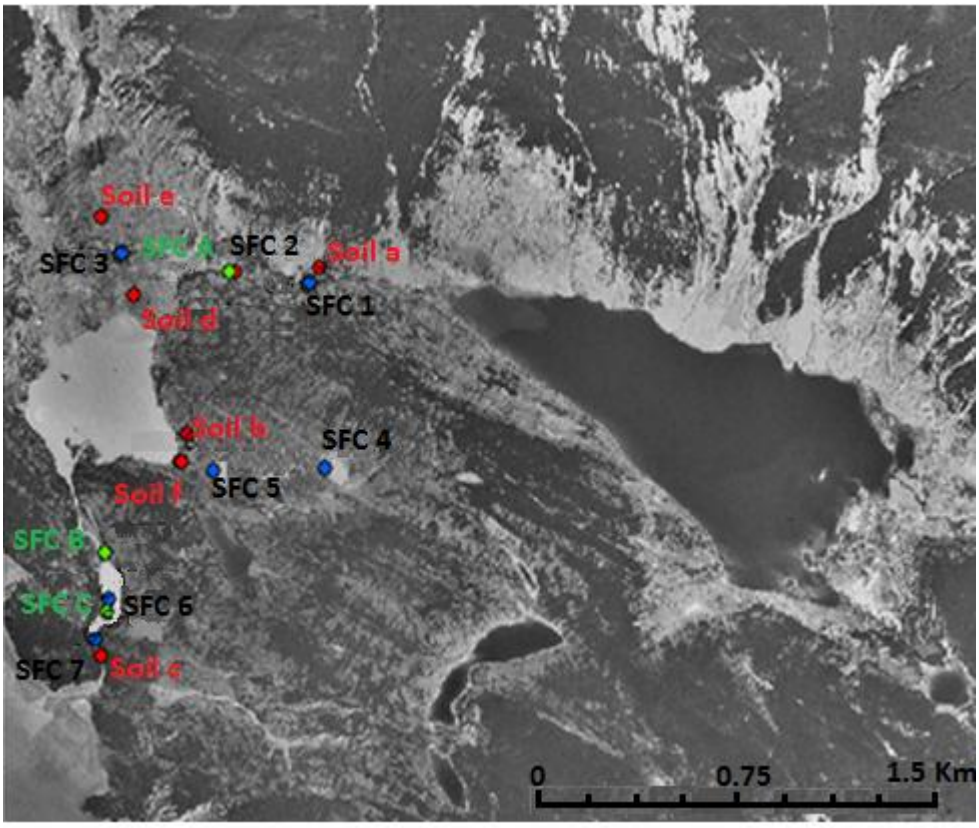

Figure 2-12: *Aerial photograph of Stone Fly Creek (SFC). Dots represent sampling sites; blue: water; green: riparian; red: wider catchment soil and water.*

Figure 2-13: *SFC, left: SFC- B; Fore ground stream bank and floodplain, background A. sinuatta thicket, Right: lower stream channel sample site.*

2.2 Sampling strategy

One of the study objectives was to investigate riparian soil development over the GBNP chronosequence. This study defines the riparian zone functionally as the area of direct interaction between the aquatic and terrestrial environment (figure 2-15). This includes the stream bed, banks and floodplain that may be submerged only part of the year, this interaction extends outwards and upwards from the stream to the overhanging vegetation, ending at the hill slope terrace (Swanson, 1982). In particular the aim was to compare near stream versus floodplain areas over different ages. Floodplains were defined as an area of low lying ground adjacent to the river channel, formed mainly of river deposited sediments and prone to flooding ending at the upland terrace (Naiman, 2005). Near stream locations were deemed to be the un-vegetated interface areas between the channel and floodplain, equivalent to bank full discharge (figure 2-15).

Along each river three riparian locations were selected, and then three representative sampling areas ($1m^2$) were chosen from within the two defined areas of interest (near stream and floodplain). From these sampling areas composite soil samples were collected (composed of three soil cores taken to ca 10cm depth) in addition to leaf samples. These samples were collected between June – August in 2010.

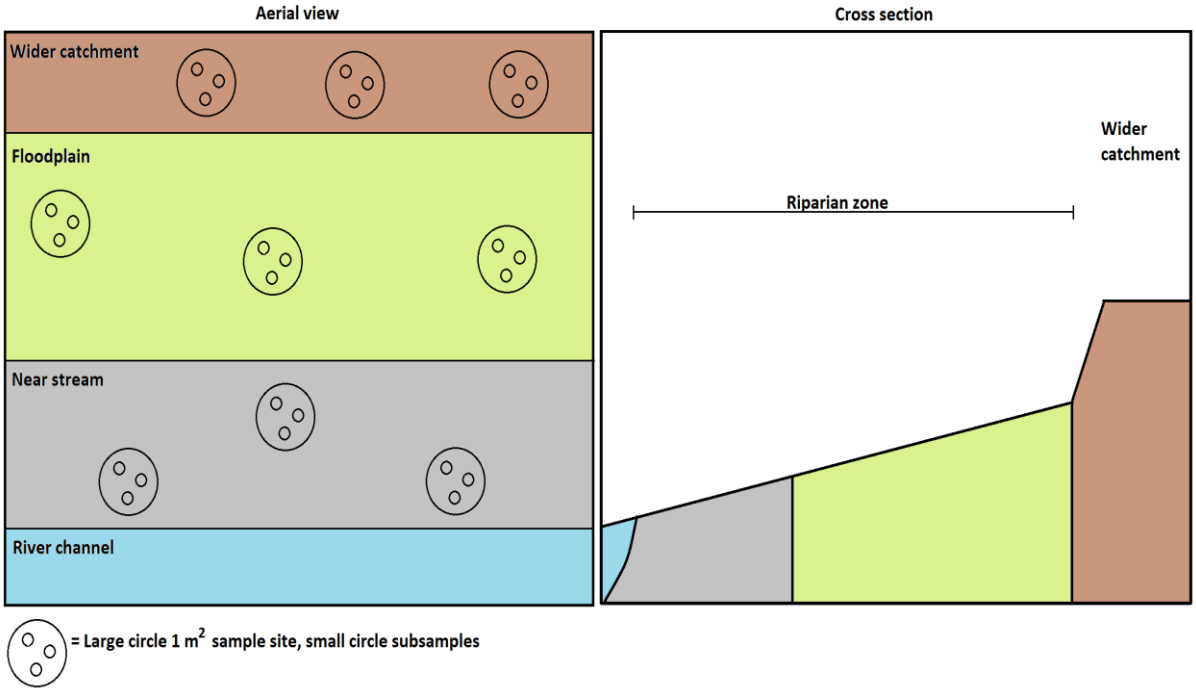

Figure 2-14: *Aerial and cross section view of riparian zone and wider catchment sampling locations.*

In addition to this riparian development another objective was to assess the change in soil physico-chemical properties and microbial activity with primary succession in the wider catchment. These areas were defined as locations beyond the hill slope terrace that marked the edge of riverine influences (Swanson, 1982). The sample collection strategy in these areas was the same as that for the sites within the riparian zone (figure 2-15).

Site selection in the wider catchment was based on LandSat imagery of our study streams which helped identify areas of interest. Analysis of these images indentified the shifting vegetation types over time with primary succession (Table 2-2). Therefore sampling was focused according to the variables of interest, which were dominant vegetation and slope. This enabled the range of available vegetation types and topographies to be sampled across the chronosequence, helping to answer the research question. Samples were collected between June – August in 2011 with some additional samples collected in 2012.

Table 2-2: *Percent watershed land cover (Mature= mixed P. sitchensis and T. heterophylla forest; other = glacier, water, unidentified). Data provided by Klaar et al, submitted.*

Stream	% Sediment	% *D. drummondii*	% *A. sinuatta*	% *P. trichocarpa*	% *P. sitchensis*	% Mature	% Other
SFC	13.2	15.5	41.9	16.8	4.6	2.4	5.1
WPC	18.3	11.3	24.8	23.1	8.0	4.9	9.6
IVS	3.8	17.2	8.13	25.7	31.6	4.0	9.6
NFS	0.6	23.9	13.2	28.2	21.6	5.8	6.7
BBS	0.2	8.26	6.4	24.1	39.9	18.4	2.6
RPC	3.4	10.1	7.9	25.0	43.4	8.1	2.1

A further study objective was to assess the water chemistry of each study watershed to assess if any changes could be linked to the changing watershed vegetation (Table 2-2). Surface water samples were collected from the river mouth and at points further upstream at tributary sub catchments. For this analysis the LandSat data was again used to determine catchment as well as sub catchment vegetation type. Samples were collected as grab samples between June – August over a three year period (2010-2012).

Hyporheic water samples were also taken from the main river channel of each stream between June – August in 2010 in order to determine whether there was a change in chemistry over the stream chronosequence and across a gradient of channel steepness which was determined using a dumpy level and staff.

2.3 Soil Analysis

2.3.1 Soil properties

Soils were transported from the field to the laboratory within 3 days of collection. Upon arrival processing was carried out as soon as possible whilst soil was kept cool in the interim. The following laboratory methods were carried out when possible at the field laboratory station in Glacier Bay National Park, however some analyses required the transport of samples to the University of Birmingham, UK and other laboratories where stated.

Field moist soils were sieved through a coarse-mesh screen (2 mm openings) to remove stones and large pieces of detritus. The resulting coarse fraction was discarded, and the soil well mixed, each composite sample was kept separate from the other replicate samples. The soil samples were analysed for soil bulk density, moisture content and organic matter content (by Loss on Ignition) following the methods described in Pinay (2003) and Templar (2003) respectively.

Soil grain size was determined using a laser Melvern Mastersizer instrument. Subsamples of dried soils (0.1-1g) were weighed out into 50ml centrifuge tubes and amended with 0.1 M NaClO solution for organic matter removal and placed in a water bath at 80°C (Anderson, 1963). The sodium hypochlorite method was preferred to the hydrogen peroxide (H_2O_2) method as this has been linked to the degradation of clay minerals (Douglas and Fiessinger, 1971). These were then centrifuged for 5 minutes at 3646G using a MSE Mistral 1000, after which the organic matter was decanted away. This procedure was repeated until the liquid after centrifuging was clear, and determined to free of organic matter.

After removal of organic matter, samples were amended with 50ml dispersion solution, then decanted into 600ml of dispersion solution already circulating within the laser Melvern mastersizer taking care to make sure that no sediment remains in tube. The instrument was set at 2000 pump speed with 15- 25 optical obscuration range; the sample was exposed to a 1 minute ultra-sonic burst of 10.50 Hz for greater dispersal. If the laser obscuration measurements appeared stable then grain size measurement was started, passing wet sample through a laser to determine grain size distribution according to the Mie scattering measurement principle.

2.3.2 **Isotopic analysis**

Soil total nitrogen (TN) and total carbon (TC) were measured using an elemental analyzer (Vario PYRO cube, Elementar, Hanau, Germay) coupled to an IRMS (Isoprime, micromass, Manchester, UK). Firstly the sample was burned at 1120 ° C in a combustion furnace. The gases formed by this combustion (CO, CO_2, N_2O, NOx) were then passed through a reduction furnace (850°C), here the nitrogen forms are reduced to N_2. Produced gases were then passed through molecular sieves, which allowed for the separation of the various gases. After this an elemental analyzer measured C and N content, and then an IRMS measured the isotopic ratios of the gases produced during sample combustion. Precision was 0.2 ‰ for ^{13}C and 0.3 ‰ for ^{15}N.

Natural abundance isotopic ratios are a useful to for the study of microbial processes. Isotopes are atoms with an equal number of protons and electrons but different number of neutrons. Isotopic fractionation is the enrichment of one isotope in favour of another in a physical or chemical reaction. For a given chemical reaction these isotopes have slightly different reaction equilibrium constants (k), which results in reaction products having a different isotopic ratio to the source. This difference is expressed as the fractionation factor alpha (α):

$$\alpha = k\,N_h / k\,N_l \qquad (2.1)$$

Where N_h and N_l are the heavy and light isotopes.

Thus, the isotopic enrichment factor (ε) for the reaction can be deduced by:

$$\varepsilon = 1000^{*} (\alpha - 1) \quad (2.2)$$

Nitrogen has two stable isotopes in the biosphere, the more abundant ^{14}N, and the more scarce ^{15}N (Table 2-3). The ratios between these two isotopes vary as a result of fractionation in physical, chemical and biological processes (Högberg, 1997).

Microorganisms utilizing N for metabolic reactions will preferentially use the lighter ^{14}N isotope, and this is referred to as the fractionation factor (‰). All of those microbial processes shown in Figure 2-15 fractionate in favour of the lighter ^{14}N and ^{18}O to a certain extent (Baggs, 2008). In soil systems micro-organisms are efficient at assimilating and metabolising the lighter isotopes, leading to an enriched $^{14}N/^{15}N$ ratio in the remaining soil N pool. As a result the N isotopic signature can be used to differentiate between these different processes (Högberg, 1997; Evans, 2007). The isotopic ratio of N containing molecules, and is a powerful tool for the exploration of nitrogen dynamics within the environment (Hobbie, 2000).

The degree of isotopic fractionation of a substance is expressed by its $\delta^{15}N$ value, which is determined by the following equation:

$$\delta_{\text{sample}} (‰) = \left(\left(R_{\text{sample}} - R_{\text{standard}} \right) / R_{\text{standard}} \right) \times 1000 \quad (2.3)$$

Where R is $^{15}N/^{14}N$ of a sample and a reference material, respectively.

The $\delta^{15}N$ of soil is affected by the interconnected factors of climate, time, cultivation, topography and parent material (Admunson *et al.*, 2003; Evans, 2007). Therefore, the level of fraction ($\delta^{15}N$) of these isotopes in the soil sediment, leaf biomass, gas emissions, and dissolved compounds from an area can be used as a tracer or integrator of microbial activities (Robinson, 2001).

Carbon has two stable isotopic species ^{13}C and ^{12}C (Table 2-3), contained in the atmosphere as bio-available CO_2. Plants are depleted in ^{13}C compared to the atmospheric standard concentration due to fractionation factors associated with photosynthesis. This is caused by the diffusion of CO_2 from the atmosphere into the

stomata airspace which has an apparent fractionation ($\Delta\delta$) of ~4.4% due to the slower motion of the heavier ^{13}C containing molecules. Additionally the photosynthetic enzymes used to acquire C discriminate further depending on the photosynthetic pathway (C3, C4 or Crassulacean Acid metabolism (CAM)). The total $\delta^{13}C$ discrimination factors for all pathways are: for C3 median: -27 ‰, C4 median: -14 ‰, CAM: -11‰ (Evans, 2007).

Oxygen's stable isotopes are ^{16}O and ^{18}O (Table 2-3), the main source of isotopic ratio signature for O from the source water. Therefore the signature of the source water, such as precipitation input, will influence the $\delta^{18}O$ of the sample plant tissue, or system outputs and emission (e.g. NO_3 and N_2O) (Evans, 2007).

Table 2-3: *Element isotopes, their abundances and ratios.*

Element	Isotope	Abundance (%)	Difference in relative atomic mass (%)*	Reference	Isotopic ratio
Hydrogen	^{1}H	99.985	100	Vienna Standard Mean Ocean Water (VSMOW)	$^{2}H/^{1}H=0.00015575$
	^{2}H	0.0155			
Carbon	^{12}C	98.892	8.3	Vienna Pee Dee Belemnite (VPDB)	$^{13}C/^{12}C=0.0112372$
	^{13}C	1.108			
Nitrogen	^{14}N	99.635	7.1	Atmospheric nitrogen	$^{15}N/^{14}N=0.003677$
	^{15}N	0.365			
Oxygen	^{16}O	99.759	12.5 ($^{18}O/^{16}O$)	VSMOW in water	$^{18}O/^{16}O=0.002052$
	^{17}O	0.037			
	^{18}O	0.204			

* Difference in relative atomic mass = ΔM/M.

2.3.3 **Nitrogen transformations and fractionation**

The multiple microbial N transformation processes (Figure 2-15) each have their own N isotope fractionation signature (Table 2-3). N mineralisation shows no significant N isotope fractionation of the N compounds used or released by the microbe (Table 2-3). Generally studies have found fractionation factors of ±1 ‰ (Högberg, 1997).

Nitrification as has been discussed is a two-step reaction; therefore the associated isotopic fractionation of this reaction is dependent on its limiting step. The oxidation of NO_2^- to NO_3^- is fast so the slower oxidation of NH_4 to NO_2 determines the isotopic fractionation, which varies between -38 to -14‰ depending on the study (Casciotti, 2002; Mariotti, 1982; Sebilo *et al.*, 2006). In addition, laboratory studies have shown that during the conversion of NH_4^+ to NO_2^-, 2 oxygen atoms come from water and one from atmospheric O_2.

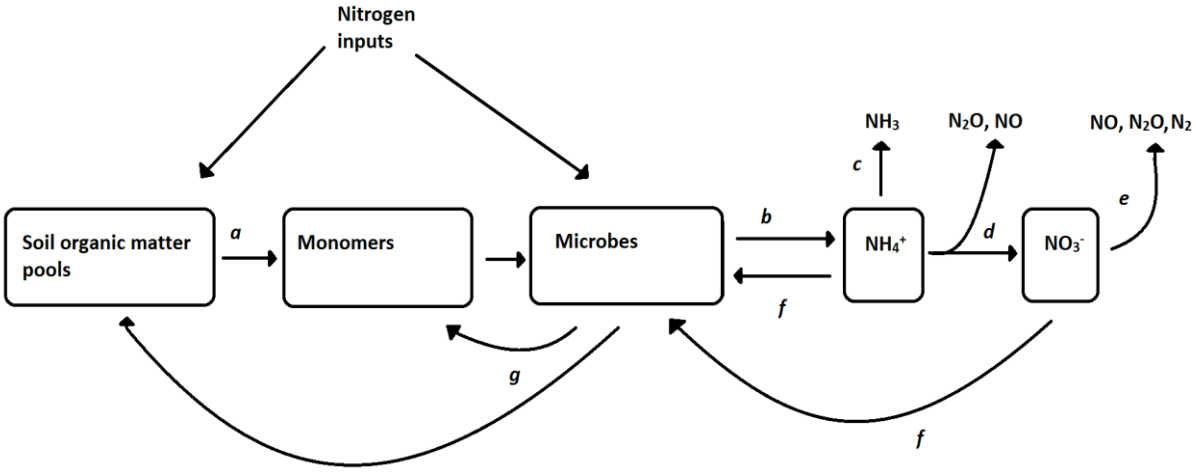

Figure 2-15: *Soil nitrogen transformations based on the conceptual model of Schimel & Bennet (2004). Nitrogen inputs can enter into the organic and inorganic pools. The transformations are: a, depolymerisation; b, gross mineralisation; c, volatilisation; d, nitrification; e, denitrification; f, microbial immobilisation; g, death of soil microbes. (Adapted from Evans, 2007).*

Table 2-4: *Observed discriminations for transformations in the nitrogen cycle. Process refers to the transformations indicated by letter in Figure 10. Values are taken from reviews by Hogberg (1997) and Robinson (2001), and Evans (2007).*

Transformation	Process	Discrimination (‰)
Gross mineralisation	*B*	0-5
Nitrification	*D*	0-35
$NH_4^+ \leftrightarrow NH_3$ equilibrium		20-27
Volatilisation	*C*	29
N_2O and NO production during nitrification	*D*	0-70
N_2O and N_2 production during denitrification	*E*	0-39
NO_3^- immobilisation	*F*	13
NH_4^+ immobilisation	*F*	14-20

Isotopic fractionation during the incorporation of oxygen from the water is negligible. Therefore, the $\delta^{18}O$ of NO_3^- derived from nitrification is defined by:

$$\delta^{18}O - NO_3^- = 2/3(\delta^{18}O - H_2O) + 1/3(\delta^{18}O - O_2) \quad (2.4)$$

The isotopic composition of air ($\delta^{18}O$-O_2) is 23.5 ± 0.3% (Kroopnick & Craig, 1972). Soil water is predominantly composed of rainfall whose isotopic composition will vary enormously in time and according to its geographical location. Denitrification has been shown to have a highly variable fractionation value (0-39‰) dependant on numerous influences such as variations in concentrations of electron donors and acceptors, temperature, and soil grain size (Table 2-4) (Hogberg, 1997).

2.3.4 ^{15}N and ^{13}C in leaves and soil

Applying isotopic ratios of $\delta^{15}N$ and $\delta^{13}C$ within the vegetation and substrate of an area can also be used as an integrator of microbial activity. These techniques are useful for tracing the N saturation and turnover time which can be influenced by mycorrhizal fungi, age of site and plant species with different sources of N.

Early successional species fix N_2 from atmosphere, over time species composition changes and $\delta\ ^{15}N$ of soils and non N-fixing plant leaves change to reflect the change in microbial processes and inputs. Comparison of the natural abundances of soil and leaf ^{15}N data can show rates of N mineralisation and nitrification as well as N pool saturation (Hobbie, 1998, 2000; M. N. Högberg, 2006; P. Högberg, 1997; Pardo, 2006).

Numerous studies lend support to the use of natural abundance measurements of stable N isotopes in plants or calculated ^{15}N enrichment factors as indicators of ecosystem N cycling and/or site N status (Emmett, 1998; Hogberg, 1990; Koba, 1997, 2000, 2003; Vervaet,, 2002). The N status of a forest determined by the C:N ratio has multiple influences upon the microbial activity of the area, and therefore on the isotopic abundance of ^{15}N within the substrate and foliage (Pardo *et al.*, 2006). With increasing N saturation and when the C:N ratio declines to below 23, bacterial N cycling processes, such as mineralisation and nitrification, will be stimulated causing changes to isotopic signatures (Pardo *et al.*, 2006).

Greater power can be given to such analysis when N is used in conjunction with other isotopic values such as $\delta^{13}C$ and $\delta^{18}O$ helping improve the interpretation of these data (Hobbie, 2000). Natural abundance of N and C isotopic ratios can be used as indicators of forest N status and C dynamics. Soil N richness and C sequestration have been shown to be closely linked, with increased soil N leading to increased soil C. The N status of a forest system can be determined by assessing the N and C dynamics of its soil and leaves. One means by which to estimate this N status is to calculatethe enrichment factor (*EF*) between $\delta^{15}N$ of the product (leaf) and substrate (soil):

$$EF = \delta^{15}N_{leaf} - \delta^{15}N_{soil} \quad (2.5)$$

In a more saturated N environments there will be a greater fractionation in the substrate leading to an increasingly positive enrichment factor (e.g. *EF* approaching zero or above). Furthermore, N enriched sites can be differentiated from N poor sites by a lower C:N ratio, higher potential net nitrification, and greater partitioning of C to the silt and clay fraction than N poor forests (located on ridges and on slopes) (Evans, 2007). The soil ^{13}C abundance can be used to determine C dynamics of forest ecosystems. This can be used to assess litter quality and SOM decomposition rates (Evans, 2007).

2.3.5 **Inorganic N analysis**

Soil NH_4^+, NO_2^- and NO_3^- were extracted using 80ml 2M KCl on a continuous wheel shaker (figure 2-16) for 1 h in 100ml HDPE bottles. Extracts were then filtered (0.2µm Millipore Nylon filter paper) and transported back to University of Birmingham, UK for colourimetric analysis using a spectrophotometer (Jenway 6800 UV/Vis). NH_4^+ was determined using the buffered hypochlorite method (Nelson, 1983) and NO_3^- using cadmium column reduction to NO_2^- coupled with the Griess reagent (Griess diazotization reaction), sample NO_2^- was also determined using this method. For all colourimetric analysis accuracy was always equal to or better than 90%, and precision was better than 1 %.

Figure 2-16: *Mechanical wheel shaker.*

2.3.6 **Potential ammonia oxidation and nitrite oxidation**

Measurements of potential rates of ammonia oxidation and nitrite oxidation were performed in order to provide insight into the integrated responses of these microbial activities to primary succession. Potential ammonia oxidation can be viewed as a proxy for the concentration of ammonia oxidising enzymes present in the soil sample. This is based on the principle that ammonia oxidation rate is proportional to ammonia-oxidizing enzymes concentration when substrates are made non-limiting for ammonia-oxidizers, when environmental conditions are made optimal for ammonia oxidation and when *de novo* synthesis occurred (Niboyet *et al*, 2010, 2011; Wertz *et al*, 2007).

Similarly potential nitrite oxidation is a measure of the concentration of soil nitrite-oxidizing enzymes (Wertz *et al*, 2007). The concentration of these enzymes in the soil reflect the *in situ* environmental constraints to which these microorganisms were exposed prior to soil sampling (Pinay *et al*, 2007; Wertz *et al,* 2007; Niboyet *et al*, 2010, 2011). Thus, potential ammonia and nitrite oxidation are thought to respond to environmental drivers on the time scale of weeks to months (Pinay *et al*, 2007; Wertz *et al,* 2007), since ammonia and nitrite-oxidizers have very slow growth rates (Poly *et al*, 2008). Rates of potential nitrification and denitrification are thought to be more

constant over time than *in situ* rates (Niboyet, 2010; McGill, 2010), and have been widely used to assess the impacts of environmental change on the size of nitrifying microbial communities (Niboyet, 2011).

Potential ammonia oxidation was determined as the reduction in ammonia concentration and increase in nitrite concentration over the experiment, as $NaClO_3$ inhibits the step wise nitrification process at this intermediate stage (Belser and Mays, 1980). Extracts were stored kept cool before analysis at the University of Birmingham. Soil slurries were made using 10 g soil (equivalent dry weight) and 25ml of 0.36 mM $(NH_4)_2SO_4$ solution containing 0.01M $NaClO_3$, in a 100ml glass jar. These were agitated on a mechanical shaker at 150 rpm for 9 hours for mixing and oxidation purposes. Samples were then extracted with using 80ml 2M KCl and stored for colourimetric analysis.

Potential nitrite oxidation rate was determined using the change in nitrite concentration over the experimental period (Niboyet et al., 2011). Soil slurries where made using 10 g (equivalent dry weight) of soil and 15ml DIW solution (0.1g $NaNO_2$/L). The slurry was then constantly shaken on an orbital mechanical shaker for 30 hours in order to maintain aerobic conditions for nitrification to occur. Solutions were then extracted using 80ml 2M KCl. After which the solution was filtered (Millipore Nylon filter paper, 0.2µm), and the extract stored until colourimetric analysis.

2.3.7 **Potential Denitrification Enzyme Activity (DEA)**

Denitrification enzyme activity was measured in the laboratory using soil slurries of two subsamples from each site sample to form two experimental groups. Samples were incubated anaerobically in 100 ml gastight jars for 4 hours in the dark at room temperature. Samples were made anoxic by alternatively vacuuming with an electric pump and flushing with oxygen free helium (He) for 15 minutes under shaking conditions (figure 2-17).

Both treatments were augmented with a solution containing C and N (10ml DI Water + 10µg NO_3-N^{-1} + 4mg Cg^{-1} (KNO_3^-)) to form a slurry suspension (Pinay *et al*., 2003). One of these groups was also treated with Acetone-free acetylene to bring the samples

atmosphere concentration to 10kPa (10% V/V) acetylene and 90 kPa He, in the other treatment group acetylene was not added (Pinay *et al.*, 2003; Robertson & Tiedje, 1987). Solutions were manually shaken every 15 minutes to ensure full mixing of soil bacteria and solution, and between the slurry and the overlaying atmosphere. Prior to sampling to ensure full sample mixing, a clean 10ml syringe used to mix the air within the septum jar by pumping the syringe 5 times. Then using a clean 10ml syringe air samples were taken at 1 and 4 hours and stored in pre evacuated 1.7ml exertainer vials until analysis. The N_2O analysis was performed on a Perkin© Elmer© Clarus 500 Gas Chromatograph (GC) with manual injection at the University of Birmingham, UK. A sub sample (2012 samples) were then analysed at the OZUR institute in Rennes, France. The accuracy and precision was better than 2%.

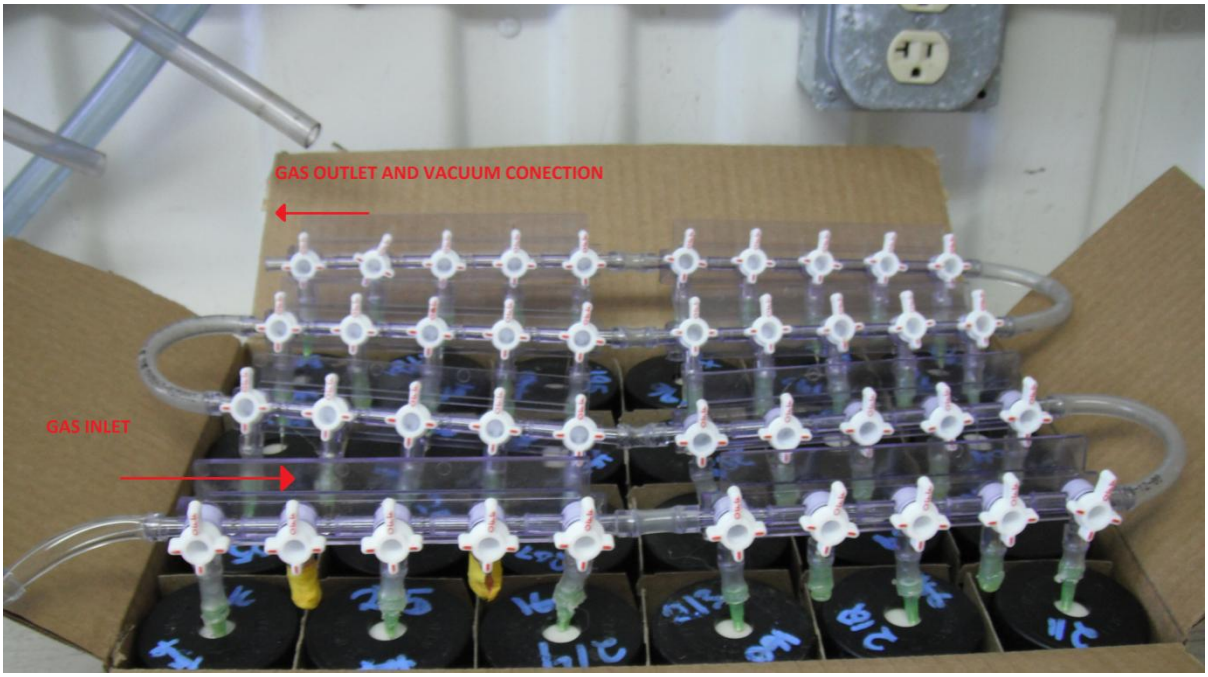

Figure 2-17: *DEA septum jars and gas manifold system.*

The equation used to determine the N_2O (mg/m^3) concentration for DEA samples was:

$$N_2O \text{ mg m}^3 = \frac{\left(\left(\frac{\text{ppm}}{10^6}\right) \times 44\right)}{\left(24 \times (10^{-3})\right)} \times 10^3 \quad (2.7)$$

Where ppm = parts per million, 44= molecular weight of N_2O, 22 = Volume of air occupied by 1 mole of gas at 20°C and 1 atmosphere pressure.

Then N_2O (ng),

$$N_2O\ ng = \left((Conc \times head\ space) + (Conc \times 0.63 \times water\ volume)\right) \times 10^6 \quad (2.8)$$

Where Conc. = N_2O (mg/m^3) concentration, 0.63 = Bunsen coefficient.

Flux (Q) over experiment period was calculated as,

$$Q = (T1 - T2) \div (4 - 1) \quad (2.9)$$

Where T = N_2O (ng) at sampling time 1 (after 1 hour) and 2 (after 4 hours).

Daily rates of N_2O (ng d^{-1} g^{-1}) emission where calculated thus,

$$N_2O\ (ng\ d^{-1}g^{-1}) = \frac{(Q \times 24)}{soil\ dry\ weight\ (g)} \quad (2.10)$$

This procedure provides a potential rate of denitrification activity under non-limiting factors (Smith and Tiedje, 1979), which can be used as an index of denitrifying population density. Although absolute rates are not very informative per se, the relative rates within soil profiles and among sites are useful for comparing denitrifying population abundance (Pinay *et al*, 2003).

Problems associated with denitrification analysis using the acetylene block technique have however been highlighted. Groffman *et al* (2006) provides an excellent review of these problems which include 1) slow diffusion of C_2H_2 into fine textured sediments, 2) enhanced soil respiration, 3) rapid decomposition of C_2H_2 by soil microorganisms, 4) contamination of C_2H_2 with other gases (Groffman *et* al, 2006). Other major concerns have also been raised are that this technique can inhibit nitrification causing soil cores to become nitrate limited especially in systems with limited nitrate pools, and also that C_2H_2 is a incomplete inhibitor of N_2O production (Groffman *et* al, 2006). Such issues raise concerns that these experiments are giving unreliable results about denitrification activity and related greenhouse gas emissions in the terrestrial environment.

However such concerns are counter balanced when considering the usefulness of these techniques as they are simple to carry out allowing a large number of samples to be run,

which is advantageous due to the high spatial and temporal variation of microbial activities and the difficulties associated with remote field work sample collection. Moreover in relation to this study, DEA assays measure denitrification potential, which is still a valid use of acetylene applications to compare terrestrial sites, to evaluate controlling factors and as an indirect measure of functional microbial community (Groffman *et al*, 2006).

2.3.8 **Net mineralization and nitrification incubations.**

Net N mineralisation and nitrification were measured using the aerobic incubation method described by Hart *et* al (1994). Subsamples of field moist soils equivalent to 10g dry-soil were placed into plastic cups. These samples were then incubated for 28 days to determine the net change in inorganic N form. Containers were covered with perforated polythene film to minimise water loss yet maintain gas exchange. The soil system + container was then weighed prior to and after each week of incubation to monitor water loss. If water loss was high (>5 % relative change in water content), deionised water was added to return the soil to its initial water content. The soil was not mixed during this process to minimise disturbance which can increase the rate of N transformation processes (Hart *et al*, 1994).

Soil samples were incubated in the dark at room temperature at the field laboratory. After the incubation period the soil was extracted using the KCl method described previously. Net N mineralisation was calculated by subtracting the initial quantity of soil inorganic N from the post-incubation quantity. Similarly, net nitrification was calculated as the change in nitrate pool size over the incubation period.

2.4 Surface and hyporheic water sampling

Surface water discharge measurements were taken using an electromagnetic flow meter (Aqua RC2), discharge (Q) was calculated according to Manning's equation:

$$Q\ (m^3\ s) = A\ (m^2) \cdot v\ (m\ s) \qquad (2.11)$$

Where A = area (m^2), v = velocity.

Level trolls (Insitu 300) were installed at the outlet of each study streams to measure discharge throughout the year and calibrated with manually taken discharges whenever possible over a range of flows.

Hyporheic samples were collected using a PVC pipe (1.5 m x 10cm I.D.) with a perforated base (bottom 5 cm with a 1 cm lip to minimise breakage) which was hammered into the river bed using a sledge hammer and a pointed steel driver. Piezometers were driven to a depth of ca. 10 cm and then pumped using a hand pump and modified Nalgene flask to remove the disturbed water present and to make sure that surface water was removed. Piezometers were then left for 40 minutes to settle and then sampled using the hand pump and Nalgene© flask.

Surface water temperature (°C) and conductivity (µS) were measured using a Hanna Hi 933100 conductivity meter and probe, and pH using a Hanna Hi 8424 N pH meter. Surface and hyporheic water samples for chemical analysis were filtered using a Nalgene© filter kit and hand pump, firstly through 1.2µm Whatman (47mm) filter paper to remove particulates, and then through a Millipore 0.2µm nylon filter paper for preservation purposes.

The filtered water was decanted in acid washed and deionised rinsed Nalgene© bottles and stored in a cooler during field work. Upon return from the field samples were either immediately stored in a fridge (for DOC analysis) or freezer (for N analysis) then sent for analysis as soon as possible.

DOC samples were sent to the University of South East Alaska, Juneau within 3 days of collection. Concentrations of DOC (determined by non-purgeable organic carbon analysis) and total dissolved N (TDN) were measured via high temperature catalytic oxidation on a Shimadzu TOC/TN-V analyzer. All DOC and TDN data are reported here as the mean of three to five replicate injections, for which the coefficient of variance was always <2%. Surface water inorganic N was determined using the colourimetric methods previously described, substituting deionised water for KCl solution when making reagent blanks.

The complete surface and hyporheic water data set can be found in Appendix C.

2.5 Statistical analysis

Statistical analysis was performed using SPSS software (version 20). All data were tested for normality and homogeneity of variance all non-normal data was then log transformed. Non-parametric statistics were used to analyse these data because it was not normally distributed even after transformation. The non-parametric tests used were the Kruskal-Wallis test (for more than two groups of samples) or the Mann-Whitney *U*-test (between two groups of sample).

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3: Soil and microbial development in the riparian zone of watersheds of different ages

3.1 Introduction

A better understanding of how riparian systems develop and change over time is required in order to inform appropriate environmental modelling and management in a changing global environment. Riparian zones are important ecotones that mediate fluxes of nutrients and organic matter between terrestrial, riverine and marine ecosystems (Burt & Pinay, 2005). Riparian vegetation has been shown to play a pivotal role in the physical and biological development of these areas, with trees and plants increasingly considered 'ecosystem engineers' of fluvial environments (Gurnell, 2013).

Classically, models of floodplain development have considered vegetation development to be relatively passive processes controlled by reach scale hydrogeomorphological factors (Bendix & Hupp, 2000; Robertson & Augspurger, 1999; Salo, *et al*, 1986). However, over the past decade research has highlighted the importance of riparian vegetation in controlling river characteristics at the landscape scale through flow resistance, sediment accretion and reinforcement effects (Bennett and Simon, 2004).

The growth of plants and in particular trees in the riparian zone alters fluvial depositional mechanisms influencing channel morphology (Nanson & Knighton, 1996; Tooth & Nanson, 1999; Griffin and Smith, 2004, Smith, 2004) and sediment accumulation, changing soil moisture, fine sediment content and root system architecture (Gurnell & Petts, 2002, 2006; Gurnell, 2013). Sediment development caused by changing sediment accretion levels has been shown to influence vegetation colonisation and survival in riparian zones by altering the physical characteristics of sediments. These same mechanisms will also enhance riparian sediments for the development nutrient retention and microbial cycling.

Riparian sediment development over time, with vegetation colonisation and growth, will change the physical and chemical environment and alter conditions for microbial activity which are controlled by such variables. For example, nitrogen mineralisation is regulated by physical environmental factors such as soil temperature, pH, moisture and organic matter quality and quantity (e.g. lignin content). Nitrification requires well drained aerobic sediments for oxygen (O_2) respiration with a supply of organic matter and ammonia from the decomposition of plant and animal detritus (Burt *et al*., 2010; Naiman *et al*., 2002). In the terrestrial environment, nitrification leads to the greater N mobility and availability to primary producers. This is due to its change in form from the cation, ammonium (NH_4^+), which is impeded by negatively charged soil particles, to the mobile anion nitrate (NO_3^-) (Grimm *et al.*, 2003).

Denitrification will be most productive in fine textured sediments (> 60% silt + clay content) in permanently or frequently inundated areas with a long water residence time (Pinay, 2000; Pinay, 1993). These stagnant conditions allow microbial respiration to occur, removing oxygen O_2 to produce a anoxic environment, which is strongly related to a water filled pore space (WFPS) of c.a. 60% (Amha & Bohne, 2011; Groffman, 1987; Groffman & Tiedje, 1991; Hynes & Knowles, 1983; Klein, 1994). Furthermore, a supply of bio available NO_3^- is needed to act as a supply of O_2, as well as sufficient supply of C.

Optimum N cycling occur within an area of fluctuating environmental conditions where subsurface flow paths containing NO_3^- intercept C-rich, anoxic substrates (Grimm, 2003). Such areas are biogeochemical 'hot spots' that allow aerobic and anaerobic conditions to occur simultaneously or in close spatial and temporal proximity due to variation in water level or the presence of anoxic/oxic micro sites within the sediment (McClain *et al*., 2003). These areas contain an active denitrifying and nitrifying bacterial community in close proximity (Burt *et al*., 2002). The close relationship between these two mechanisms is termed coupled nitrification-denitrification. Rates of coupled nitrification-denitrification activity are highest at the interfaces between favourable conditions for both nitrifiers and denitrifiers, particularly areas with frequent alternation between aerobic and anaerobic conditions (Reddy and Patrick, 1975; Wrage, 2001).

The influence of plants on these important soil variables will change continuously over time due to annual growth cycles, and also in response to external drivers, such as climate, hydraulic and fluvial fluctuations and extremes, and over longer term growth trajectories (Gurnell, 2013). Understanding how this change occurs in an environment undergoing rapid change is important as much ecological theory is based on ecosystems in a steady state. Glacier Bay National Park, Alaska (GBNP) provides an opportunity to study an environment free from anthropogenic influences undergoing rapid primary succession. Here, the interplay between floodplain vegetation development and sediment accretion can be examined over a successional chronosequence.

The influences of vegetative succession by the development of tree species and coarse woody debris input on fluvial processes over time has been demonstrated in this area (Klaar & Milner, 2009; 2011). This study expands upon existing research on the role of vegetation development and microbial N cycling, usually constrained to small-scale studies located in single glacial forefields, into a larger scale study over a long term successional trajectory.

The main aims of this study were to:

1) Show change over time of riparian sediment properties, such as physical characteristics, nutrient retention and potential microbial activity.

2) Using space for time comparison, determine the time frame for riparian sediment development from abiotic to biotic driven systems.

We hypothesize that as riparian zones change over time from physically to biologically driven environments, N retention and microbial cycling will increase.

Moreover, the difference between near stream and floodplain areas will become more distinct as floodplain and watershed vegetation development alters nutrient inputs via litter fall and subsurface flow from the surrounding catchment and through river deposition.

3.2 Methods

3.2.1 Sample collection

The sampling strategy was in accordance that that stated in section 2.2.

3.2.2 Laboratory analysis

Soil physico-chemical properties were determined according to the methods described in section 3.3.1, potential nitrification in section 2.3.2, potential denitrification in section 3.3.3 and net mineralisation and nitrification in section 2.3.4.

3.2.3 Statistical analysis

Statistical analysis was performed using SPSS software (version 20). All data were tested for normality and homogeneity of variance all non-normal data was then log transformed. Non-parametric statistics were used to analyse these data because it was not normally distributed even after transformation. The non-parametric tests used were the Kruskal-Wallis test (for more than two groups of samples) or the Mann-Whitney *U*-test (between two groups of sample).

3.3 Results

3.3.1 Physical soil characteristics

The soil physico-chemical properties (0-10cm) of each riparian location (near steam and floodplain) are summarised in Table 3-2 and the results of the Kruskal-Wallis test are given in Table 3-3. Potential denitrification (without C_2H_2) and $\delta^{15}N$ were not included in the statistical analysis due to many samples being below detection limits.

Stone Fly Creek (SFC) had the largest percent fine grain size content for near stream and floodplain locations (mean 33.6% and 52.8%) (Figure 3-1, Table 3-1). Within the remaining study streams near stream areas showed a fine sediment content lower that SFC, with a gradual, though not significant, increase in silt + clay content along the

chronosequence, which is also evident to a lesser extent in the floodplain areas. Sediment grain size becomes less variable in floodplain areas over time, as opposed to near stream locations that show no temporal trend (Table 3-1).

Soil organic matter (OM) content constitutes a very low percentage of overall soil weight in near stream (0.8 – 2.1%) and floodplain (1.4 – 6.6%) areas. OM increased over time, with a significant (Kruskal-Wallis; $\chi^2 = 15.1$, df = 5, $P < 0.01$) increase in floodplain sediments between the second youngest and oldest watershed (Figure 3-1). Percent soil moisture increases in floodplain sediments during mid to late succession (141 + years). Conversely, bulk density decreases with increasing site age for floodplain sediments, with near stream areas remaining similar (ca 1.5 g cm^3) throughout.

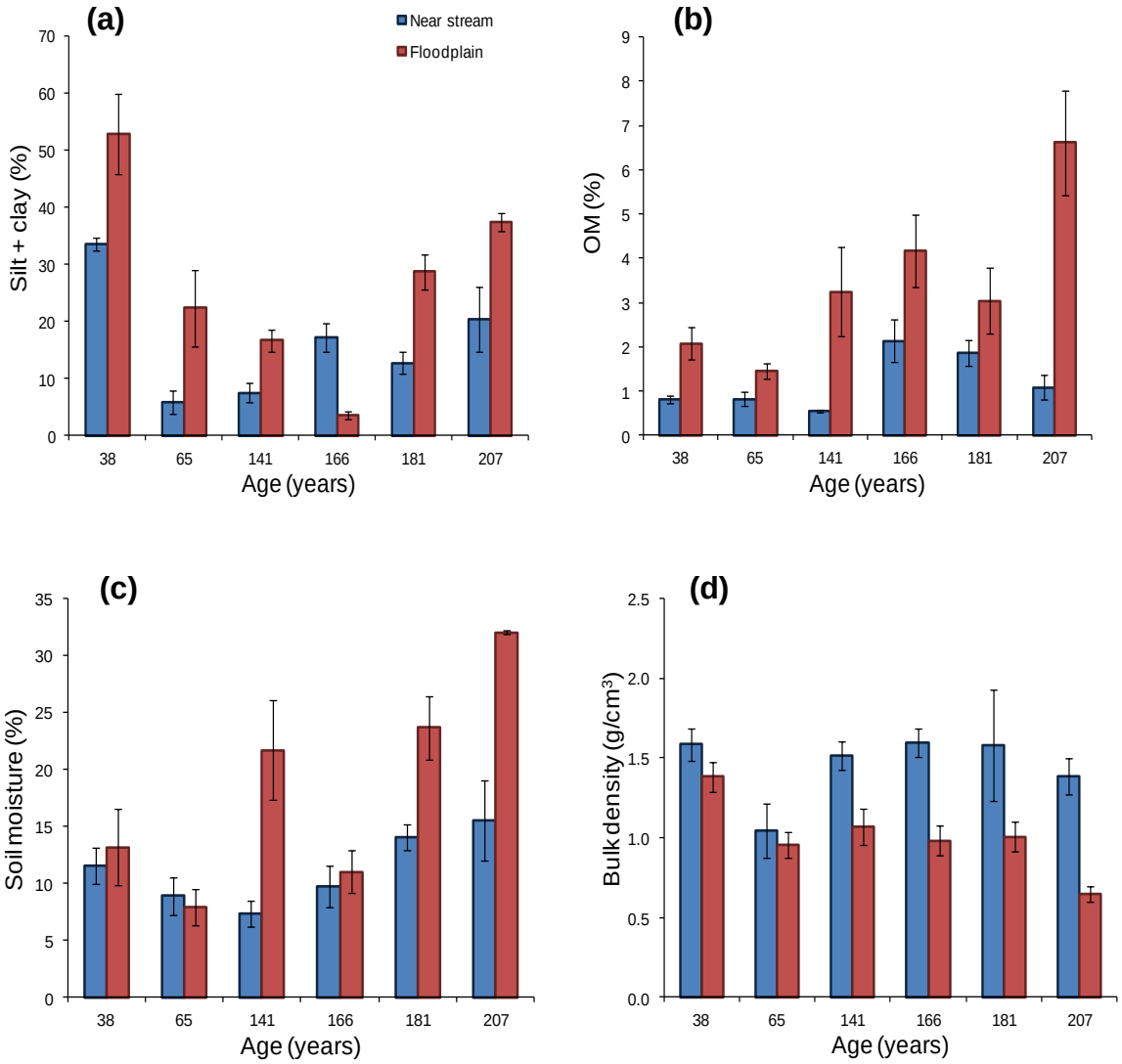

Figure 3-1*: Mean (S.E±) comparison of soil physical properties at near stream and floodplain areas: a) percent silt + clay; b) organic matter (%); c) Soil moisture content (%); d) bulk density (g/cm³).*

Sediment nutrient content (% C and N) was higher in the floodplain areas and showed an increasing trend with age in both riparian positions, though these increases were not significant. Due to the very low N content, particularly of near stream young sediments, it was not possible to analyse these for $\delta^{15}N$ values. Those values that were obtainable showed an enriched N pool in floodplain soils increasing with age. Furthermore, soil $\delta^{13}C$ values showed that near stream sediments were less negative than those found in the floodplain (Figure 3-2; Table 3-1).

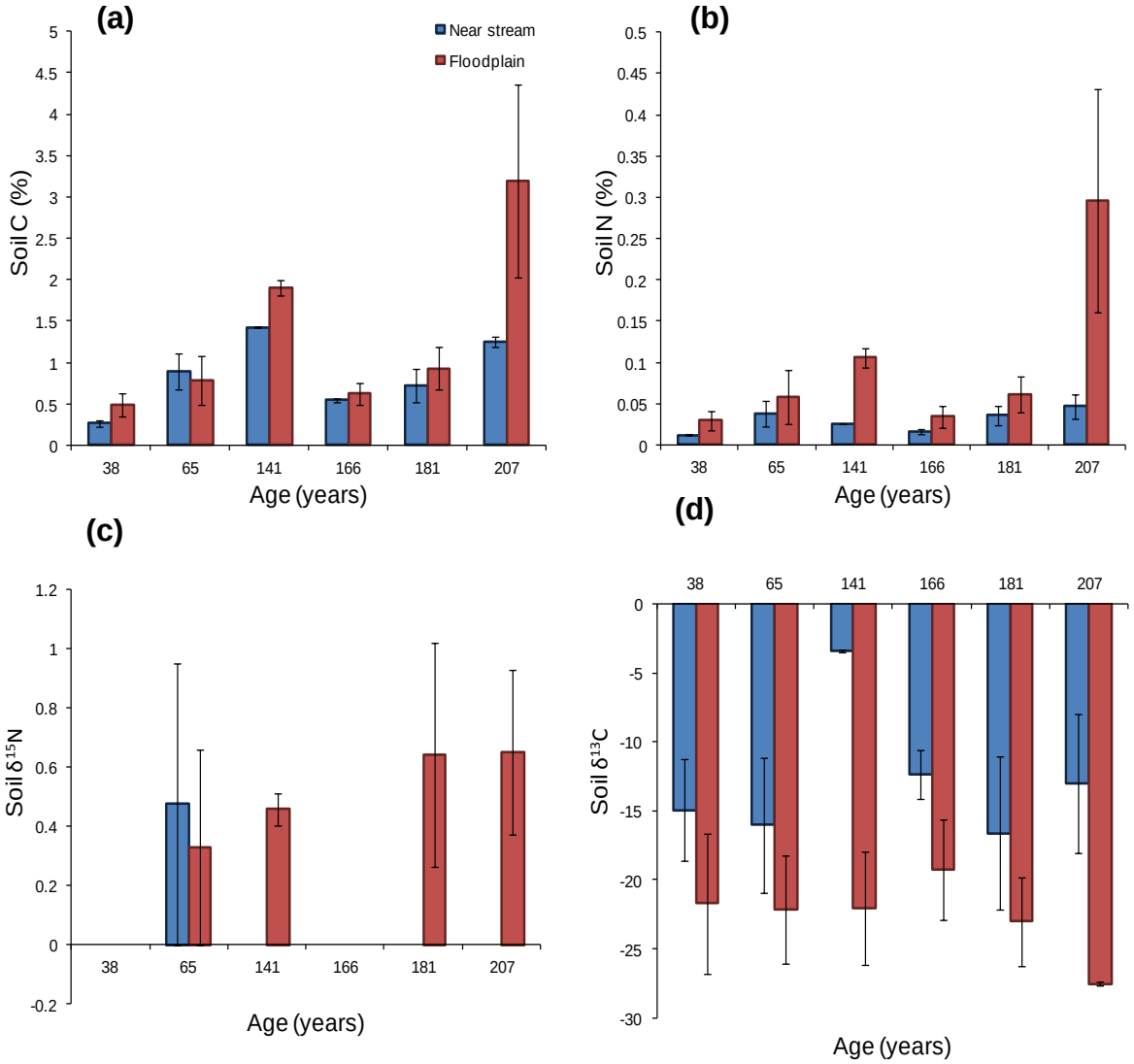

Figure 3-1: *Mean (S.E±) comparison of soil properties at near stream and floodplain areas: a) C (%); b) N (%); c) $\delta^{15}N$; d) soil $\delta^{13}C$.*

Table 3-2: *Soil property means (S.E.±) and coefficient of variation (standard deviation/mean). Significant statistical differences (using Mann-Whitney U-test) between riparian position are displayed as suffix symbol (+), and over ages (using Kruskal-Wallis) as letters, values with the same symbol are significantly different.*

	Age (years)	Near stream			Floodplain		
		mean	S.E (±)	CV	**mean**	S.E(±)	CV
Silt + clay content (%) (*n = 9*)	38	**33.6^{ab}**	1.1	0.09	**52.8^{ab}**	7.0	0.4
	65	**5.8^{+a}**	1.9	1.02	**22.4^{+a}**	6.7	0.9
	141	**7.5^{+b}**	1.7	0.70	**16.7^{+b}**	1.9	0.3
	166	**17.2^{+}**	2.5	0.43	**3.6^{+c}**	0.7	0.6
	181	**12.8^{+}**	1.8	0.43	**28.7^{+}**	3.1	0.3
	207	**20.3**	5.6	0.83	**37.5^{c}**	1.6	0.1
Organic matter (%) (*n = 9*)	38	**0.8^{+}**	0.1	0.3	**2.1^{+}**	0.4	0.5
	65	**0.8^{+ac}**	0.2	0.6	**1.4^{+a}**	0.2	0.3
	141	**0.6^{+b}**	0.02	0.1	**3.2^{+}**	1.0	0.7
	166	**2.1^{+ab}**	0.5	0.6	**4.2^{+}**	0.8	0.6
	181	**1.9^{c}**	0.3	0.4	**3.0**	0.7	0.7
	207	**1.1^{+}**	0.3	0.7	**6.6^{+a}**	1.2	0.5
N (%) (*n = 6*)	38	**.012**	.001	0.16	**.03**	.011	0.74
	65	**.38**	.16	0.72	**.057**	.32	0.67
	141	**.25**	.0	0.00	**.11**	.01	0.98
	166	**.17**	.003	0.35	**.034**	.013	0.16
	181	**.036**	.011	0.53	**.062**	.022	0.64
	207	**.047^{+}**	.014	0.54	**.29^{+}**	.13	0.61
C (%) (*n = 6*)	38	**.27^{a}**	.041	0.27	**.49**	.21	0.5
	65	**.89**	.21	0.42	**.78**	.29	0.66
	141	**1.42^{a}**	.005	0.00	**1.91**	.091	0.07
	166	**.54**	.016	0.05	**.63**	.13	0.37
	181	**.72**	.19	0.48	**.93**	.26	0.48
	207	**1.2^{+}**	.064	0.09	**3.2^{+}**	1.2	0.63
δ^{15}N (*n = 6*)	38	**.00**	-	n.a	**.00**	-	n.a
	65	**.47**	.47	n.a	**.33**	.33	n.a
	141	**.00**	.00	n.a	**.46**	.054	n.a
	166	**.00**	-	n.a	**.00**	-	n.a
	181	**.00**	-	n.a	**.64**	.38	n.a
	207	**.00**	-	n.a	**.65**	.28	n.a
δ^{13}C (*n = 6*)	38	**-14.9**	3.66	n.a	**-21.7**	4.89	n.a
	65	**-16.02**	4.89	n.a	**-22.16**	3.88	n.a
	141	**-3.4**	.11	n.a	**-22.0**	4.08	n.a
	166	**-12.4**	-19.3	n.a	**-19.25**	3.8	n.a
	181	**-16.6**	5.5	n.a	**-23.0**	3.2	n.a
	207	**-12.5^{+}**	.13	n.a	**-27.5^{+}**	.13	n.a

Table 3-3**:** *Comparison of physicochemical properties' variance within riparian locations over chronosequence. df; degrees of freedom between groups of samples, χ^2; test statistic, P; probability level. Non-parametric Kruskal-Wallis test has been used.*

Top soil (0-10cm)	**Near stream**			**Floodplain**		
	df	χ^2	*P*	df	χ^2	*P*
Silt + clay content (%)	**5**	26.8	<0.01	**5**	27.6	<0.01
Soil Organic matter (%)	**5**	23.2	<0.01	**5**	15.1	<0.01
TN (%)	**5**	9.3	>0.05	**5**	10.2	>0.05
TC (%)	**5**	13.3	<0.05	**3**	11.3	<0.05
$\delta^{13}C$	**5**	4.4	>0.05	**5**	6.4	>0.05
C:N	**5**	4.8	>0.05	**5**	6.8	>0.05
Nitrate	**5**	4.8	>0.05	**5**	17.7	<0.05
Ammonium	**5**	10.3	>0.05	**5**	9.4	>0.05

3.3.2 **Soil inorganic N**

The predominant form of inorganic N across all sites was ammonium, with the highest concentrations found in floodplain soils, increasing with site age. Concentrations were highly variable across riparian sites, but decrease in variability from the early successional peak at 65 years towards the older streams (Table 3-3).

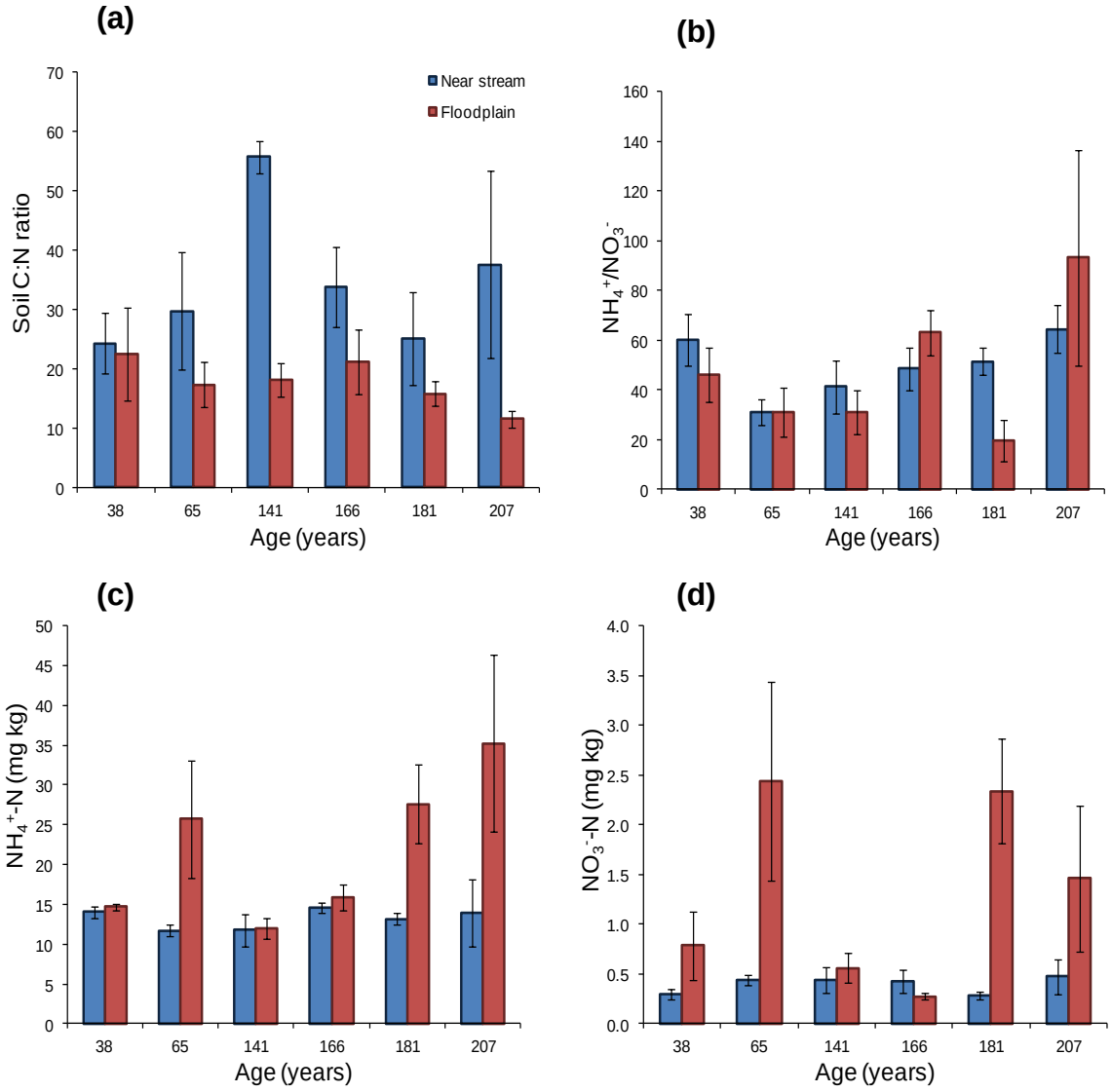

Figure 3-2: *Mean (S.E1±) comparison of soil N at near stream and floodplain areas: a) C:N ratio; b) NH_4^+/NO_3^- ratio c) NH_4^+-N; d) NO_3^--N.*

NO_3^- concentrations were comparatively lower than NH_4^+ across the chronosequence, but again highest in the floodplain areas. Near stream soil C:N ratios were higher than floodplain areas across the chronosequence, but showed no other trends with age, whereas floodplain soils decreased with age (Figure 3-3).

Table 3-4: *Soil inorganic N and C:N ratio means (SE±) and coefficient of variation (standard deviation/mean). Significant statistical differences (using Mann-Whitney U-test) between riparian position are displayed as suffix symbol (+), and over ages (using Kruskal-Wallis) as letters, values with the same symbol are significantly different.*

	Age (years)	Near stream			Floodplain		
		mean	S.E (±)	CV	**mean**	S.E(±)	CV
NH_4-N (mg/kg)	38	**14.06**	0.76	0.16	**14.68**	0.38	0.078
	65	**11.69**	0.72	0.18	**25.68**	7.34	0.86
	141	**11.76**	2.03	0.52	**12.01**	1.30	0.26
(*n = 9*)	165	**14.61**	0.62	0.13	**15.92**	1.62	0.31
	181	**13.17^{+}**	0.71	0.16	**27.59^{+}**	7.35	0.86
	207	**13.91**	4.19	0.91	**35.24**	11.05	0.94
NO_3-N (mg/kg)	38	**0.29**	0.051	0.51	**0.79^{a}**	0.34	1.30
	65	**0.44**	0.051	0.35	**2.43**	1.00	1.23
	141	**0.43**	0.13	0.86	**0.56**	0.15	0.65
(*n = 9*)	166	**0.43**	0.11	0.81	**0.28^{b}**	0.03	0.37
	181	**0.28^{+}**	0.036	0.38	**2.34^{+ab}**	0.53	0.78
	207	**0.47**	0.17	1.11	**1.46**	0.74	1.52
NO_3/NH_4 ratio	38	**60.2**	10.3	0.51	**46.3**	10.9	0.71
	65	**30.0**	5.05	0.48	**31.0**	9.9	0.96
	141	**41.2**	10.8	0.78	**30.9**	8.8	0.7
(*n = 9*)	166	**48.6**	8.4	0.52	**63.1**	8.9	0.42
	181	**51.5^{+}**	5.3	0.31	**19.7^{+}**	8.4	1.3
	207	**64.5**	28.3	1.31	**93.2**	43.4	1.4
Soil C:N ratio	38	**24.3**	5.1	0.36	**22.5**	7.8	0.6
	65	**29.8**	9.9	0.58	**17.4**	3.7	0.37
	141	**55.7**	2.7	0.07	**18.1**	2.9	0.23
(*n = 9*)	166	**33.8**	6.7	0.34	**21.2**	5.4	0.44
	181	**25.1**	7.9	0.55	**15.9**	2.0	0.22
	207	**37.6^{+}**	15.8	0.73	**11.6^{+}**	1.4	0.21

3.3.3 **Microbial N cycling**

Across the riparian zone net N mineralisation rates (change in NH_4^+ + NO_3^-) was highest in the early to mid-successional streams (38 - 141 years), with the older watersheds (166 - 207 years) exhibiting negative N mineralisation (Figure 3-4). The standard error of these data was very high and increased with age particularly in floodplain areas; this is due to the sporadic nature of microbial activity in such dynamic riparian environments (Table 3-3). Net nitrification (NO_3^- change) was positive across the chronosequence within the riparian zone but floodplain areas displayed the higher rates of nitrate production. Highest rates were found at BBS (166 years) (0.45 mg-N kg d^{-1}) but with a high standard error demonstrating the high degree of variability between samples (Figure 3-3).

Near stream potential nitrification showed no chronological trend with all sites exhibiting similar microbial rates and variability. Floodplain microbial activity increased significantly by more than 4 times in the floodplain areas between the youngest (4.35 mg-N kg d^{-1}) and oldest stream (27. 99 mg-N kg d^{-1}).

Denitrification enzyme activity (DEA) in the floodplain was approximately 5 x higher at the oldest steam, RPC (2.49 mg N_2O-N kg d^{-1}) when compared to the youngest, SFC (0.5 mg N_2O-N kg d^{-1}) (Table 3-3). DEA rates across the chronosequence did not show a gradual increase with age however, a sharp increase was observed in the oldest floodplain (Figure 3-5). This mirrored the peak floodplain rates of nitrification seen at RPC within the floodplain. Variability of potential denitrification data was consistently high across the chronosequence in both near stream and floodplain areas (Table 3-3). Also higher amounts of N_2O were produced under inhibited conditions (due to presence of C_2H_2) with most N_2O produced without the inhibitor in mid successional floodplains (Figure 3-5).

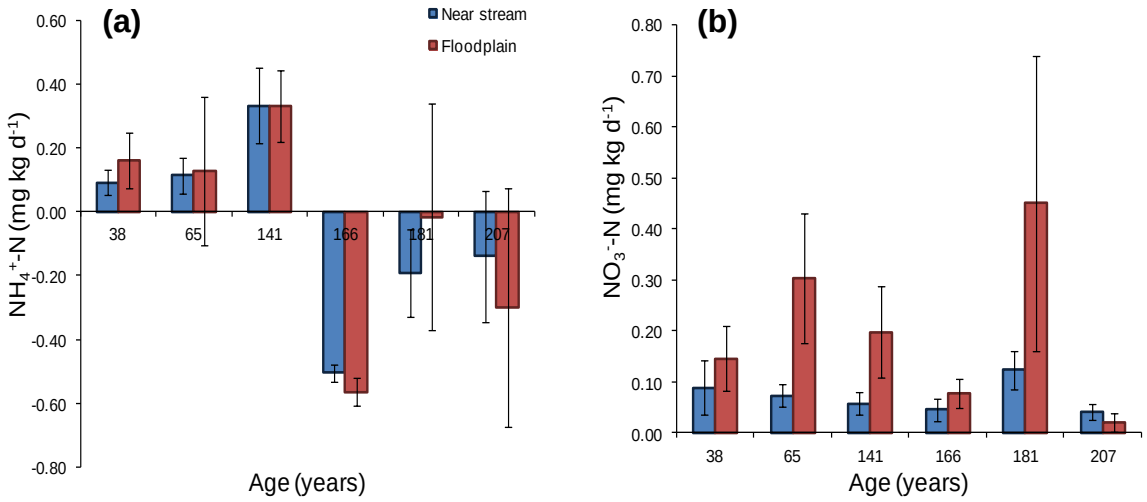

Figure 3-3: *Comparison of mean (SE±) a) net mineralisation; b) nitrification over chronosequence and riparian position.*

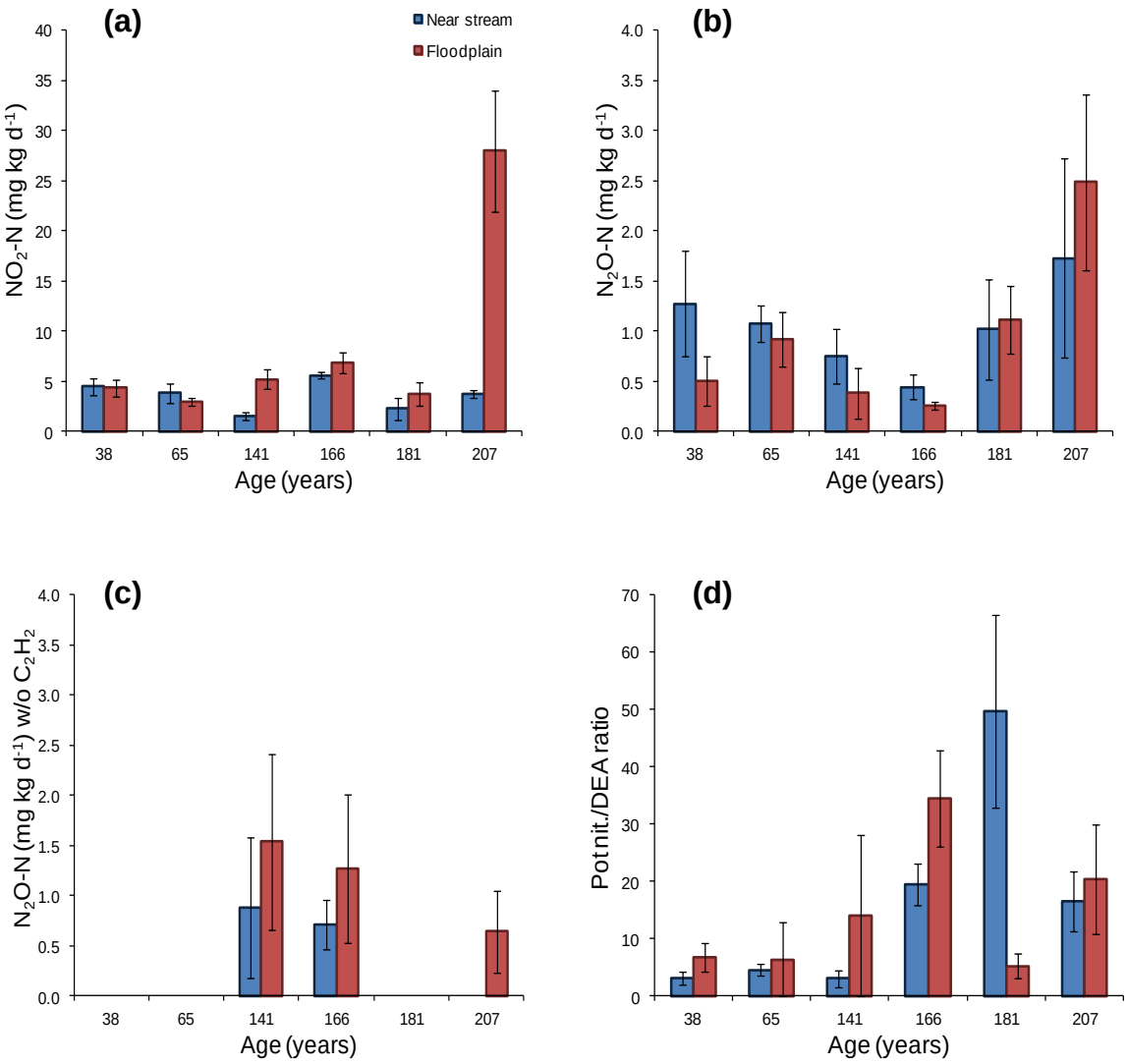

Figure 3-4: *Comparison of mean (1.SE±) a) potential nitrite oxidation (NO_2^-- consumption); b) denitrification (with C_2H_2); c) denitrification (without C_2H_2); d) ratio between potential nitrification and denitrification across chronosequence and riparian position.*

It is interesting to note the higher rates of nitrification compared to denitrification across the sampling sites, with potential nitrification rates up to 40 times higher than rates of potential denitrification (Figure 3-5).

Table 3-5: *Soil property means (SE±) and coefficient of variation (standard deviation/mean). Significant statistical differences (using Mann-Whitney U-test) between riparian position are displayed as suffix symbol (+), and over ages (using Kruskal-Wallis) as letters, values with the same symbol are significantly different.*

	Age (years)	Near stream			Floodplain		
		mean	S.E (±)	CV	**mean**	S.E(±)	CV
Net mineralisation ($mg\text{-}NH_4^+\text{-}N\ kg\ d^{-1}$) (*n = 9*)	38	**0.09**	0.04		**0.16**	0.09	
	65	**0.11**	0.06		**0.13**	0.23	
	141	**0.33**	0.12		**0.33**^{a}	0.11	
	166	**-0.51**	0.03		**-0.56**^{a}	0.04	
	181	**-0.19**	0.14		**-0.02**	0.36	
	207	**-0.14**	0.21		**-0.23**	0.28	
Net nitrification ($mg\text{-}NO_3^-\text{ - N kg } d^{-1}$) (*n = 9*)	38	**0.09**^{a}	0.05		**0.15**	0.06	
	65	**0.07**$^{+b}$	0.02		**0.30**$^{+}$	0.13	
	141	**0.06**^{c}	0.02		**0.20**	0.09	
	166	**0.05**^{abc}	0.02		**0.08**	0.03	
	181	**0.12**	0.04		**0.45**	0.29	
	207	**0.04**	0.02		**0.02**	0.02	
Potential nitrification ($mg\text{-}NO_2^-\text{ - N kg } d^{-1}$) (*n = 9*)	38	**4.48**	0.85	0.57	**4.35**	0.87	0.60
	65	**3.83**	0.96	0.75	**2.99**^{a}	0.36	0.36
	141	**1.52**$^{+a}$	0.36	0.70	**5.24**$^{+}$	1.00	0.47
	166	**5.58**^{ab}	0.31	0.17	**6.84**^{b}	1.04	0.46
	181	**2.27**^{b}	1.05	1.38	**3.77**^{bc}	1.14	1.05
	207	**3.77**$^{+}$	0.43	0.34	**27.99**$^{+ac}$	6.02	0.65
Potential denitrification ($mg\text{-}N_2O\text{ - N kg } d^{-1}$) With C_2H_2 (*n = 9*)	38	**1.27**	0.53	1.24	**0.50**	0.24	1.43
	65	**1.07**	0.18	0.50	**0.92**	0.27	0.88
	141	**0.75**	0.27	1.07	**0.38**^{a}	0.25	1.58
	166	**0.45**	0.12	0.84	**0.26**^{b}	0.04	0.46
	181	**1.02**	0.50	1.47	**1.11**	0.34	1.06
	207	**1.73**$^{+}$	0.99	1.72	**2.49**$^{+ab}$	0.88	1.06
Potential denitrification ($mg\text{-}N_2O\text{ - N kg } d^{-1}$) Without C_2H_2 (*n = 9*)	38	**0.0**	-	-	**-**	-	-
	65	**0.0**	-	-	**-**	-	-
	141	**0.88**	0.7	1.95	**1.54**	0.87	1.39
	166	**0.71**	0.24	1.03	**1.27**	0.74	1.75
	181	**0.0**	-	-	**-**	-	-
	207	**0.0**	-	-	**0.64**	0.41	1.56
Pot. Nit/DEA ratio (*n = 9*)	38	**3.2**	1.1	0.17	**6.9**	2.5	0.23
	65	**6.8**	1.0	0.16	**6.4**^{a}	2.0	0.32
	141	**3.1**$^{+a}$	1.5	0.26	**14.1**$^{+}$	5.1	0.28
	166	**19.4**^{a}	3.6	0.17	**34.5**^{ab}	8.39	0.14
	181	**49.6**	2.1	0.10	**5.2**^{b}	2.2	0.42
	207	**16.5**	5.1	0.44	**20.4**	9.5	0.46

Table 3-6: Comparison of microbial activity between riparian areas. df; degrees of freedom between groups of samples, χ^2; test statistic, P; probability level. Non-parametric Kruskal-Wallis test has been used.

Top soil (0-10cm) (*n = 9*)	**Near stream**			**Floodplain**		
	df	χ^2	*P*	df	χ^2	*P*
Net mineralisation (mg-NH_4^+-N kg d^{-1})	**5**	27.1	<0.01	**5**	12.4	<0.05
Net nitrification (mg-NO_3^- - N kg d^{-1})	**5**	4.2	>0.05	**5**	8.2	>0.05
Potential nitrification (mg-NO_2^- - N kg d^{-1})	**5**	18.6	<0.05	**5**	26.2	<0.01
Potential denitrification (mg-N_2O - N kg d^{-1}) With C_2H_2	**5**	3.6	>0.05	**5**	18.5	<0.05
Pot. Nit/DEA ratio	**5**	14.6	<0.05	**5**	16.9	<0.01

3.3.4 **Foliar data**

Isotopic data displayed differences between vegetation types within the riparian positions over time, and within sites and different vegetation species. The $\delta^{15}N$ values of nitrogen fixing species *D. drummondii* and *A. sinuatta* fell within the expected range for atmospheric N fixing plant species (0‰ to -6‰) (Robinson, 2001). At the youngest site (SFC, 38 years), *A. sinuatta* and *Salix* spp. $\delta^{15}N$ values were very closely matched this similarity decreased with age. The range of *P. sitchensis* foliar $\delta^{15}N$ (-4 – 1 ‰) and C:N ratio (35 – 50) was higher than other sampled vegetation. Similar patterns for these data were also found in the riparian vegetation.

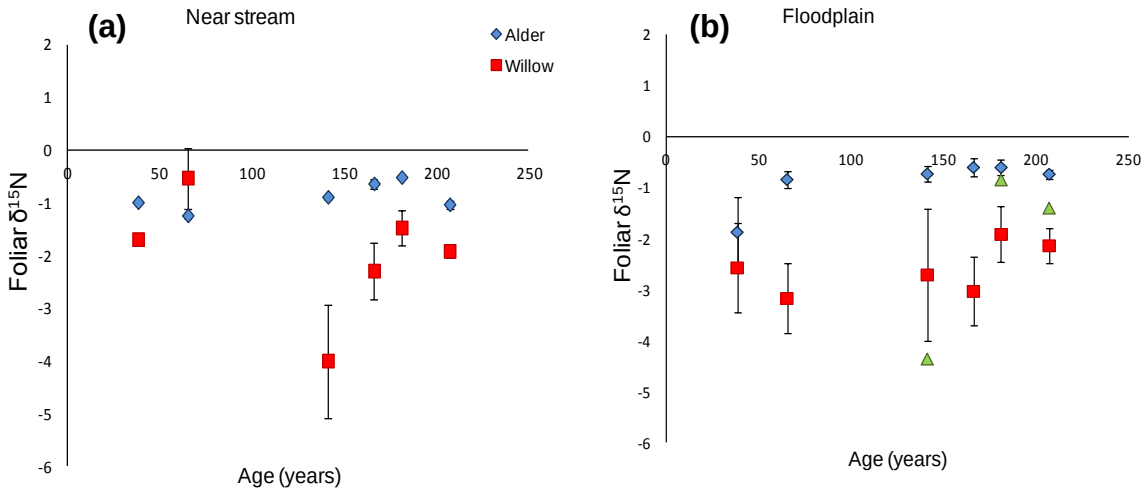

Figure 3-5: *Relationship between age and foliar δ15 between riparian (mean 1S.E ±) a) near stream and b) floodplain locations. A. sinuatta* (alder), *Salix spp.* (willow).

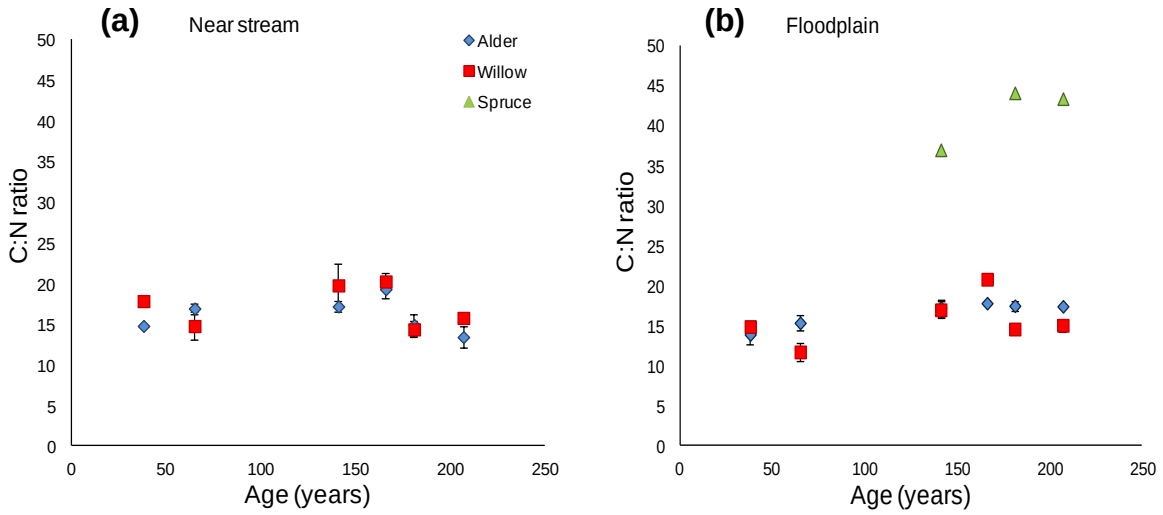

Figure 3-6*: Relationship between age and foliar C:N ration between riparian (mean 1 S.E ±) a) near stream and b) floodplain locations. A. sinuatta* (alder), *Salix spp.* (willow), *P. sitchensis* (spruce).

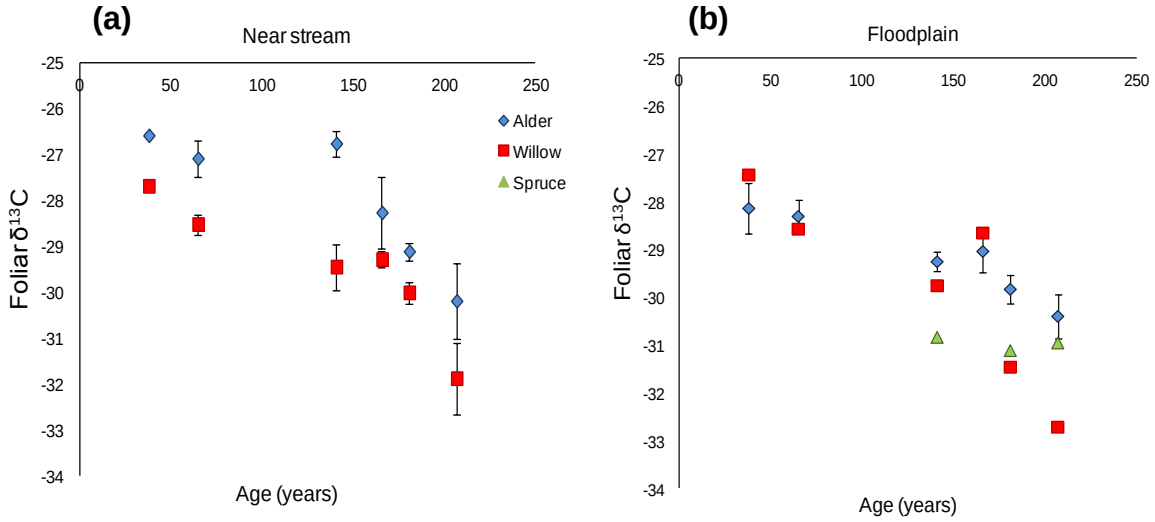

Figure 3-7: *Relationship between age and foliar ^{13}C between riparian (mean 1 S.E ±) a) near stream and b) floodplain locations. A. sinuatta* (alder), *Salix spp.* (willow), *P. sitchensis* (spruce).

Across both riparian locations and vegetation types there is a clear decrease in foliar $\delta^{13}C$ over the chronosequence particularly for mid successional ages onwards (141+ years).

3.4 Discussion

3.4.1 Physical characteristics

With increasing site age there was an increase in percent soil OM and moisture of floodplain sediments along with a decrease in bulk density. This was due to the accumulation and decomposition of vegetative matter over time improving the soil layer for plant and microorganisms. The floodplains of the older streams displayed higher amounts of organic matter, percent C, N and inorganic N concentrations compared to younger sites, as these systems develop from abiotic to biotic controlled systems.

Fine sediment content increased between the second youngest and the oldest site, indicating increased accretion of fine sediments as vegetation development increases floodplain roughness. However, this is not the case for the youngest watershed which displayed high fine sediment content across the riparian zone. During this early successional phase (0-50 years) there is a larger percentage of exposed sediments and the presence of a glacial ice remnant near the middle section of this watershed. Deposits of unconsolidated, un-vegetated glacial outwash in this watershed will be eroded easily supplying large quantities of fine suspended sediment for deposition on to the river floodplain. These factors caused the lack of clear sediment sorting found here with the high fines portion found across the riparian zone compared to the older streams. With increasing age the distribution of sediments became more distinct within the riparian zone of the remaining watersheds.

Vegetation development stabilises the river banks, consolidating sediments and increasing biological controls on these environments, mediating sediment input and river transport. As a consequence river transport and depositional patterns change, altering riparian near stream and floodplain areas (Chapin & Walker, 1994; Gurnell & Petts, 2006; Milner, 2007; Naiman, 2005). Silt and clay content of sediments is an important factor for soil nutrient retention and microbial activities such as denitrification, which typically requires fine textured sediments that retain moisture

and nutrients (Pinay, 2003). These physical characteristics indicate a shift in floodplain processes during mid to late succession as shown by the changing nutrient content.

3.4.2 **Microbial net N transformations**

Net N incubations showed that over a 28 day period early to mid age sites (38-141 years) displayed positive rates of N production for both N mineralisation ($NH_4^+ + NO_3^-$) and N nitrification (NO_3^-). The floodplains of the two youngest sites (38 and 65 years) support early successional plant species dominated by nitrogen fixing deciduous *A. sinuatta*. High levels of extractable soil N with peak levels of NO_3^- were found in the floodplain areas of WPC (65 years). Various authors have identified that most N accumulation occurs during the early period of *A. sinuatta* domination and mainly in the form of NH_4^+ in Glacier Bay (Bormann & Sidle, 1990; Crocker & Major, 1955). *A. sinuatta* leaf litter decomposes and mineralises quickly, providing readily bio-available N allowing rapid soil N turnover in *A. sinuatta* dominated sites, as shown by the positive net mineralisation and nitrification levels (Hobbie, 1998).

In these older riparian zones net N mineralisation became negative, that is there was less total inorganic N after the incubation period than at the start. Net nitrification rates in these samples remained similar to the younger watersheds; however soil NH_4^+ after incubation was substantially lower resulting in the negative N production rates. This change in N mineralisation corresponds to the change in dominant vegetation during this time period.

Vegetation succession in eastern arm of Glacier Bay National Park follows the classical successional trajectory from early stands of N fixing *A. sinuatta* to later *P. trichocarpa* to the mixed *P. sitchensis*-*T. heterophylla* forests (Chapin & Walker, 1994; Milner, 2007). Dominance by Sitka *P. sitchensis* occurs in catchment >150 years and has consequences for soil status and microbial activity. This shift from *A. sinuatta* and *P. trichocarpa* to *P. sitchensis*, changes vegetative inputs to lignin rich needles which decompose slowly, providing labile and more recalcitrant forms of C slowing organic matter decomposition and release of NH_4^+ (Vitousek & Hobbie, 2000; Hättenschwiler *et al.*, 2005; Staelens *et*

al., 2011). Furthermore, needles are retained by the plant for longer periods than one year, resulting in the decline of leaf tissues in the soil layer (Hobbie, 1998). Both processes change the soil N pool from the early bio-available labile form to refractory N. Slower decomposition during net incubations experiments would result in the samples becoming NH_4^+ limited creating the observed negative N production during mineralisation incubations, where soil N reduced over the incubation period.

Dominant vegetation type has been shown to play a driving role in the development of microbial communities during primary succession (Bardgett & Walker, 2004; Chapin & Walker, 1994; Edwards et al, 2006; Knelman *et al.*, 2012; Tscherko et al, 2005), having strong influences on important environmental variables, such as moisture, temperature, shading, cation exchange capacity, nutrient retention and pH (Walker and del Moral, 2003; Knelman, 2012). In forested areas as dominant tree species provide the majority of nutrient and organic matter inputs to the soil, they are a key regulating variable for microbial processes (Knelman *et al*., 2012; Scott & Binkley, 1997). Therefore, this change in watershed and floodplain vegetation cover alters the physical soil environment as well as the incoming nutrients to the riparian zone and is reflected in soil N cycling processes.

Foliar $\delta^{15}N$ and C:N ratios reflect soil quality and N pool status of the sample sites. By comparing these factors over time we can infer how the soil quality, N status, and nature of the soil N pool available (bio-available or refractory) develop over time. Precipitation $\delta^{15}N$ was not measured in this study; however it is generally accepted to be between 0 and -10% for this region (Hobbie, 1998; Nadhoffer and Fry 1994). In the older floodplains (181-207 years) *P. sitchensis* foliar $\delta^{15}N$ was consistently higher than that of *Salix* spp.. This can be attributed to the preference of *P. sitchensis* trees for utilizing NH_4^+ instead of NO_3^- as a source of N (60% and 40%) unlike *Salix* spp. which has a ruderal lineage so generally utilises NO_3^- effectively (Hobbie, 1998; Ingestad, 1979; Lee and Stewart, 1978). Nitrification in these areas will discriminate against molecules containing the heavier ^{15}N isotope and enriching the remaining NH_4^+ pool, leading to higher ^{15}N values of *P. sitchensis*. This also increased over time between mid

(141 years) to late successional stages (207 years) indicating that the slower N mineralisation in late succession would lead to less N available for uptake and a recycling mechanism of enriched N within *P. sitchensis* forests between vegetation and soil N pools (Hobbie, 1998). Also *Salix* spp. $\delta^{15}N$ showed a slight increase suggesting that soil NO_3^- is also becoming more enriched as the lighter isotope is lost through denitrification, uptake and mobilisation. This decrease in the rate of nitrogen turnover is in agreement with the hypothesis that the fraction of refractory N increases with age, the more labile bio-available N being used up during early succession (Hobbie, 1998). The high *P. sitchensis* C:N ratios in older sites are indicative of N limitation particularly 166- 207 years.

3.4.3 **Potential microbial transformations**

Despite the low levels of net N transformations across the riparian zone and watershed age there is potential nitrifying activity present, particularly in older floodplain areas. As shown by the potential nitrite oxidation experiment, N consumption rates increase over age and riparian position with clear peak rates in the oldest floodplain. High potential nitrification rates and low levels of extractable soil NO_3^- over the chronosequence indicate large amounts of microbial NO_3^- immobilisation. This has received considerable attention as a mechanism for stabilising soil N and thus reducing N losses (Stark & Hart, 1997). Although we did not directly determine microbial NH_4^+ verses NO_3^- immobilisation, we speculate that soil microbes in GBNP assimilate substantial amounts of NO_3^- for growth. Based on the presence of significant potential nitrification levels, yet relatively low *insitu* concentrations of soil NO_3^-, which is the predominant form of N available to soil heterotrophic microbes.

Potential denitrification activity is highly variable throughout the river watersheds across the riparian locations. Rates between floodplain and near stream areas were not significantly different, although rates in the oldest floodplain areas were significantly higher than those in some of the younger floodplains. The ratio between potential nitrification and denitrification clearly shows that nitrification is out performing denitrification by up to ca. 40 x. This indicates that although more N may be produced in

the older sites the low extractable amounts of NO_3^- suggests efficient uptake by plants and large amounts of heterotrophic microbial immobilisation. Fractionation in the remaining soil N pool can be seen with the increasing $\delta^{15}N$ of soil, *P. sitchensis*, and to a lesser extent *Salix* spp. leaves.

3.4.4 **Near stream – floodplain comparison**

The data shows that with increasing age there is a change in many soil properties, however between near stream and floodplain areas significant differences within the same age was rare. Despite increased age riparian sites remained similar, indicating an influence dampening that of increasing site age.

The cause of this may be that riverine systems are very dynamic in their long term behaviour. Geomorphological processes such as lateral channel shifts and streamside landslides result in disturbance to riparian areas (Swanson, 1994). Geomorphological processes can transport soil, sediment and woody debris down hillslope and through stream systems in steep, mountainous, forest landscapes. These movements can operate in sequence down gravitational flow paths, forming a cascade of disturbance processes that alters stream and riparian ecosystems (Nakamura *et* al, 2000). The affected stream and riparian landscape can be viewed through time as a network containing a shifting mosaic of disturbance patches – linear zones of disturbance created by the cascading geomorphological processes (Nakamura *et* al, 2000).

Ecological disturbance ranges in severity from the major effects of flooding and debris flows, which completely remove alluvium, riparian soil and vegetation along steep narrow, low order channels, to minor localized patches of trees toppled by floating logs along the margins of larger channels. These disturbance regimes can eat away at the surrounding area, leaving a zero-age class substrate in its wake (Nakamura *et* al, 2000; Swanson, 1994). These interacting geomorphic processes would hinder successional soil development and may be responsible for the lack of a clear separation over time of the different riparian locations investigated.

3.5 Conclusions

Riparian floodplain soil has shown a change over time with vegetation development, as shown by changes to sediment physical characteristics (fine sediment, bulk density, OM), nutrient content (% N, C and C:N ratio) and microbial activity (net and potential). Although not all of these changes were significant there are clear trend of soil development for vegetation and potential microbial activity over time. There was a clear increase in nitrifier microbial activity during succession as shown by net N turnover rates and furthermore there is a significant increase in potential microbial nitrification and denitrification rates between the youngest and older sites. The increasing rates of nitrification to denitrification in these areas are indicative of increasing N availability coupled with effective removal of N from the soil by plants and microbes.

Variation in physico-chemical data decreased over time for certain variables as floodplain areas stabilise, shifting from physically (SFC) to biologically (older sites) driven environments during mid to late successional stages of development. This is also demonstrated by the increasing soil OM and fines content, and the decrease in bulk density in the older late successional sites. This led to floodplains becoming significantly different with increasing stream age, with physical and biological properties becoming increasingly different over time, such as nutrient content (N and C) and microbial activity (potential experiments), confirming the hypothesis set out in the introduction.

This research demonstrates that riparian areas change over time with vegetation succession as an important factor, altering this environment over a relativity short period of time (ca. 207 years), increasing nutrient retention and microbial activity. Future research should consider using isotope tracers for the measurement of *in situ* rates of N cycling in such systems to unravel some of the interesting patterns discovered in this study such as the shift to negative net mineralisation with increased age.

3.6 References

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4: Catchment processes: the influences of vegetation, slope and age.

4.1 Introduction

There is an urgent need to understand and predict change in rapidly changing ecosystems (Milner, *et al*, 2007). Most current ecological theory and understanding is based upon research which comes from more stable ecosystems, where structure and composition are not rapidly changing, and inputs of materials and nutrients are roughly equal to outputs (Chapin & Matson, 2002; Milner *et al*., 2007). In contrast, ecosystems undergoing rapid change are environments with shifting compositions and a less balanced energy budget. Within a changing global environment, understanding such ecosystems is useful for predicting how Earth ecosystems will change in the future with shifting environmental drivers. Such a driving influence is the retreat of glacial ice sheets in sub arctic regions due to global warming. Glacier Bay National Park (GBNP), Alaska provides an excellent environment in which to study such rapidly changing ecosystems on recently exposed sediments, experiencing rapid primary succession. Over the past 250 years this area has experienced a gradual, well documented glacial recession, leading to the creation of watersheds of known ages. This study examined six of these watersheds aged between 38 to 207 years old. This series of watersheds allows a space for time substitution that has been used for multiple studies, which revealing the patterns of chronological development within the GBNP area (Crocker & Major, 1955; Hobbie, 1998; Milner *et al*., 2007).

The focus of this study was on the development of watershed soil nutrient content over time, particularly carbon (C) and nitrogen (N) concentrations, form and ratio (C:N), as well as their isotopic signature ($\delta^{15}N$ and $\delta^{13}C$) within soil and overlying vegetation. Furthermore net and potential microbial activity assays were performed to determine potential activity, turnover time and emission (N_2O) from these systems over time, and with changing vegetation cover. Vegetation development over time was considered a driving variable for soil and microbial development as vegetation type can alter soil abiotic and biotic conditions, thereby influencing microbial activity and therefore soil C

and N cycling (Zeng, 2009). A number of studies have been undertaken linking vegetation to microbial populations and soil status, however most are conducted in well developed productive soil systems (Grayston, 1998; Wardle, 1999). Those studies that have focused on primary succession of new landscapes, have concentrated entirely on the early stages of primary succession (Bardgett, 2004). This study also aimed to link underlying soil physic-chemical properties to the dominant vegetation, and then use vegetation cover as a proxy for these processes at the watershed scale. Present vegetation cover was determined using satellite imagery, which was also able to track changes over time within the study watersheds using historical satellite images over 22 years.

For this method to work other potentially important physical watershed characteristics were tested to determine their influence upon soil characteristics. Site slope steepness was examined as this will affect soil physical and chemical characteristics, through interaction with water movement that can influence soil particles (grain size distribution) and nutrient leaching (Swanson *et al.*, 1988). Steepness will influence the gravitational movement of material effecting water retention time, which regulates contact time between nutrients and microorganisms. The interactions of geomorphology, hydraulic residence times and substrate permeability create biogeochemically reactive interface zones (Grimm *et al*., 2003). Longer water retention results in greater removal of nutrients, even in areas with low microbial capacity (Grimm *et al*., 2003; McClain *et al*., 2003; Pinay *et al.*, 2003). Therefore it would be expected that shallow slope gradients will allow longer retention times, and conversely high sloping areas will have shorter retention times, resulting in differing microbial activity in these areas. Also the water travel time can influence nutrient cycling, as demonstrated by Pinay & O'Keefe (2008) within the hyporheic zone of an Alaskan river. Increasing ground water travel time through sediments decreased water NO_3^- and increased N_2O emissions, as the longer the contact time between NO_3^- containing ground water and sediments increased the time that soil microbes had to denitrify the NO_3^- (Pinay & O'Keefe, 2008). Therefore this principle should apply to soil in areas of varying slope and water retention. Site slope should be reflected within the physical

(sediment grain size), chemical (inorganic N pool), and microbial population (population size and activity) of the soil and within site vegetation. Essentially hydraulic residence time and flow path are the important factors for reactions on water and soil chemistry, as length of contact is only important if flow paths bring together critical reactants (McClain *et al.*, 2003).

The objectives of this study were to:

1. Quantify watershed soil nutrient content (N and C) and microbial community size and activity across GBNP chronosequence.
2. Determine the driving factors behind any differences at these sites (age, slope, forest cover).
3. Use satellite imagery data to scale up soil physico-chemical properties to the catchment scale for present (2009) and past environments (22 years), and calculate the rate of change for these watersheds over this time frame.

This thesis aims to test a number of hypotheses (H_x) that are:

1. The primary driving influence for change in microbial communities will be vegetation type, altering nutrient input and storage within soils over time.
2. With increasing watershed age soil nutrient content and microbial communities will increase.
3. Demonstrate that satellite imagery of vegetation could potentially be a useful tool to estimate catchment scale soil physico-chemical properties and their change over time.

4.2 Methods

4.2.1 Sample collection

The sampling strategy was in accordance that that stated in section 2.2.

4.2.2 **Laboratory analysis**

Soil physico-chemical properties were determined according to the methods described in section 3.3.1, potential nitrification in section 2.3.2, potential denitrification in section 3.3.3 and net mineralisation and nitrification in section 2.3.4.

4.2.3 **Statistical analysis**

Statistical analysis was performed using SPSS software (version 20). All data were tested for normality and homogeneity of variance all non-normal data was then log transformed. Non-parametric statistics were used to analyse these data because it was not normally distributed even after transformation. The non-parametric tests used were the Kruskal-Wallis test (for more than two groups of samples) or the Mann-Whitney *U*-test (between two groups of sample).

4.3 Results

4.3.1 Soil properties under successional vegetation

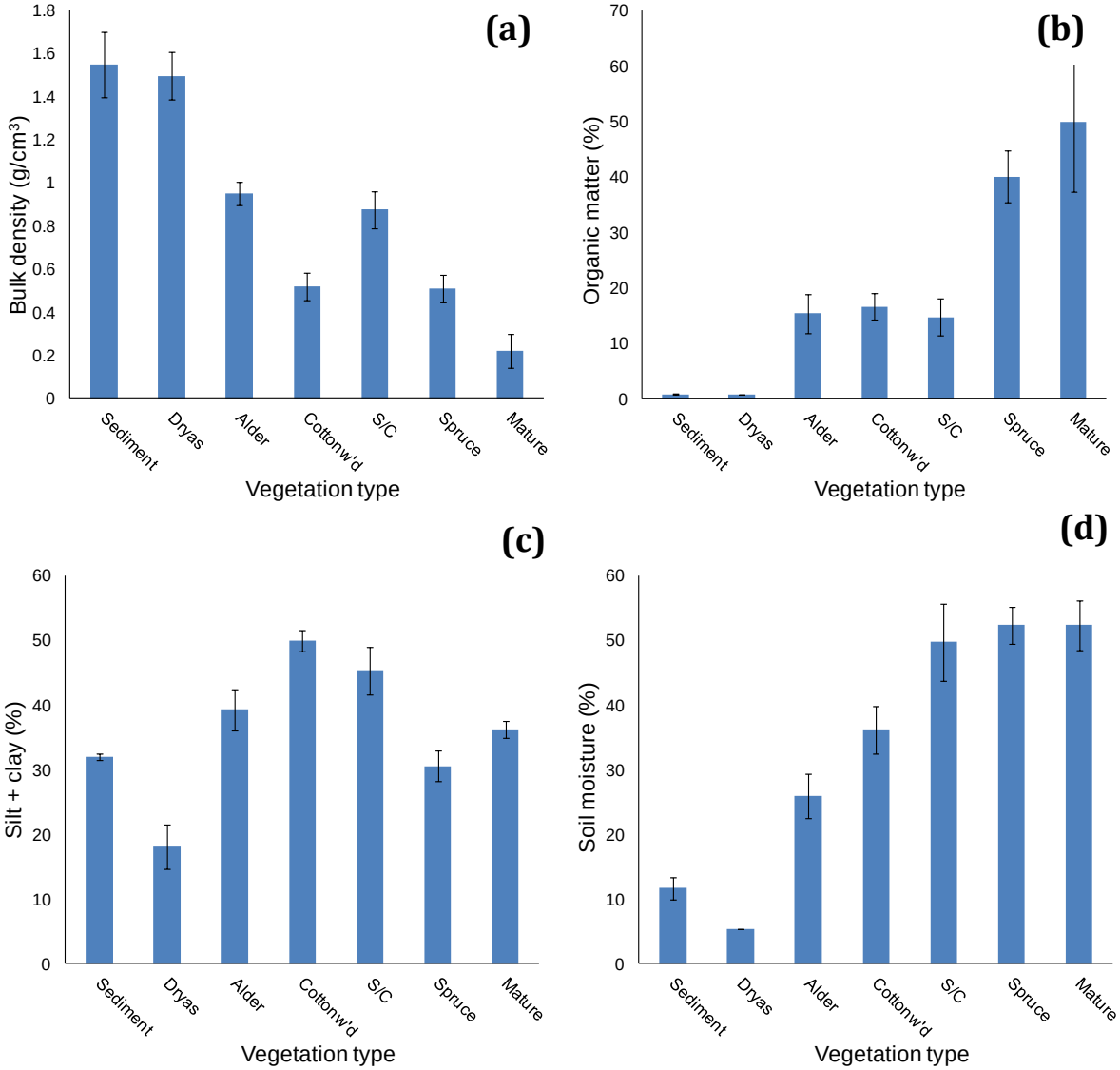

Figure 4-1: *Soil physical properties associated with dominant vegetation types. Mean values with S.E (±) a) Bulk density; b) percent organic matter; c) percent silt and clay content; d) percent soil moisture.*

There was a clear change in soil characteristics associated with the different dominant vegetation types. Exposed sediments and *D. drummondii* showed significantly higher bulk density, lower soil organic matter and moisture content than later successional vegetation cover (mixed *P. sitchensis* and *T. heterophylla* forest) (Figure 4-1; Table 4-1).

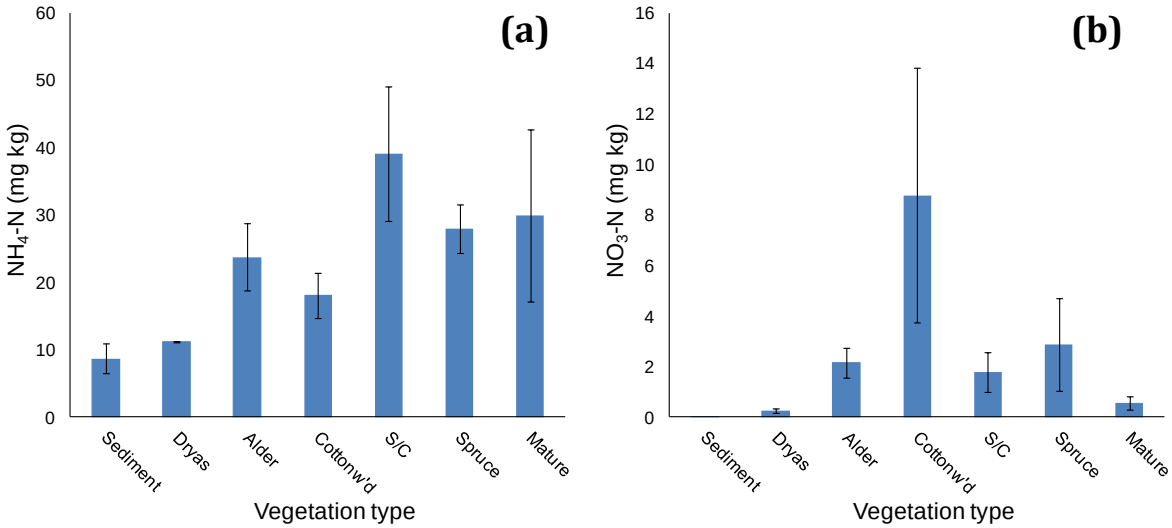

Figure 4-2: *Soil inorganic N, a) NH_4-N and b) NO_3-N (mean S.E±).*

Soil inorganic N was extremely low or below detection in young bare sediments but increased with vegetation development. NH_4^+ was the dominant form of inorganic N across all sites, with higher concentrations in later the successional *P. trichocarpa* and *P. sitchensis* forests, peaking under mixed *P. sitchensis-P. trichocarpa* forest (though not significantly). Soil NO_3^- was low across all sites but showed significantly higher concentrations in mid successional *P. trichocarpa* (Kruskal-Wallis, df = 7, χ^2 = 24.7, P <0.05) (Figure 4-2; Table 4-1).

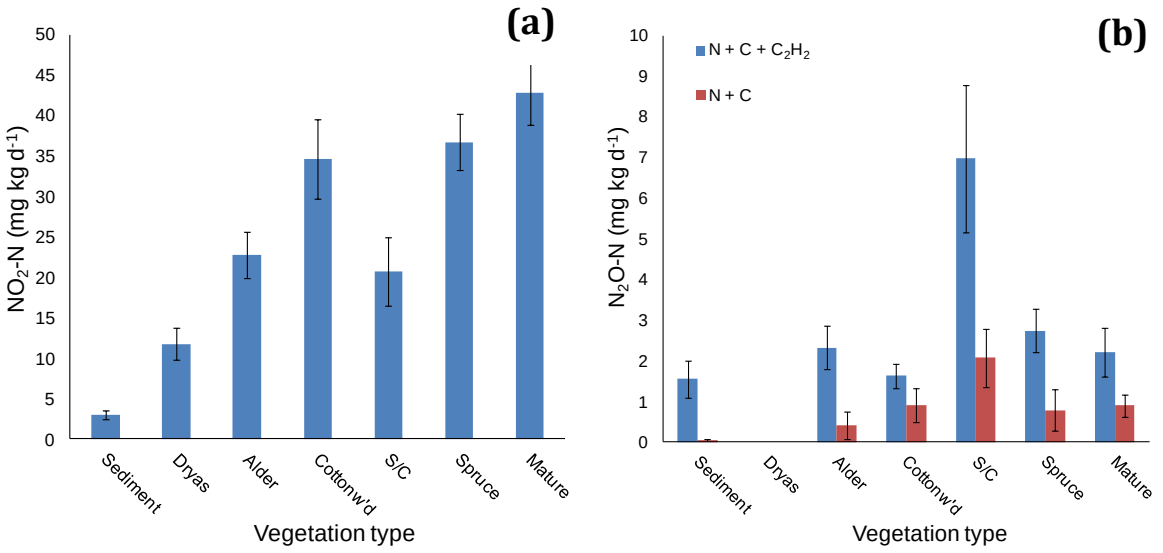

Figure 4-3: *a) Potential nitrite oxidation; b) potential denitrification associated with dominant vegetation types (mean ± S.E).*

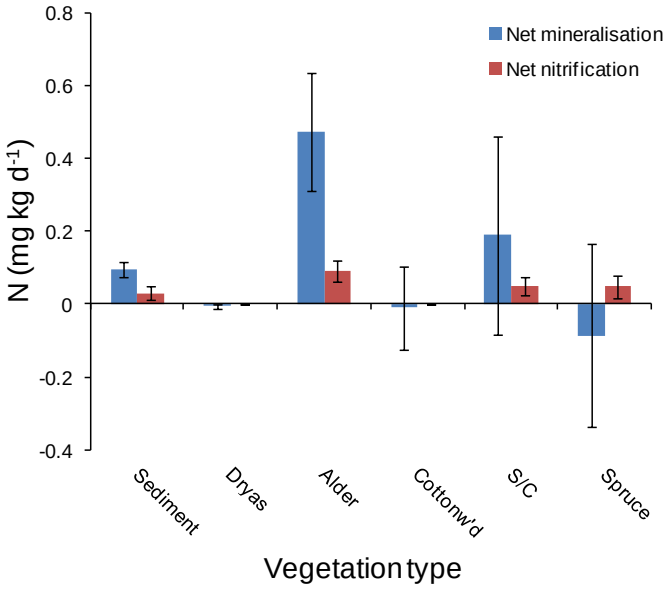

Figure 4-4: *Net mineralisation and nitrification against vegetation type (mean ± S.E).*

Both potential experiments showed low microbial populations in the newly exposed bare sediments, increasing with size as vegetation developed (Figure 4-3). Potential denitrification showed a pronounced significant peak in mid successional stands of mixed *P. sitchensis-P. trichocarpa* (Figure 4-3; Table 4-1). Net mineralisation showed low rates in sediment and *D. drummondii*, and peaked in *A. sinuatta* dominated areas, becoming increasingly negative and variable with increasing *P. sitchensis* dominance (Figure 4-4). Net nitrification also peaked in stands of *A. sinuatta* vegetation; however there was no significant difference between vegetation types (Kruskal-Wallis, df= 4, , χ^2 = 11.13, P= <0.05).

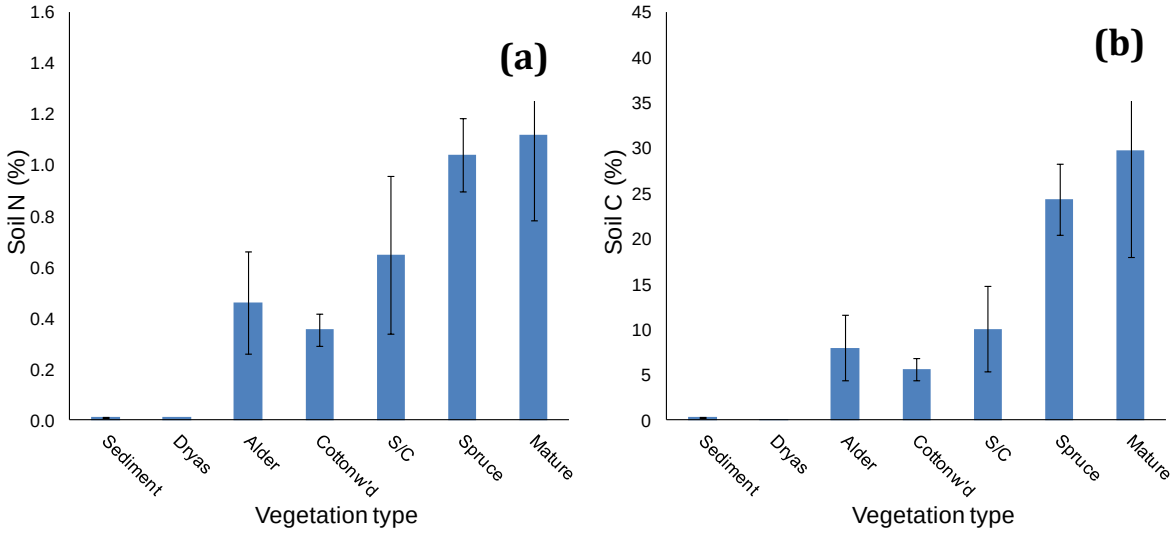

Figure 4-5: *a) Soil percent N; b) percent C associated with dominant vegetation type (Mean ±S.E).*

Soil percent C and N (% soil weight) was extremely low in early vegetation covers (sediment and *D. drummondii*) increasing significantly with later successional vegetation (Kruskal-Wallis, df = 7, χ^2 = 16.7, P<0.05, Kruskal-Wallis, df = 7, χ^2 = 14.4, P<0.05 respectively) (Figure 4-5). Percent C increased more in relation to N in later *P. sitchensis* and mature forests as shown by the soil C:N ratio, though this was not significant (Figure 4-6; Table 4-2).

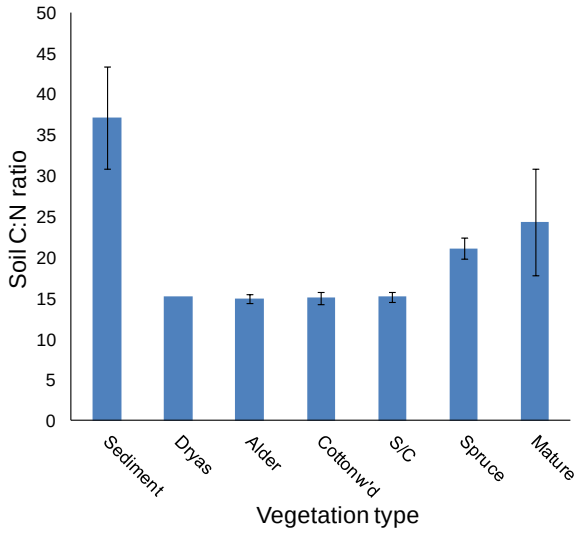

Figure 4-6: *Soil C:N ratio associated with dominant vegetation types (Mean ±S.E).*

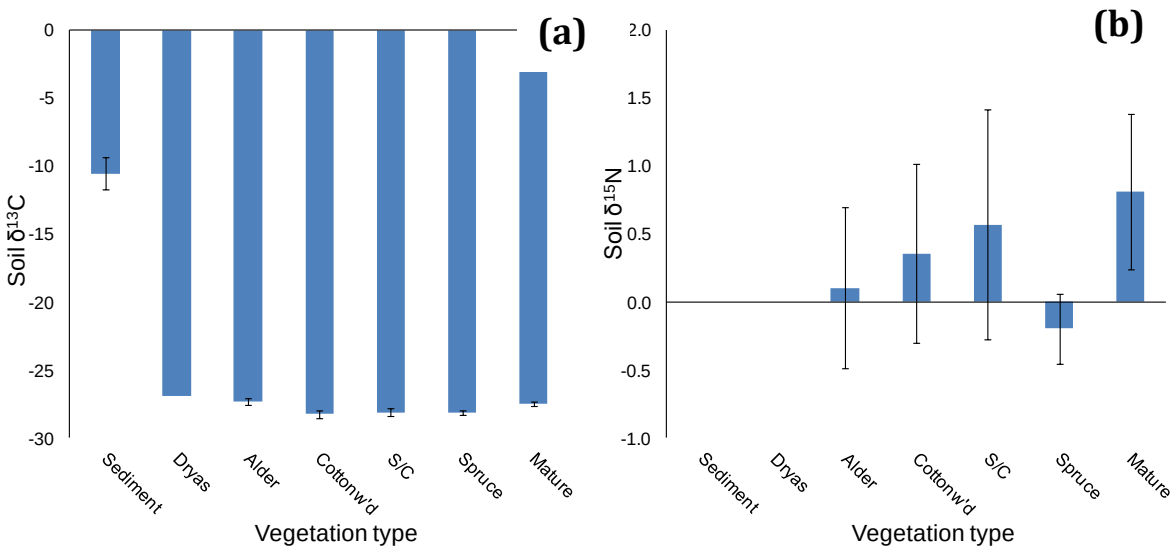

Figure 4-7: *a) Soil δ¹³C; b) δ¹⁵N associated with dominant vegetation types (Mean ±S.E).*

Soil $\delta^{15}N$ of young sites with no vegetation and *D. drummondii* cover were below detection limits, due to the extremely low N (%) in these samples. $\delta^{15}N$ values were variable across the range of the remaining vegetation types, with *P. sitchensis* displaying a mean negative value (Figure 4-7).

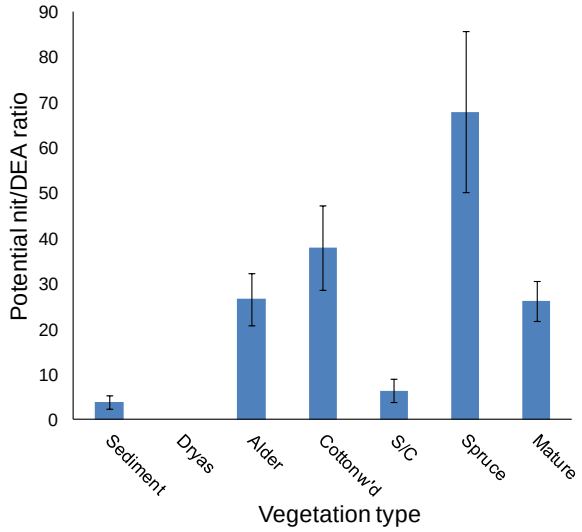

Figure 4-8: *Ratio between potential nitrification and DEA associated with dominant vegetation types (Mean ± 1S.E).*

Potential denitrification was similar over the different vegetation types, with peak concentrations associated with older successional vegetation types (Figure 4-3). However potential nitrite oxidation (nitrification) increased significantly over these vegetation types in relation to DEA, increasing availability of NO_3- (Figure 4-8).

Table 4-1: *Soil characteristics associated with dominant vegetation types. Results are given as means (±SE), values with the same suffix letter are significantly different at the P<0.05 level (by Kruskal-Wallis with multiple comparisons). n.a = below detection limit.*

	Vegetation type													
	Sediment	S.E (±)	**Dryas**	S.E (±)	**Alder**	S.E (±)	**Cottonw'd**	S.E (±)	**Sp/Cot**	S.E (±)	**Spruce**	S.E (±)	**Mature**	S.E (±)
	(n = 3)		**(n = 3)**		**(n = 39)**		**(n = 18)**		**(n = 12)**		**(n = 51)**		**(n = 9)**	
Bulk density (g/cm^3)	**1.55**^{a}	(0.15)	**1.50**^{b}	(0.11)	**0.95**^{c}	(0.06)	**0.52**^{ac}	(0.06)	**0.88**^{d}	(0.09)	**0.51**^{abc}	(0.06)	**0.22**^{abcd}	(0.08)
Silt + clay content (%)	**31.98**	(0.45)	**18.16**^{a}	(3.43)	**39.29**	(3.21)	**50.01**^{ab}	(1.64)	**45.32**	(3.63)	**30.61**^{b}	(2.35)	**36.21**	(1.29)
Soil Moisture (%)	**11.65**^{a}	(1.69)	**5.30**^{b}	-	**25.93**^{c}	(3.46)	**36.21**	(3.71)	**49.74**^{a}	(6.00)	**52.35**^{abc}	(2.80)	**52.38**^{a}	(3.80)
Organic matter (%)	**0.81**^{a}	(0.12)	**0.69**^{b}	-	**15.32**^{c}	(3.57)	**16.60**^{a}	(2.34)	**14.72**	(3.31)	**40.08**^{abc}	(4.69)	**49.94**^{abc}	(12.54)
NH_4-N (mg/kg)	**8.73**	(2.24)	**11.21**	(0.14)	**23.75**	(5.01)	**18.08**	(3.42)	**39.08**	(9.98)	**27.91**	(3.56)	**29.89**	(12.77)
NO_3-N (mg/kg)	**0.00**^{a}	-	**0.25**	(0.09)	**2.17**^{a}	(0.60)	**8.78**^{a}	(5.04)	**1.78**^{a}	(0.80)	**2.87**^{a}	(1.85)	**0.55**	(0.26)
Pot. Nit. (NO_2-N mg kg d^{-1})	**2.95**^{a}	(0.51)	**11.76**	(2.01)	**22.76**^{a}	(2.88)	**34.64**^{a}	(4.87)	**20.71**	(4.17)	**36.72**^{a}	(3.48)	**42.86**^{a}	(4.01)
DEA (with C_2H_2)	**1.55**	(0.46)	**0.00**^{a}	-	**2.32**^{b}	(0.53)	**1.62**	(0.29)	**6.97**^{ab}	(1.81)	**2.73**	(0.53)	**2.20**	(0.60)
DEA (without C_2H_2)	**0.03**	(0.03)	**-**	-	**0.40**	(0.33)	**0.90**	(0.43)	**2.07**	(0.72)	**0.89**	(0.28)	**-**	-
Potential nit/DEA	**3.82**	(1.43)	**-**	-	**26.63**	(5.76)	**37.90**	(9.25)	**6.44**	(2.48)	**67.89**	(17.76)	**26.16**	(4.43)

Table 4-2: *Soil percent N and C and isotopic signatures associated with dominant vegetation types. Results are given as means (±SE), values with the same suffix letter are significantly different at the P<0.05 level (by Kruskal-Wallis with multiple comparisons). n.a = below detection limit.*

	Vegetation type													
	Sediment	S.E (±)	**Dryas**	S.E (±)	**Alder**	S.E (±)	**Cottonw'd**	S.E (±)	**Sp/Cot**	S.E (±)	**Spruce**	S.E (±)	**Mature**	S.E (±)
	(n = 3)		**(n = 1)**		**(n = 15)**		**(n = 8)**		**(n = 4)**		**(n = 20)**		**(n = 3)**	
% N	**0.01**[a]	0.00	**0.01**	-	**0.46**	0.20	**0.36**	0.06	**0.65**	0.31	**1.04**[a]	0.14	**1.12**	0.34
% C	**0.29**[a]	0.03	**0.14**	-	**7.98**[b]	3.62	**5.63**	1.20	**10.06**	4.75	**24.36**[ab]	3.93	**29.76**	11.78
$\delta^{15}N$	**n.a**	-	**n.a**	-	**0.11**	0.59	**0.36**	0.66	**0.57**	0.85	**-0.20**	0.26	**0.81**	0.57
$\delta^{13}C$	**-10.54**[a]	1.20	**-26.96**	-	**-27.31**	0.27	**-28.23**[a]	0.28	**-28.11**	0.31	**-28.13**[a]	0.15	**-27.48**	0.16
Soil C:N ratio	**37.18**[a]	6.31	**15.17**	-	**14.97**[ab]	0.58	**15.03**	0.80	**15.18**	0.62	**21.14**[b]	1.25	**24.38**	6.53

4.3.2 **Slope comparison.**

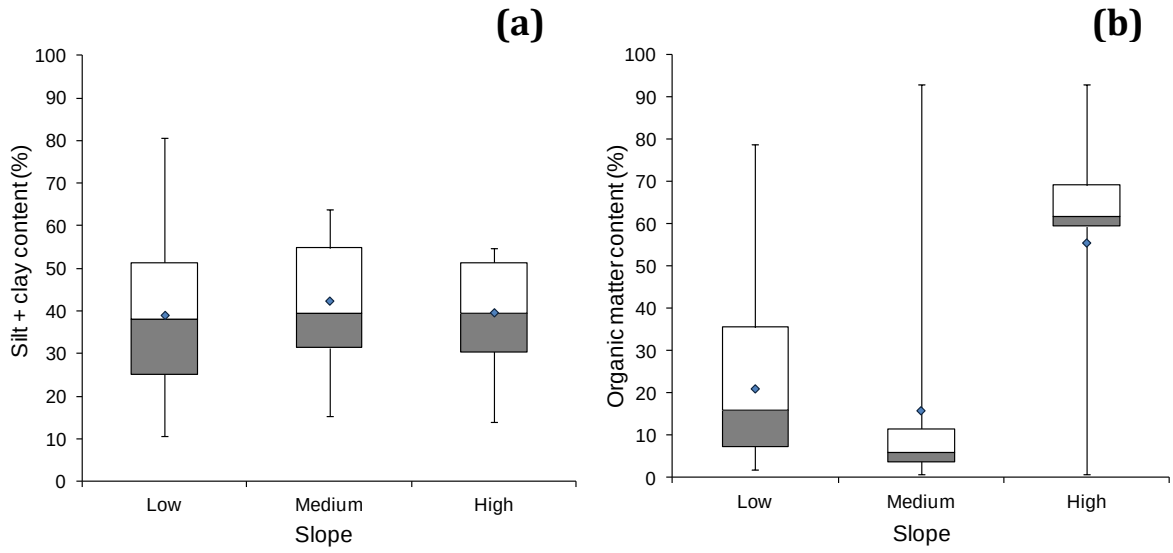

Figure 4-9: *a) Soil grain size; b) organic matter across slope gradient. (Diamond is the mean, solid line the median, clear box is the 75th and shaded box is the 25th percentile and whiskers indicate the minimum and maximum values).*

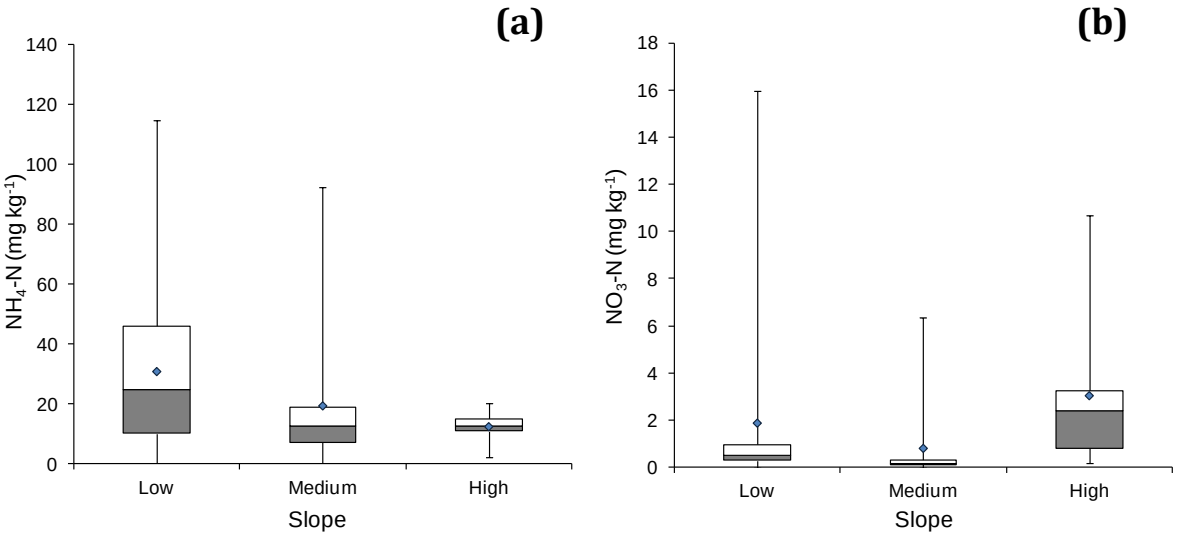

Figure 4-10: *a) Soil NH_4^+; b) NO_3^- concentration across slope gradient. (Diamond is the mean, solid line the median, clear box is the 75th and shaded box is the 25th percentile and whiskers indicate the minimum and maximum values).*

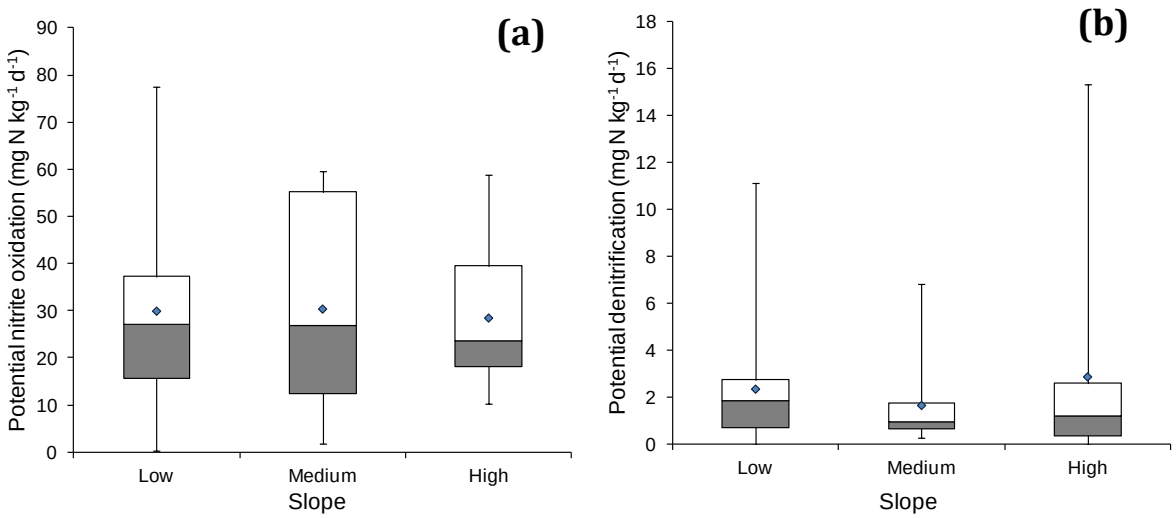

Figure 4-11: *a) Potential nitrite oxidation; b) DEA across slope gradient.*

Slope steepness has little apparent influence on soil properties when independent of the site age and vegetation cover, with only NH_4^+-N concentration showing any trends, with higher concentrations in the low sloping areas (Figure 4-10). A greater range and higher concentrations was found where slope was low (Table 4-2).

Table 4-3: *Soil properties across slope gradient and vegetation types (S.E is 1 standard error ± of the mean). Values with the same suffix letter are significantly different to the P<0.05 level (by Kruskal-Wallis with multiple comparisons).*

Soil variable	Slope gradient					
	Low (*n = 75*)		Medium (*n = 33*)		High (*n = 30*)	
	Mean	S.E	Mean	S.E	Mean	S.E
Bulk density (g/cm^3)	0.73	0.05	0.72	0.08	0.59	0.08
Soil Moisture (%)	41.77^a	2.31	25.07^{ab}	3.52	52.40^b	4.58
Silt + clay content (%)	39.22	2.28	40.91	2.60	35.88	3.37
Organic matter (%)	21.86^a	2.40	15.36^{ab}	4.68	49.83^b	7.51
NH_4-N (mg/kg)	30.49^a	3.49	17.20^a	4.09	11.28^a	1.34
NO_3-N (mg/kg)	4.33^a	1.61	0.75^a	0.24	1.87	0.70
Potential nitrite oxidation (mg kg d^{-1})	30.50	2.71	26.86	3.42	31.10	3.68
Potential denitrification (mg kg d^{-1})	3.04	0.48	2.21	0.50	2.61	0.64
N (%)*	0.64	0.11	0.29^a	0.13	1.26^a	0.20
C (%)*	13.08	2.76	5.70^a	3.41	29.13^a	4.81
$\delta^{15}N$*	-0.04	0.27	1.36	0.54	-0.19	0.29
$\delta^{13}C$*	-26.71	0.81	-26.17	1.46	-28.08	0.22
C:N ratio*	18.37	1.22	17.73^a	2.58	22.53^a	1.99

** n = low 25, medium 11 and high 7*

NH_4^+ concentrations in low sloping areas were significantly higher than those found in medium to high sloping sites, NO_3^- showed no difference between low and high slope sites (Table 4-3). Interestingly medium sloping areas showed significantly lower percent N, C, OM, and C:N ratio than high sloping areas, though low and high showed no significant difference. There were no significant patterns in the soil data that showed an

influence of slope on soil physical characteristics (bulk density and grain size) nor the microbial activity and population size (Table 4-3).

These data were independent of vegetation type, however dominant vegetation type does influence soil variables, therefore slope within the dominant vegetation types must be considered.

4.3.3 **Slope and vegetation type**

The following tables display these data according to dominant vegetation type across the gradient of slope steepness.

Table 4-4: *Soil characteristics and statistical differences associated with A. sinuatta vegetation at different slopes. (Kruskal- Wallis one way, significant at 0.05).*

Soil characteristics for *A. sinuatta*	**Slope (*n*)**						**Statistical comparisons**		
	Low (18)		**Medium (12)**		**High (9)**		P Low-medium	P Low-high	P Medium-high
	Mean	S.E	Mean	S.E	Mean	S.E			
Bulk density (g/cm^3)	0.95	0.13	1.01	0.07	0.95	0.06	ns	ns	ns
pH	6.20	0.34	6.86	0.24	5.73	0.09	ns	ns	ns
Soil Moisture (%)	19.26	3.64	14.01	3.76	51.96	7.99	.002	.021	ns
Silt + clay content (%)	33.67	6.08	40.76	6.04	40.62	3.96	ns	ns	ns
Organic matter content (%)	4.70	0.89	5.96	2.42	41.36	9.68	ns	ns	ns
NH_4-N (mg/kg)	42.02	11.23	12.67	2.83	8.63	1.56	ns	.009	ns
NO_3-N (mg/kg)	2.44	1.07	1.47	0.59	5.05	2.88	ns	ns	ns
Potential nitrite oxidation (mg kg d^{-1})	26.04	6.17	16.96	4.71	22.83	2.97	ns	ns	ns
Potential denitrification (mg kg d^{-1})	3.93	1.53	2.84	1.25	4.92	1.83	ns	ns	ns

The steepness of *A. sinuatta* covered areas did not influence soil physical characteristics significantly, except soil moisture which increased with slope gradient, nor potential microbial activities. Nutrient concentration was affected as mean soil NH_4^+ content is higher in low slope areas compared to medium and high slope (Table 4-4).

Table 4-5: *Soil characteristics and statistical differences associated with P. trichocarpa vegetation at different slopes. (Kruskal- Wallis one way, significant at 0.05).*

Soil characteristics for *P. trichocarpa*	**Slope (*n*)**						**Statistical comparisons**		
	Low (12)		**Medium (6)**		**High**		P Low-medium	P Low-high	P Medium-high
	Mean	S.E	Mean	S.E	Mean	S.E			
Bulk density (g/cm^3)	0.55	0.08	0.44	0.10	-	-	ns	-	-
pH	5.93	0..2	6.38	0.23	-	-	ns	-	-
Soil Moisture (%)	42.89	2.46	22.85	7.73	-	-	.025	-	-
Silt + clay content (%)	49.21	2.37	51.61	1.45	-	-	ns	-	-
Organic matter content (%)	20.93	2.35	6.46	1.26	-	-	.003	-	-
NH_4-N (mg/kg)	16.33	3.68	21.56	7.47	-	-	ns	-	-
NO_3-N (mg/kg)	12.96	7.36	0.41	0.13	-	-	.025	-	-
Potential nitrite oxidation (mg kg d^{-1})	30.36	6.32	43.22	6.66	-	-	ns	-	-
Potential denitrification (mg kg d^{-1})	1.43	0.34	1.73	0.46	-	-	ns	-	-

Areas of high slope were not sampled under *P. trichocarpa* or *P. sitchensis*/*P. trichocarpa* vegetation as the rarity and inaccessibility of these sites made their sampling impractical. The only soil variables that were significantly different between low and medium sloping areas were soil NO_3^-, OM and moisture, all increasing with decreasing slope (Table 4-5).

Table 4-6: *Soil characteristics and statistical differences associated with P. sitchensis/P. trichocarpa vegetation at different slopes. (Kruskal- Wallis one way, significant at 0.05).*

Soil characteristics for *P. sitchensis*/*P. trichocarpa*	**Slope (*n*)**						**Statistical comparisons**		
	Low (9)		**Medium (3)**		**High**		P Low-medium	P Low-high	P Medium-high
	Mean	S.E	Mean	S.E	Mean	S.E			
Bulk density (g/cm^3)	0.94	0.11	0.69	0.03	-	-	ns	-	-
pH	6.32	0.21	6.99	0.36	-	-	ns	-	-
Soil Moisture (%)	59.58	4.21	20.23	0.63	-	-	.013	-	-
Silt + clay content (%)	50.25	2.96	30.52	6.26	-	-	.012	-	-
Organic matter content (%)	17.11	3.58	3.96	1.14	-	-	.032	-	-
NH_4-N (mg/kg)	48.52	11.61	10.74	6.01	-	-	.033	-	-
NO_3-N (mg/kg)	2.32	1.01	0.16	0.04	-	-	.033	-	-
Potential nitrite oxidation (mg kg d^{-1})	22.07	5.42	16.64	4.27	-	-	ns	-	-
Potential denitrification (mg kg d^{-1})	3.28	0.74	1.63	0.47	-	-	.ns	-	-

Under the mixed *P. sitchensis P. trichocarpa* forest both forms of inorganic N decreased with increasing slope, as did soil OM, moisture and the fine sediment content (Table 4-6).

Table 4-7: *Soil characteristics and statistical differences associated with P. sitchensis vegetation at different slopes. (Kruskal- Wallis one way, significant at 0.05).*

Soil characteristics for *P. sitchensis*	Slope (*n*)						Statistical comparisons		
	Low (21)		Medium (3)		High (9)		P Low-medium	P Low-high	P Medium-high
	Mean	S.E	Mean	S.E	Mean	S.E			
Bulk density (g/cm^3)	0.63	0.08	0.41	0.07	0.27	0.03	ns	.031	ns
pH	6.35	0.19	5.39	0.02	4.76	0.11	ns	.001	ns
Soil Moisture (%)	47.15	3.21	39.52	7.33	62.69	5.00	ns	.019	ns
Silt + clay content (%)	31.05	2.50	52.69	0.00	-	-	.013	ns	ns
Organic matter content (%)	31.33	4.13	23.83	6.34	79.89	9.35	ns	.003	ns
NH_4-N (mg/kg)	31.34	3.96	2.85	2.85	16.98	1.33	.021	ns	ns
NO_3-N (mg/kg)	3.10	2.15	0.02	0.02	1.86	0.51	ns	.044	.008
Potential nitrite oxidation (mg kg d^{-1})	35.77	4.23	35.43	5.10	37.78	6.29	Ns	ns	ns
Potential denitrification (mg kg d^{-1})	3.17	0.73	2.50	0.98	1.86	0.42	ns	ns	ns

NO_3^- was low throughout *P. sitchensis* forest and decreased further as slope increased, NH_4^+ followed a similar pattern but concentrations were higher (Table 4-7). Slope did not significantly affect potential microbial activity.

It is evident from these data that inorganic N was influenced by slope, although not uniformly across vegetation type. Other important factors such as microbial activity were not influenced by slope. Soil percent N, C, C:N ratio and isotopes (^{15}N and ^{13}C) was not significantly different between high and low slope within individual vegetation types.

4.3.4 **Change over chronosequence**

This section examines these data to determine if there is significant change in the soil characteristics of particular forest types over time. Slope has been ignored as only one variable (NH_4^+) displayed any statistically significant difference based on slope steepness.

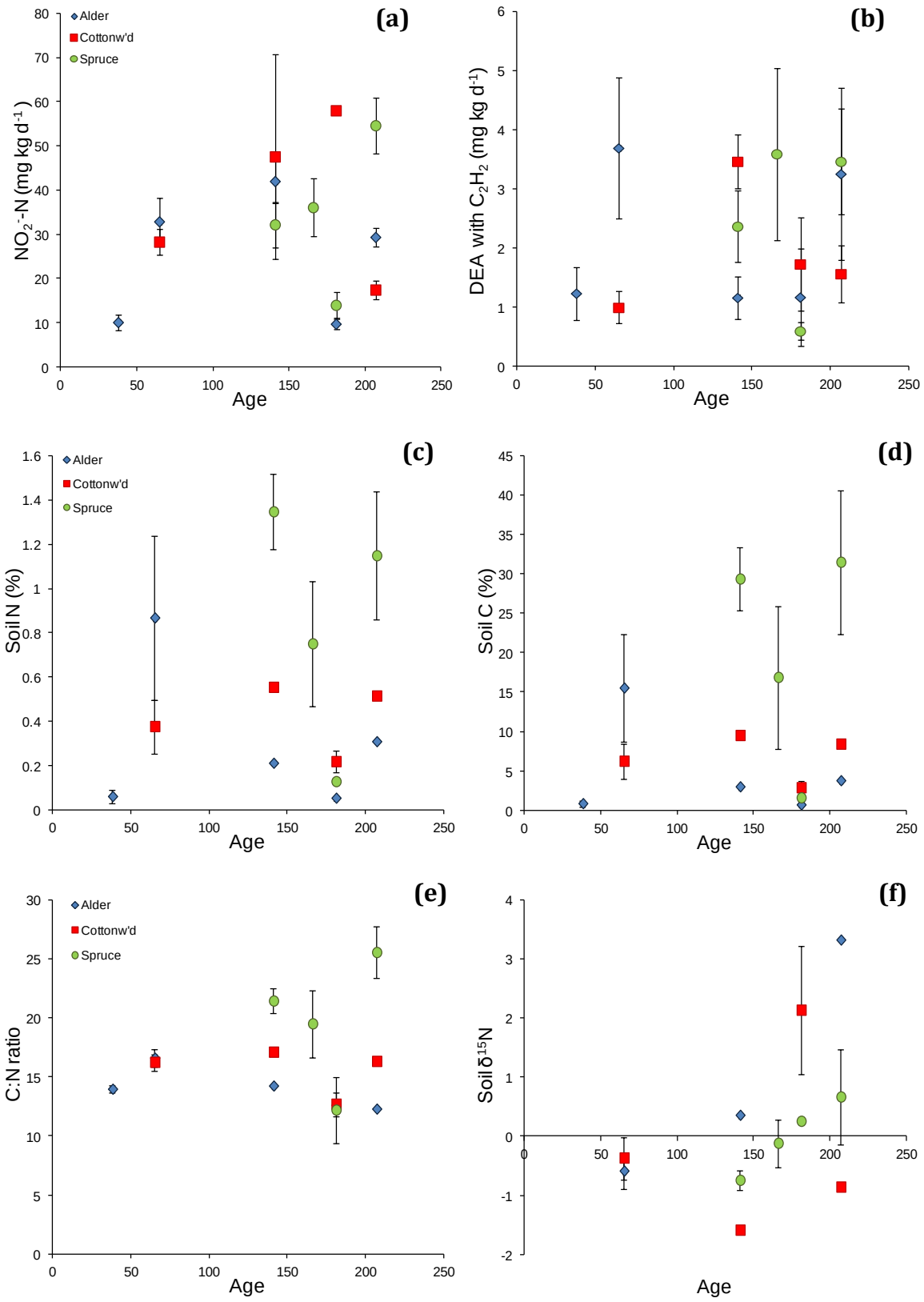

Figure 4-12: *Mean (S.E ±) soil properties associated with forest types and watershed age; a) potential nitrite oxidation; b) DEA with C2H2; c) soil percent N; d) soil percent C; e) C:N ratio; f) Soil δ15N.*

There are trends over time for some of the parameters; however these are not significantly different within forest types (Figure 4-12). Microbial activity increases from the early sites (0-50 years) to the older, as does soil N and C as well as an enrichment in soil $\delta^{15}N$ particularly in the two older watersheds.

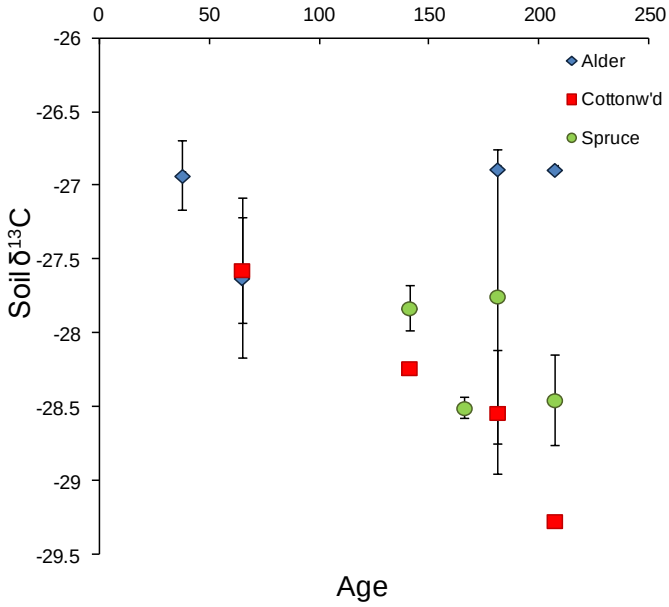

Figure 4-13: *Soil $\delta^{13}C$ (‰) over time beneath dominant vegetation types.*

Decreasing $\delta^{13}C$ was found in soil samples with increasing age beneath all dominant vegetation types except stands of *A. sinuatta* vegetation.

4.3.5 **Foliar data**

Foliar variables were examined with relation to slope and vegetation type. Of particular interest are nutrient concentrations, isotopic signatures and ratios.

Table 4-8: *Mean foliar variables for dominant vegetation types across slope gradient (1 S.E ±). Values with the same suffix letter are significantly different to the P<0.05 level (by Kruskal-Wallis with multiple comparisons).*

		Slope					
Vegetation (*n*)	**Foliar variable**	**Low**	(S.E ±)	**Medium**	(S.E ±)	**High**	(S.E ±)
Salix spp. *n* = 15 (low), 5 (med), 8 (high)	C:N ratio	16.01[a]	(0.97)	13.99	(1.94)	11.77[a]	(0.80)
	$\delta^{15}N$	-1.96	(0.23)	-3.29	(0.37)	-2.61	(0.63)
	$\delta^{13}C$	-29.86	(0.35)	-29.24	(0.13)	-28.43	(0.39)
	TN	3.09	(0.18)	3.48	(0.51)	4.14	(0.26)
	TC	47.34	(0.50)	46.70	(0.90)	47.86	(0.39)
A. sinuatta *n* = 22 (low), 7 (med), 9 (high)	C:N ratio	16.29	(0.84)	15.98	(0.69)	15.33	(0.69)
	$\delta^{15}N$	-0.94	(0.12)	-1.07	(0.09)	-0.94	(0.11)
	$\delta^{13}C$	-30.12	(0.35)	-29.58	(0.58)	-29.62	(0.46)
	TN	3.07	(0.15)	3.09	(0.13)	3.22	(0.13)
	TC	47.93	(0.29)	48.94	(0.47)	48.84	(0.73)
P. trichocarpa *n* = 6 (low), 1 (med)	C:N ratio	19.26	(2.33)	17.83	(1.49)	N.A	
	$\delta^{15}N$	-3.81	(0.39)	-4.66	(0.44)	N.A	
	$\delta^{13}C$	-30.83	(0.50)	-30.37	(0.32)	N.A	
	TN	2.39	(0.27)	2.97	(0.49)	N.A	
	TC	43.72	(2.35)	52.19	(4.27)	N.A	
P. sitchensis *n* = 15 (low), 2 (med), 5 (high)	C:N ratio	44.63	(1.88)	53.35	(6.31)	46.20	(3.01)
	$\delta^{15}N$	-1.61	(0.49)	-0.66	(1.15)	-0.97	(0.16)
	$\delta^{13}C$	-31.62	(0.35)	-32.13	(1.03)	-31.35	(0.13)
	TN	1.10	(0.04)	0.92	(0.10)	1.05	(0.09)
	TC	48.02	(0.33)	47.91	(0.47)	47.96	(1.27)

The compiled foliar data across all study streams revealed that only *Salix* spp. foliar C:N ratio showed a significantly different result between low and high sloping areas, the remaining data showed no significant change with site slope (Table 4-8). The following figures present these data, merged to exclude slope, displayed over the chronosequence.

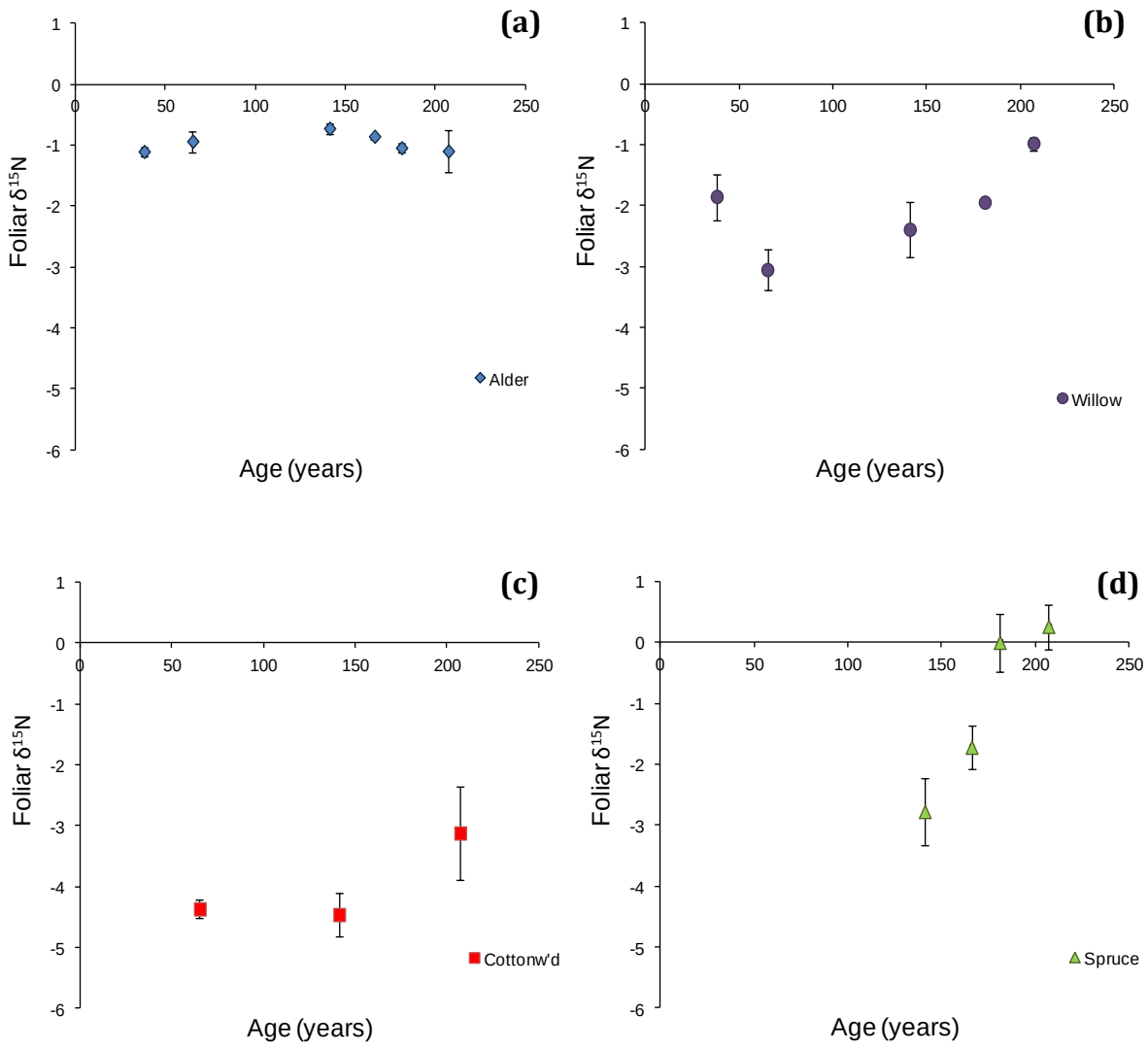

Figure 4-14: *Mean (1 S.E ±) foliar δ15N values for the sampled vegetation types (A. sinuatta (a), Salix* spp. *(b), P. trichocarpa (c) and P. sitchensis (d)) over time.*

A. sinuatta $\delta^{15}N$ remained consistent across watershed age whereas *Salix* spp. showed an initial decrease at 65 years, but gradually increase towards the oldest site (207 years). *P. sitchensis* $\delta^{15}N$ mirrored this increase with age, with negative values at mid successional sites increasing to low positive enriched $\delta^{15}N$ values in the two oldest streams (Figure 4-14).

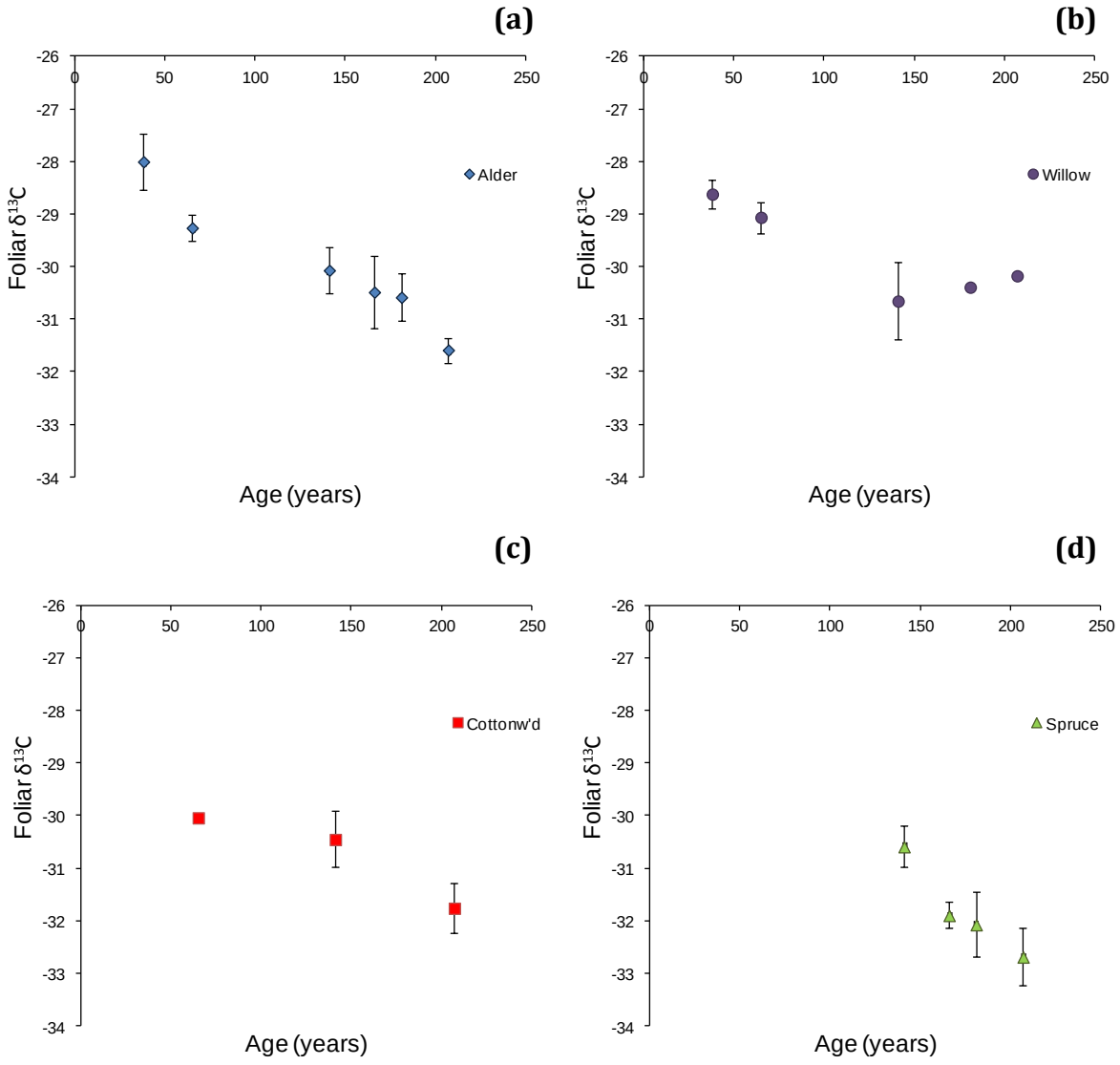

Figure 4-15: *Mean (1 S.E ±) foliar δ¹³C values for the sampled vegetation types (A. sinuatta (a), Salix* spp. *(b), P. trichocarpa (c) and P. sitchensis (d)) over time.*

A. sinuatta foliar $\delta^{13}C$ decreased over the chronosequence between the early (38 and 65 years) and the oldest successional site (207 years). This pattern was repeated within *Salix* spp. and *P. sitchensis* during the early successional stage but levelled out at older sites (Figure 4-15).

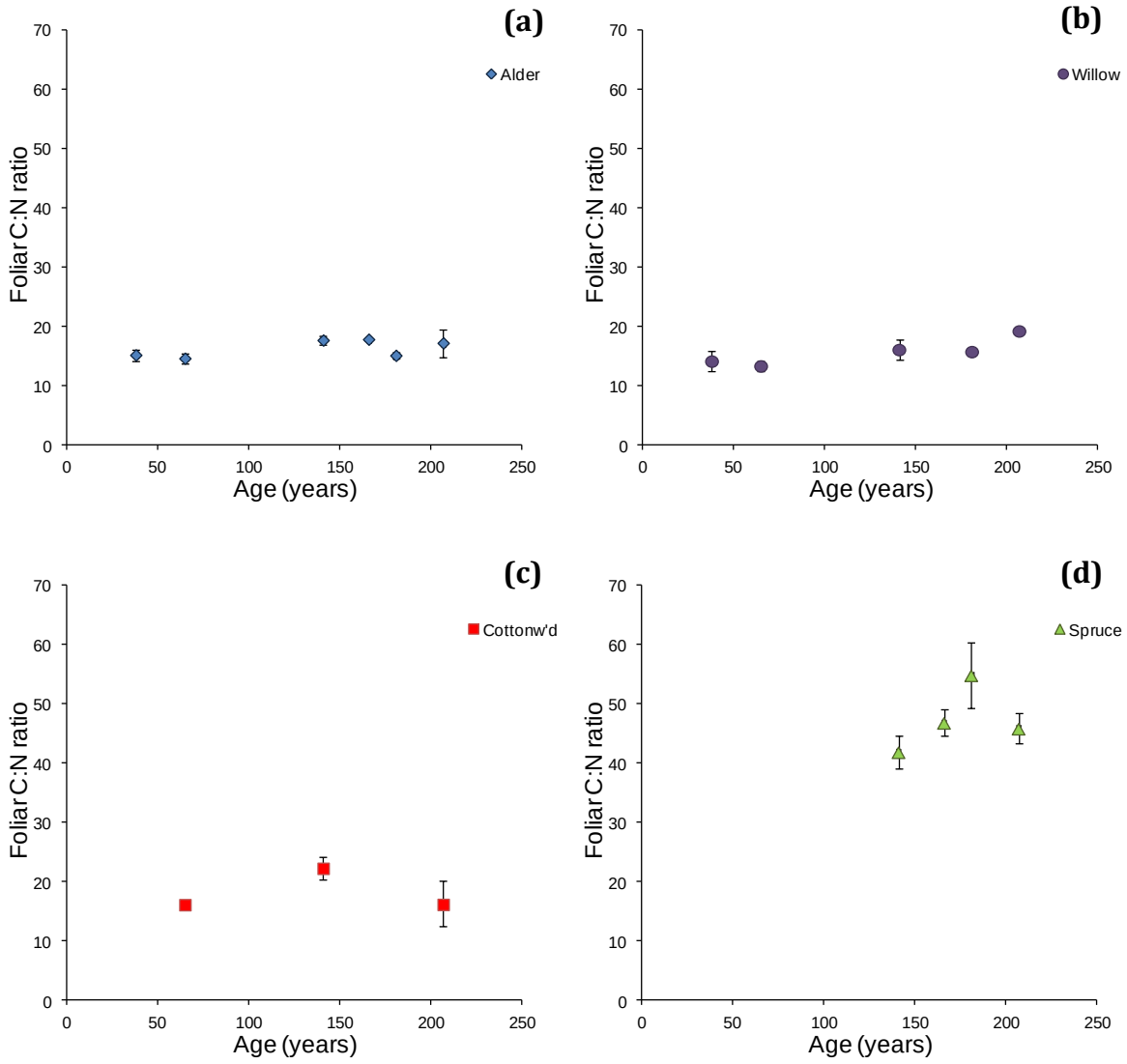

Figure 4-16: *Mean (1 S.E ±) foliar C:N ratio values for the sampled vegetation types (A. sinuatta (A), Salix* spp. *(B), P. trichocarpa (C) and P. sitchensis (D)) over time*

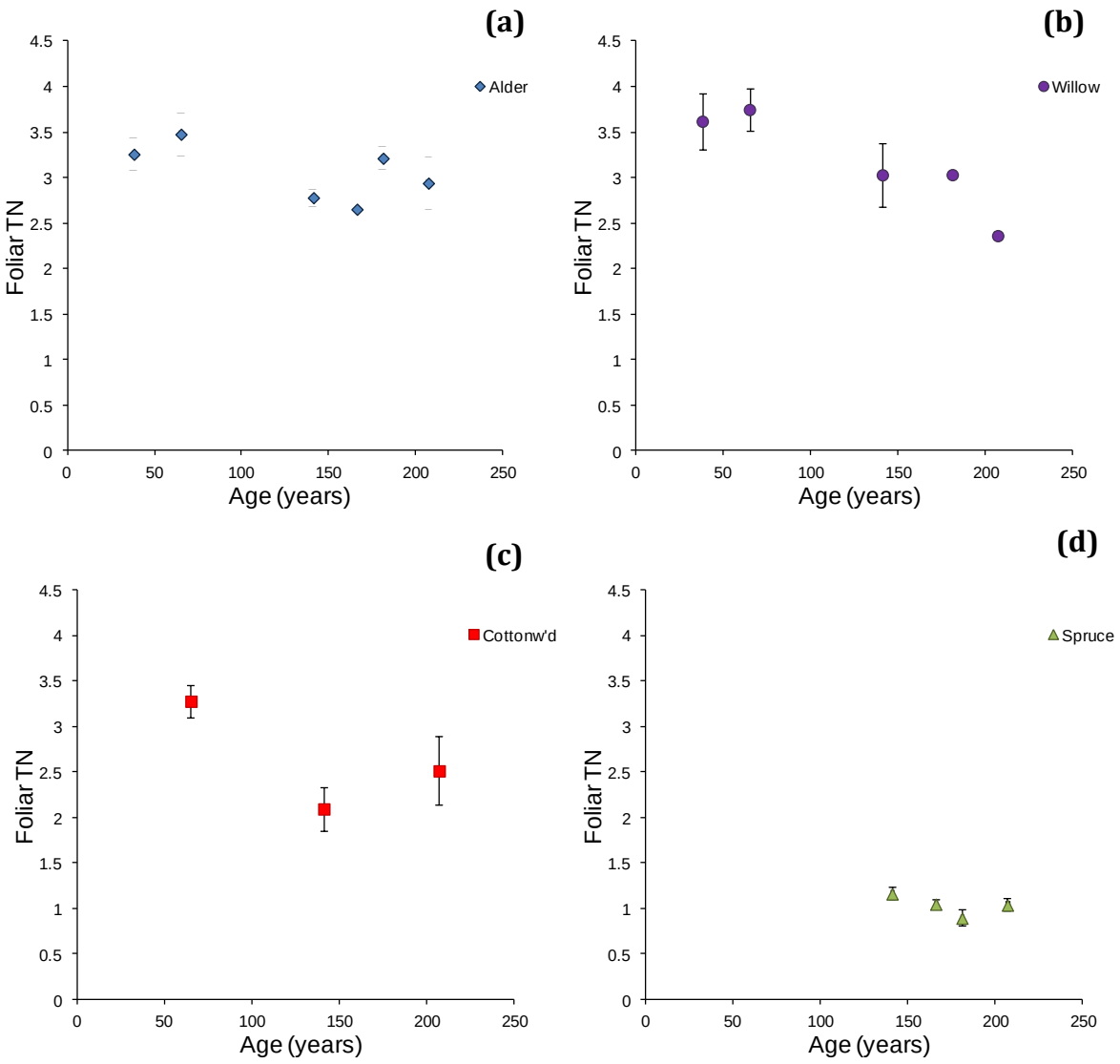

Figure 4-17: *Mean (1 S.E ±) foliar TN (%) for the sampled vegetation types (A. sinuatta (a), Salix* spp. *(b), P. trichocarpa (c) and P. sitchensis (d)) over time*

Foliar C:N ratios remained consistent over the chronosequence, except for *P. sitchensis* which showed a increasing trend with age, as well as the highest foliar values for any of the sampled vegetation (Figure 4-15). Foliar TN decreased over the chronosequence, though this was not significant (Figure 4-17). Forest N status was evaluated using the ^{15}N enrichment factor *(EF) of* foliar samples:

$$EF = \delta^{15}N_{foliar} - \delta^{15}N_{soil} \quad 4.1$$

The ^{15}N abundance in the soil surface layer (c.a. 10cm) was used to calculate the enrichment factor.

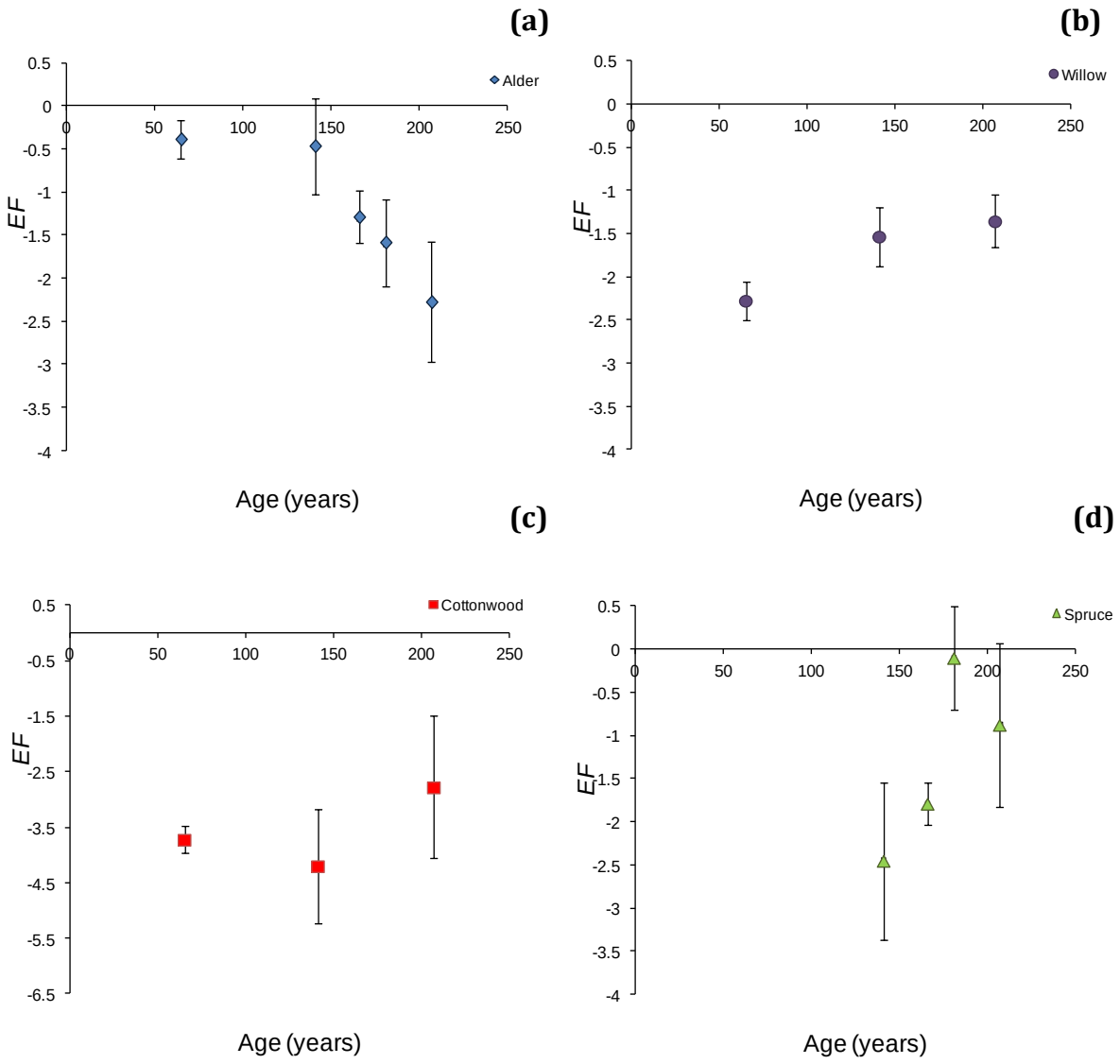

Figure 4-18: *Mean (1 SE±) enrichment factors (EF) over the chronosequence for sampled vegetation (A. sinuatta (a), Salix* spp. *(b), P. trichocarpa (c) and P. sitchensis (d)).*

EF increased with age within the non N-fixing vegetation types, N-fixing *A. sinuatta* became more negative as soil $\delta^{15}N$ became more enriched relative to its atmospherically derived foliar ^{15}N (Figure 4-18). Differences in enrichment factor did not vary significantly with age or site slope gradient.

4.3.6 **Catchment scale processes**

Mean values of soil nutrient (N g^{-1} km^2), organic matter (%) content and experimental derived microbial cycling rates (N- g^{-1} km^2 d^{-1}) were coupled with 22 years of catchment vegetation type information obtained using satellite remote sensing imagery (Figure 4-19). Using these data it was possible to estimate change in associated nutrient storage, turnover rates and emissions over a 22 year period from 1987 to 2009 using 5 satellite images taken during this period.

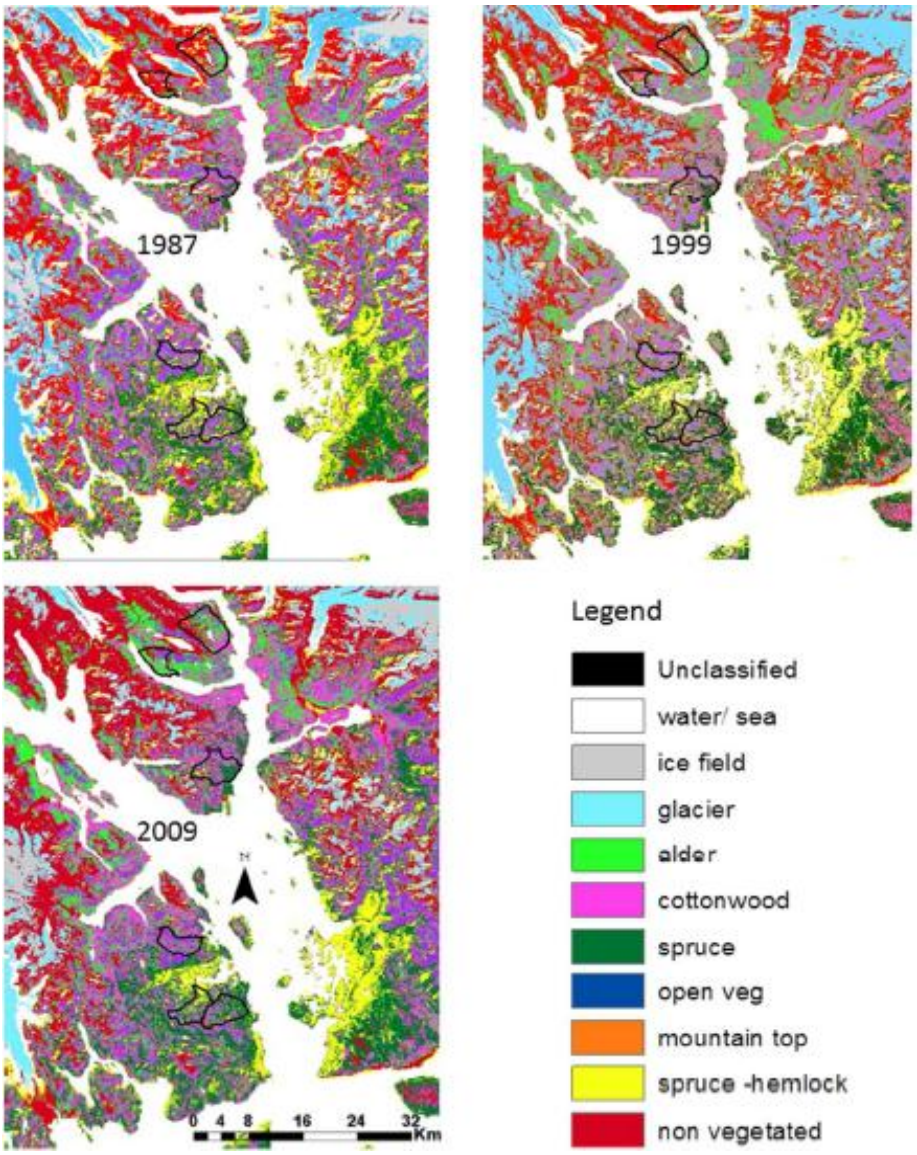

Figure 4-19: *Vegetation types of GBNP for three years over a 22 year period, study catchments are highlighted. (Klaar, et al., submitted).*

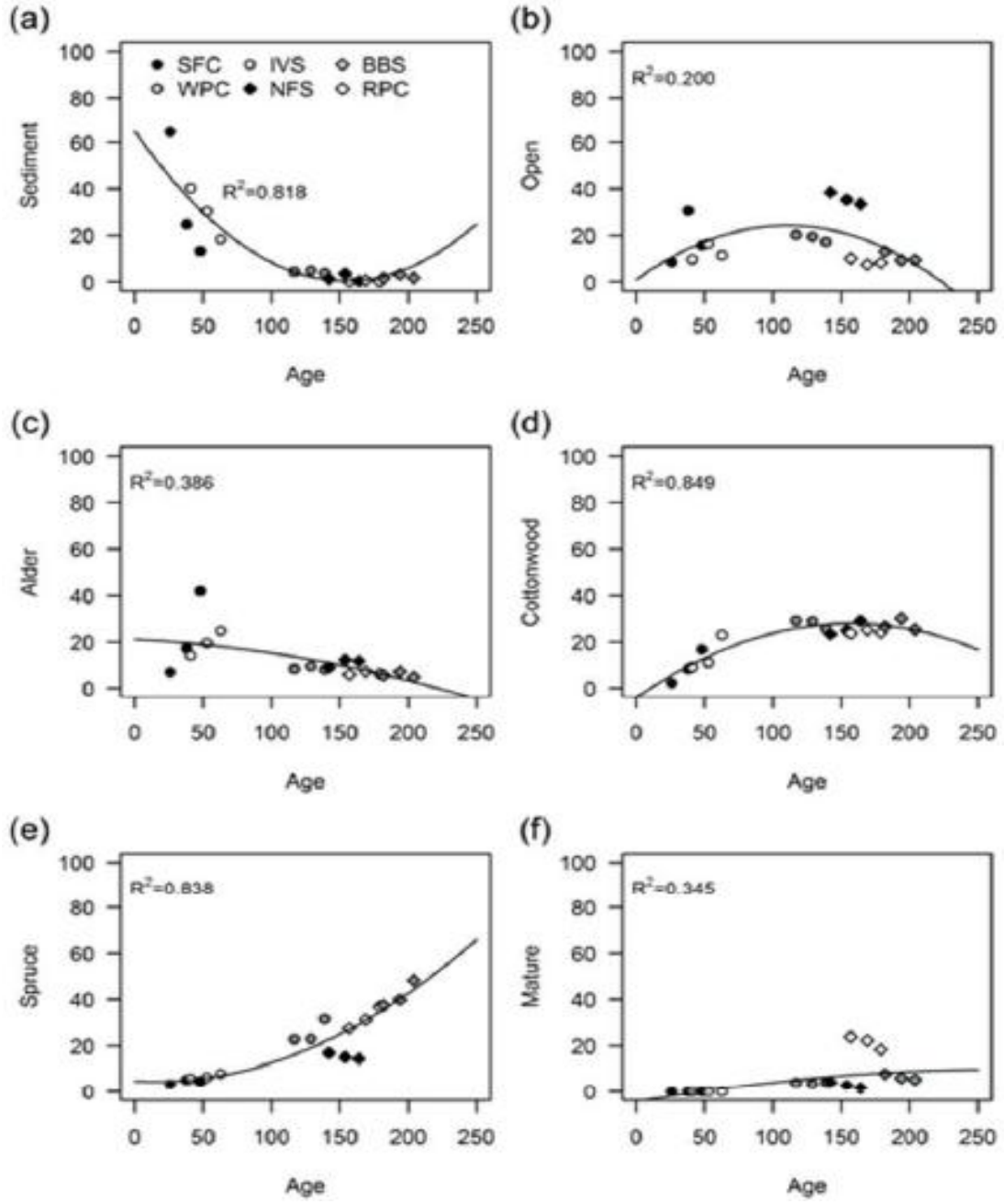

Figure 4-20: *Catchment vegetation type (%) change over the chronosequence for a) Sediment; b) open; c) A. sinuatta; d) P. trichocarpa; e) P. sitchensis; f) mature (Klaar, et al, submitted).*

The vegetation cover of these catchments changed over the past 22 years with vegetation succession, with newly exposed sediments near the top of the bay colonised by *A. sinuatta* vegetation, and *P. sitchensis* + *T. heterophylla* increasing in the older sites (Figure 4-19 and 4-20) (Klaar, *et al*, submitted). Vegetation change and soil development over time will influence the nutrient characteristics of the soil and the

microbial cycling occurring in these soils as inputs and physical characteristics change over this time period. These broad influences were then use to estimate soil processes occurring within a certain vegetation types, using spatial extent to scale up these processes to the watershed scale (Figure 4-21).

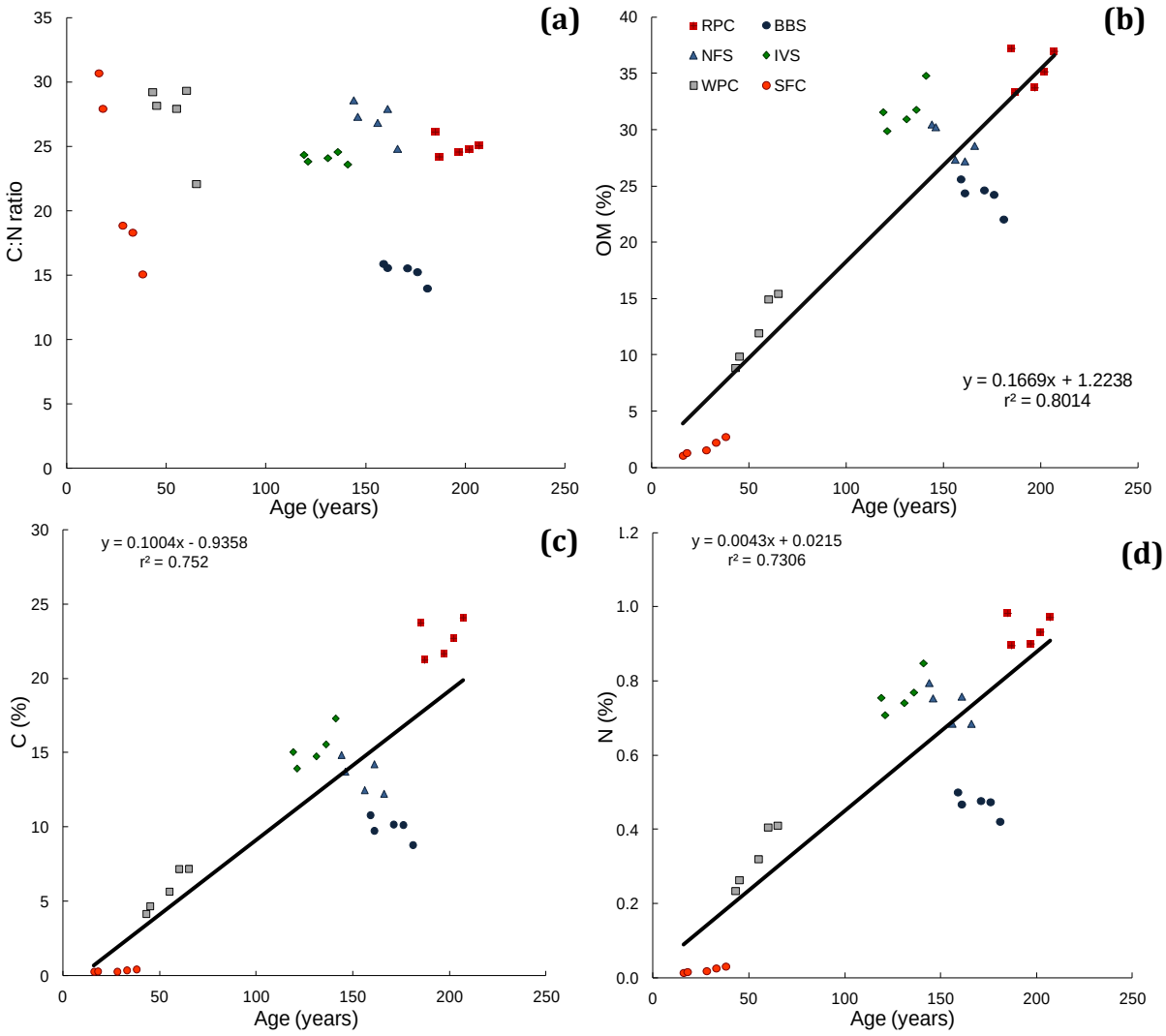

Figure 4-21: *Estimated change over 22 year period within study watersheds with linear regressions for soil: a) C:N ratio (no significant regression); b) organic matter content (%); c) C (%) and d) N (%). (RPC: Rush Point Creek; BBS: Burg Bay South; NFS: North Fingers Stream; IVS: Ice Valley Stream; WPC: Wolf Point Creek; SFC: Stone Fly Creek).*

The estimated percent soil OM, N and C increased over time as vegetation changed, although two of the mid-late successional watersheds deviated from this trend. Soil C:N ratio showed no distinct pattern with over age (Figure 4-21).

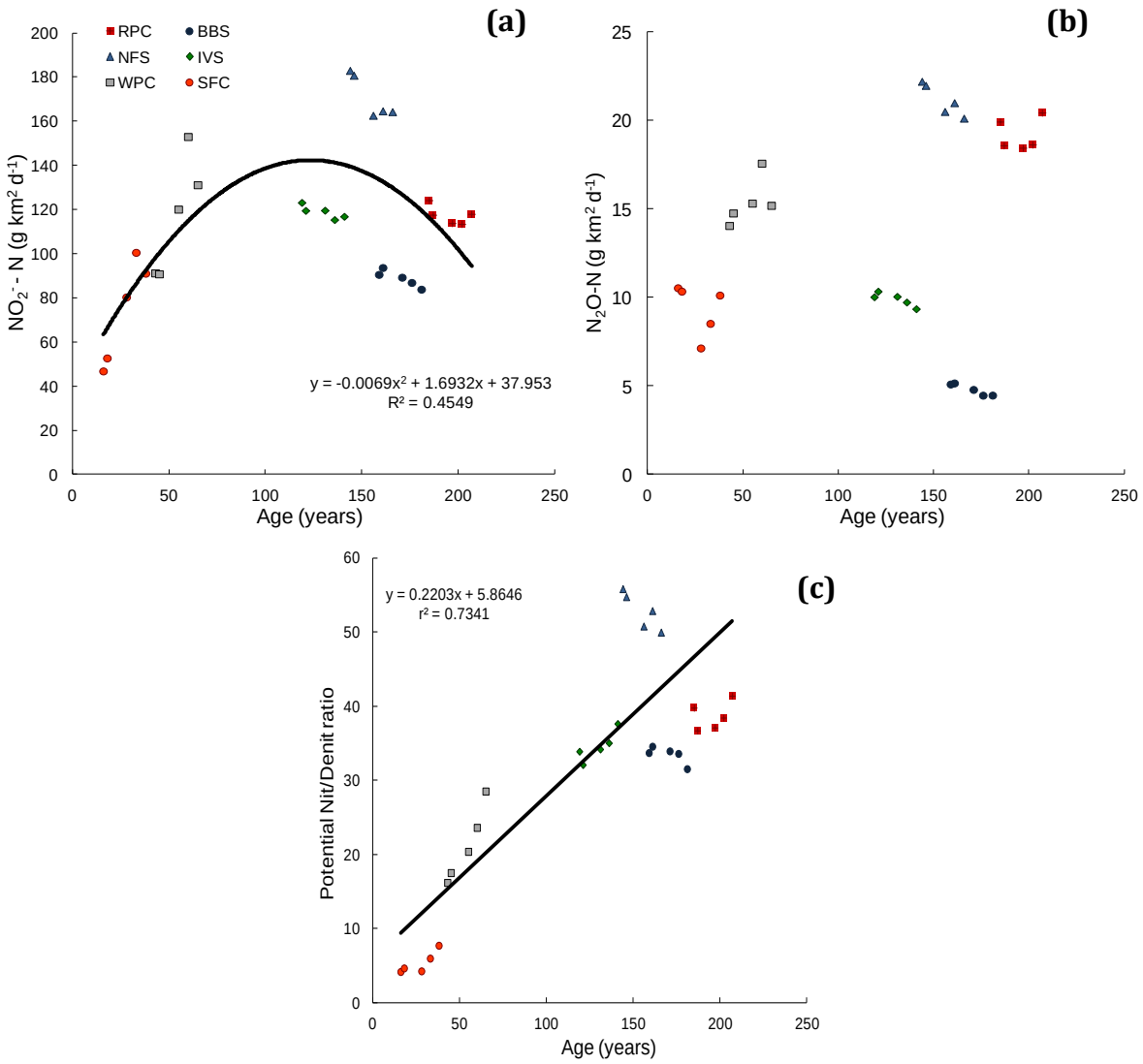

Figure 4-22: Estimated change over 22 year period for a) potential nitrite oxidation (polynomial regression); b) denitrification potential; c) ratio between potential nitrification and denitrification (linear regression). (RPC: Rush Point Creek; BBS: Burg Bay South; NFS: North Fingers Stream; IVS: Ice Valley Stream; WPC: Wolf Point Creek; SFC: Stone Fly Creek).Potential nitrite oxidation rates increased markedly during early successional vegetation development, peaking at 166 years before decreasing in the older watersheds, showing a humped shape response to succession. Potential denitrification enzyme activity is sporadic over the chronosequence, but does show consistently higher rates in the oldest stream catchment (RPC). Availability of NO_3^-, as shown by the ratio of potential nitrification against potential denitrification (DEA)

increased with stream age (Figure 4-22). The slump during mid-late succession (NFS and BBS) is reflected across OM, soil C and N and within the potential microbial data.

Table 4-9: *Statistical data for regression analysis (ANOVA).*

Modelled variable	R^2	F
OM (%)	0.802	136.678
C (%)	0.752	84.910
N (%)	0.731	75.948
Potential nitrification	0.454	8.782
NEA/DEA ratio	0.741	63.508

4.4 Discussion

4.4.1 Catchment scale processes

Landsat images of GBNP have been used to indicate vegetation change over the past 22 years (1987-2009) (Klaar, et al, submitted). This study has utilised these data to provide change in vegetation type within our study watersheds over this time period for use as a proxy of underlying soil development after glacial recession. This is made possible due to the close link between vegetation and soil systems as indicated in this study and earlier research, confirming H_1 and H_3 (see Bardgett & Walker, 2004; Chapin & Walker, 1994; Edwards *et al*., 2006; Knelman *et al*., 2012; Tscherko *et al*, 2005).

By linking dominant vegetation with underlying soil variables this thesis has quantified the rate of change within recently deglaciated areas over c.a. 200 years. Regressions of the predicted variables showed a significant linear increase in soil N, C and OM (%) over time. Particularly rapid rates of accumulation were predicted during early successional stages, as bare sediment is colonised by vegetation leading to the input and decomposition of organic matter. The deviation from this trend in mid to late

successional ages was caused by the low values found in the samples from these stream ages, particularly in BBS, within the dominant *P. sitchensis* forests compared to the other ages. Interestingly NO_3^- availability over time, shown by the ratio between potential nitrification and denitrification, in these mid- late successional sites fitted closely with the linear increase displayed by the other sites, suggesting that NO_3^- availability is similar despite the differences in soil N and C (%). Potential nitrite oxidation and denitrification also showed a rapid increase during early successional *A. sinuatta* development, stabilising to a plateau in the older streams. However denitrification enzyme activity (with and without C_2H_2) showed lower values than nitrification and is more variable throughout the later successional periods, due to the sporadic nature of this process. This thesis demonstrates the utility of such a method, confirming H_3 that such an approach could be successfully applied to the watershed scale.

4.4.2 **Driving influences on soil properties**

Vegetation cover is the dominant factor driving changes in soil properties and microbial activity over time. Over the different stream ages within a particular forest type, soil variables did not differ significantly, meaning that site age was not a clear influence independent of vegetation type. Also differences in slope between sites did not influence most soil properties or potential microbial activities; with the exception of soil inorganic N. Slope gradient did significantly influence the amount of inorganic N with higher concentrations found in areas of low slope compared to high. This difference in N retention is clear across age and vegetation type and is most likely due to the highly mobile nature of NO_3^- in the environment, moving to low sloping areas with water movement. Unexpected patterns were also observed with larger amounts of soil OM, moisture and fine grain size fractions found in the some medium sloping sites. Slope did not significantly influencing the majority of soil physical, chemical and microbial variables meaning this is not a confounding variable in the scaling up of watershed processes using Landsat imagery.

Percent soil N, C and OM was significantly influenced by vegetation type supporting H_1 that vegetation change over time, with vegetation colonisation and successional

development, would alter soil characteristics. Comparable soil physico-chemical properties have been reported in similar forest types in Alaska (D'Amore, 2010) and other regions around the world (Degrange, 1998; Tscherko, 2004). Young newly exposed glacial sediments are physically driven environments subject to physical and chemical weathering that leads to the leaching of organic matter and nutrients (Gurnell, 2011; Luizao *et al.*, 2004; Milner, 2007). Bacteria, lichen and finally plant colonisation stabilise sediment structure thereby reducing erosion, with many of these pioneer species fixing N from the atmosphere. This is incorporated into the soil layer by decomposition and mineralisation, thereby improving soil resources for further colonisation and development (Bardgett, 2004; Milner *et al*., 2007). Continued colonisation over time increases vegetation cover and root system development, further stabilizing sediments and leading to the accumulation and decomposition of soil OM (Milner *et al.* 2007). Results indicate early sediments had a high bulk density and lower percent N, C and OM in early successional vegetation due to lack of input and leaching (Crocker & Major, 1955; Högberg *et al.,* 2006). These variables increased over time, primarily associated with the colonisation of N fixing species, such as *A. sinuatta*, which alter soil N dynamics by supplying nitrogen rich organic matter to the soil system which over time improves soil quality (Chapin, 1994; Milner *et al*., 2007; Naiman, 2005). It is clear that nutrient availability increases between these vegetation types also with a high C:N ratio found in bare sediment and the lowest under *A. sinuatta* vegetation.

4.4.3 **Vegetation change and microbial activity**

Potential nitrite oxidation was used to estimate the potential activity of nitrifying bacteria as this process is carried out by two closely related bacteria by the step wise oxidation of NH_4^+ to NO_2^-, followed by to oxidation of NO_2^- to NO_3^- (Wrage *et al*, 2001). Hence measuring one activity will give a good estimate of total nitrification potential (Niboyet *et al*., 2011). Unsurprisingly the lowest rates were found in the early successional sites of bare sediment, due to low N and C concentrations, highest levels were found in the oldest successional vegetation types of *P. sitchensis* and mature forest types. This is because of the well aerated nature of soil associated with coniferous

forests enabling greater levels of the aerobic nitrification processes (Ambus & Beier, 2006) and also the greater availability of NH_4^+-N found in these areas.

Denitrification enzyme activity (DEA) assays produce non limiting conditions - that is anaerobiosis, nitrate and C supply - to force the metabolism of these bacteria for a short period of time to prevent population growth (Smith & Tiedje, 1979). Assays can be used to determine a soils maximum denitrification capacity, providing an indirect evaluation of the denitrifying community present, and provides information on their physiological capacity (Pinay *et al*., 2003). This study found significant denitrification activity across the chronosequence, varying between age, slope and dominant vegetation cover. Lowest rates were found in the early successional land covers, sediment and *D. drummondii*, of the youngest stream SFC (38 years). This is because these relatively young soils lacked the necessary chemical (N and C) and physical (anoxia) conditions required to sustain denitrifying bacterial populations, particularly in areas with no vegetation cover. Exposed sediments lack OM and N input from vegetation, and had a coarse sandy texture resulting in the leaching of essential nutrients. Contrasting these DEA rates with established stands of *A. sinuatta* at SFC, and those of the second youngest stream WPC (65 years), showed significantly larger microbial communities within these *A. sinuatta* covered sites. Early successional vegetation have altered the soil system, as demonstrated by increasing levels of OM and inorganic N in *A. sinuatta* stands, increasing soil C and N, accordingly thereby improving soil conditions for the occurrence of DEA. Denitrification community size peaks at the mid successional age (141 years) with mixed *P. trichocarpa* and *P. sitchensis* forest.

Lower levels of DEA occurred in the later successional immature *P. sitchensis* and *T. heterophylla* forest types compared to nitrification, which in some areas outstripped DEA by up to 40 x. The disconnect between these N cycling processes may have been driven by physical differences in soil properties as in the mid successional stage deciduous vegetation (*P. trichocarpa*, *A. sinuatta*) dominates the forest resulting in a compact, moist soil layer more conductive to episodic fluctuations in oxic/anoxic conditions that also have a supply of labile C and N from the overlaying vegetation which is more conducive to denitrification activity, as shown in this and previous

research (Ambus & Beier, 2006; Chapman, 2006; Orwin *et al*., 2010; Wardle, 1999). Whereas older sites are able support larger activity of nitrifying bacteria, as these immature *P. sitchensis* forest usually have more aerated soil (lower bulk density) supplying recalcitrant forms of C and N which is less favourable to denitrification (Ambus & Beier, 2006). As expected in the uninhibited treatment N_2O production is lower than in the presence of C_2H_2 which inhibits the N_2O reductase enzyme blocking it from complete reduction to N_2. The changing microbial activity and nutrient content of soils over time supports that second hypothesis that these variables would change over time, in conjunction with vegetation development.

The ratio between potential nitrification and denitrification showed that over time there is an increase in the availability of NO_3^- to the soil system potential nitrification out strips potential denitrification. There is a higher potential for NO_3-N production in *P. sitchensis* forest compared to earlier systems, as is reflected by the changing isotopic ratios (^{15}N and ^{13}C) of the soil and vegetation. Soil $\delta^{15}N$ under *P. sitchensis* is negative overall but over time when samples are split by site age, these values increase to low positives. This enrichment could be due to the loss of the light N isotope via DEA, leaching and immobilisation by plants and bacteria over time leading to the enrichment of soil $\delta^{15}N - NH_4^+$ available for *P. sitchensis* uptake, and furthermore this enriched *P. sitchensis* needles feeding back into the soil system. These data confirm the second hypothesis (H_2) that over time soil microbial communities would develop, increasing in conjunction with vegetation development.

4.4.4 **Foliar and soil indicators**

As *A. sinuatta* fixes atmospheric nitrogen you would expect its foliar N concentration and isotopic signature to remain stable and consistent across topographical and chronological gradients as shown. The enrichment factor (EF) between *A. sinuatta* foliar and soil $\delta^{15}N$ became more negative with increasing age, as the soil N pool became more enriched compared to the atmospheric signal confirming the changing soil N content of H_2. The EF associated with *P. sitchensis*, increased with site age indicating a more N

availability and therefore more fractionation in older sites (Michener & Lajtha, 2007). This is not reflected in increased insitu levels of soil N however due to its efficient rapid removal from the soil by plant and microbial uptake.

Increased N availability will result in N isotope fractionation as microbes are able to discriminate against the heavier isotope resulting in an enriched soil pool, whereas in N poor environments the whole pool is utilized by microbes. Denitrification enriches $\delta^{15}N$ in the remaining soil NO_3^- which is then available for plant uptake and assimilation into biomass causing the ^{15}N enrichment (Garten, 1993; Sebilo *et al*., 2006). Comparing foliar $\delta^{15}N$ of *Salix* spp. and *P. sitchensis* should give an indication of the soil pool of NO_3^--N and NH_4^+-N respectively, through *Salix* spp.'s preference for NO_3^- and *P. sitchensis*'s preference for NH_4^+ (20% NO_3^- and 80% NH_4^+) (Hobbie, 1998; Ingestad, 1979; Lee and Stewart, 1978). *Salix* spp. leaves were collected from within the dominant vegetation species of each study stream where possible, and so are present across all successional phases of development. The lowest $\delta^{15}N$ value was recorded at WPC (65 years) with a similarly low value recorded at the SFC (38 years). After which foliar values increases over time becoming more positive, yet remaining negative throughout with highest values recorded at RPC (207 years). IVS (141 years) exhibited the greatest potential DEA in stands of mixed *P. sitchensis*/*P. trichocarpa* than any over stream yet *Salix* spp. foliar $\delta^{15}N$ remains negative in this area as did *P. sitchensis*. This is a further indication, along with the potential microbial data, that nitrification is outstripping DEA, producing more NO_3^- than can be denitrified, and diluting the denitrification signal in the soil N available for plant uptake in these mid successional sites.

The low levels of soil NO_3^- with high levels of NH_4^+ can be explained by a number of interacting processes removing the excess NO_3^- from the environment quickly, including DEA, plant and heterotrophic bacterial immobilisation and soil leaching. The relative abundance of NH_4^+ and lack of isotopic fraction observed in *P. sitchensis* needles was due to the high levels of OM decomposition with *A. sinuatta* and *P. trichocarpa* this present in these mid successional forests, though this is not directly measured here.

In the older sites where dominant vegetation changes to *P. sitchensis* and mature vegetation *P. sitchensis* $\delta^{15}N$ increases to low positive values in the oldest site RPC (207 years) along with an increase in *Salix* spp. $\delta^{15}N$. Again it appears that nitrification is outstripping denitrification at these sites, causing an enrichment of the soil NH_4^+-N pool, indicated by the increasing foliar $\delta^{15}N$ and EF of *P. sitchensis* trees preferentially using NH_4^+-N as an N source (Hobbie, 1998; Ingestad, 1979; Lee and Stewart, 1978). Within *P. sitchensis* forests foliar $\delta^{15}N$ becomes more enriched over time indicating a recycling of enrichment organic matter within the soil-vegetation system, as the heavier isotope is retained and the lighter lost from the soil system by denitrification and mobilisation. The NH_4^+ pool of these later successional forest types are of a similar size to the mid successional, though the higher C:N ratio and poorer quality of *P. sitchensis* litter will cause slower decomposition rates, slowing the release of NH_4^+ also potentially resulting in the more enriched *P. sitchensis* foliar signal. Similarly the very low concentrations of extractable NO_3^- in these forests along with low potential and insitu N_2O emissions indicate that a large amount of heterotrophic microbial immobilisation is occurring.

Foliar C:N ratio has widely been used as a index of organic material quality, plant tissue with a C:N ratio of <25 is considered of good quality and favourable for fast decomposition to occur, whereas a ratio of >25 is of lower quality and therefore slower to decompose (Myers *et al*, 1995; Luizoa, 2004). *Salix* spp., *P. trichocarpa* and *A. sinuatta* leaves are of good quality, however *Salix* spp. leaves collected from high sloping areas showed a significant trend of lower C:N ratios compared to the other gradients, suggesting these are of a higher quality for decomposition. C:N ratio data indicates that slope steepness does influence N availability. Soil under these vegetation species matched their ratio closely; however soil under *P. sitchensis* vegetation, which had a higher poor quality ratio, displayed a higher ratio, which increased with site age, indicating that over time the accumulation of poorer quality litter fall begins to change the soil quality. *P. sitchensis* C:N ratios are high compared to the other species consistent with the fact that these provide poorer quality organic matter for decomposition than

the deciduous species, as has been extensively supported in previous research (Ambus & Beier, 2006; Klemedtsson *et al*, 2005; Luizao *et al*., 2004).

The decrease in foliar $\delta^{13}C$ of each sampled vegetation type with increasing site age is an interesting trend as this variable is influenced by a number of interacting environmental factors, such as site elevation, topography, water availability, atmospheric CO_2, and N availability, with fractionation occurring during diffusion through the stomata (4.4‰) and during photosynthesis (27‰) (Garten & Taylor, 1992; Matsushima *et al*, 2012). Samples taken from sites with similar climate, topography and elevation eliminated these as causes of variation, leaving water and N availability as possible causes. Increased water supply results in more negative values as CO_2 supply through the stomata is facilitated by water availability, however this is generally observed in seasonally dry environments were plants experience drought stress (Korol, 1999; Warren, 2001). Meanwhile increased N availability in less negative $\delta^{13}C$ in plant tissue was due to rapid CO_2 consumption via carboxylation during photosynthesis (Matsushima *et al*., 2012). Regression analysis was performed plotting $\delta^{13}C$ against soil N and moisture to gain a mechanistic understanding of these influences; however neither displayed any significant correlations. These data seem to indicate that during early succession with the abundance of N fixing species and less completion due to the smaller N cycling microbial activity, N availability to plants is greater, resulting in more photosynthesis and less negative $\delta^{13}C$. Similar isotopic values have been observed in comparable forest types (Hobbie, 1998; Matsushima, 2012).

4.5 Conclusions

Data showed that over time soil properties and microbial communities developed as would be expected in primary successional habitats. There is an accumulation of OM and N in the soil layer from low levels in the young early successional sites over time as vegetation and soil layer develop. Additionally the physical nature of the soil changes from a dense coarse sediment layer, through to a more aerated finer grain size in the later successional periods. With these changing soil characteristics the potential microbial activity also change over the chronosequence from very low in early

succession to higher levels in mid to late aged sites. The slope of the site does influence soil nutrient concentrations, with higher concentrations of inorganic N found consistently at lower sloping areas. The only significant difference between potential microbial activity was observed between different vegetation types, with these influencing this by providing different inputs of nutrients to the soil system for microbial cycling.

The greatest influence on these soil properties and microbial communities was vegetation cover. We were therefore able to discount catchment topography and estimate soil properties at the catchment scale for current and past environments. Over a 22 year period we were able to use vegetation cover to predict change within a recently deglaciated environment. This method revealed that the majority of sites followed a similar successional trajectory within GBNP. Soil N, C and OM (%) showed linear increases over the chronosequence. Also potential microbial N cycling and N availability increased over time, with youngest successional ages showing the greatest increase during early vegetation colonisation. Potential microbial activities showed an initial increase during early successional period, with denitrification then becoming more variable, and nitrite oxidation levelling out with increasing age.

4.6 References

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5: Water chemistry of Glacier Bay

5.1 Introduction

Small headwater streams, defined as first order perennial streams, have a profound influence on downstream river water chemistry and quantity, contributing up to 70% of mean annual water volume and 65% of nitrogen (N) flux to second order streams and 55% and 40% in forth and higher order rivers (Alexander, 2007). Riparian vegetation attenuates fluxes of nutrients (N and P) and sediments from the terrestrial system, changing their timing, magnitude and chemical form (Burt, 2010; McClain *et al*., 2003; Naiman and Decamps, 1997) Streams are a major source of dissolved organic carbon to global oceans transporting an estimated 0.25 Gt annually (Hedges, 1997; Jiao *et al*., 2010). Fluxes of both N and C from watersheds provide nutrient subsidies to coastal marine environments increasing their productivity and forming the basis of heterotrophic food webs in these areas (Asmala *et al*., 2013). Differences in land use and cover have been shown to significantly alter riverine, estuarine and coastal water chemistry (Jickells, 1998; Sachse, 2005). Vegetative cover influences hydrological flow paths, changing the nature of terrestrially derived dissolved organic carbon (DOC) and nitrogen (DON). The bioavailability of nutrients can be altered during transport through the river system, through processes such as photochemical oxidation and biological transformation in lake systems (Mattsson, 2005; Tranvik *et al*., 1999). Episodic climatic events, such as floods, change transport processes between the terrestrial and aquatic environments. During base flow, riverine DOC/DON is derived from through flow in low mineral dominated soil horizons. Whereas during storm flow events DOM is derived from water flow through litter and upper soil horizons, greatly altering DOM bioavailability and concentration (Asmala *et al.*, 2013; D'Amore, 2010; McKnight, 1994). Changing climatic conditions will alter the nature of riverine subsidies to estuarine environments affecting productivity, nutrient cycling, gas emissions and species diversity (Asmala, 2013).

In sub-Arctic regions riverine inputs are changing as watershed ice sheets, permafrost and glacial coverage recede due to global warming (Hood, 2008). Hood, et al (2008) in their study of three adjacent river watersheds in south eastern Alaska, demonstrated river water chemistry varied with percent glacier cover (0%, 25% and 55%). Differences in glacial cover changed the available pathways and nutrient sources to surface waters. As glacier extent reduced newly exposed land surfaces were colonised by early successional N fixing plants, which altered watershed soils increasing the metabolism of DOC and DIN in the river system. Peak DIN concentrations were observed in the watershed with 25% glacial cover, which was dominated by early successional *D. drummondii* and *A. sinuatta* (Hood, 2008). How vegetation successional development will influence water chemistry over a longer period after glacial recession is still poorly understood. Understanding how surface water chemistry changes temporally following glacial recession will provide insights into the long term riverine solute yields, and downstream influences on coastal productivity and biodiversity. Glacier Bay National Park (GBNP) provides an opportunity to quantify how surface water chemistry changes over a relatively short time scale (38-207 years) since glacial retreat. Helping to determine how the development of watershed vegetation and complexity, such as the growth and development of forested wetlands and peat lands will change river water chemistry in these regions over time.

The objectives of this study were to; 1) assess the change in surface water chemistry of six streams of different ages in GBNP, 2) determine the effect of watershed and sub catchment vegetation cover on surface water chemistry. We hypothesize that differences in vegetation cover will affect water chemistry, altering riverine exports of DOC and TN as well as their bioavailability, at the watershed and sub catchment scale. Also that hyporheic water chemistry will be influenced by age, vegetation cover.

5.2 Methods

For surface and hyporheic water sampling strategy and analysis methodology see section 2.4, and for statistical analysis see section 2.5.

Annual nutrient flux (kg km^{-2} yr^{-1}) from each catchment was derived by:

$$\text{mg m}^{-3} = \text{conc.} \times 1000 \tag{5.1}$$

Where conc. = concentration (mg L^{-1}), then:

$$\text{mg km}^{-2}\,\text{s}^{-1} = \frac{(\text{mg m}^{-3} \times \text{Q})}{\text{Catchment area}} \tag{5.2}$$

Where Q = discharge (m^3 s^{-1}) and Catchment area = km^2. Then:

$$\text{kg km}^{-2}\,\text{yr}^{-1} = \frac{(\text{mg km}^{-2}\text{s}^{-1} \times 86400 \times 365)}{1000000} \tag{5.3}$$

Table 5-1: *Summary of the physical characteristics of the 6 study streams. (SFC- Stonefly Creek, WPC- Wolf Point Creek; IVS- Ice Valley Stream; NFS- North Fingers Stream; BBS- Berg Bay Stream; RPC- Rush Point Creek; Bo- Boulder; Co- cobble; Gr- gravel; QS- Quaternary deposites; Tg- tertiary intrusive (biotite granodiorite); Kg- Cretaceous intrusive (biotite-hornblende granodiorite and tonalite); Sc- Silurian-Devinian sediments and carbonates (Rendu Formation); Ss- Silurian sediments (Tidal Formation)). (Adapted from Roberson and Milner, (2006) and Hill et al, (2009)).*

Stream (I.D)	Age (years)	Reach gradient (%)	Stream length (km)	Drainage area (km^2)	Average annual discharge (m^3/s)	Average discharge (m^3/s)*	Discharge range	Av. Q per watershed area (m^3 km^2 s)	Stream order	Dom. subs.*	Geology
SFC	38			10		0.8	0.9	0.08	2	Bo	Qs^b, Kg
WPC	65	1.14	5.6	29.8	2.29	2.7	2.6	0.09	2	Bo	Qs, Kg^b
IVS	141	0.98	8.3	19.4	3.02	1.5	3.5	0.08	2	Co	Qs^b, Ss
NFS	166	1.14	8.0	16.8	5.65	1.1	1.0	0.07	2	Bo	Qs, Sc+Ss^b, Kg
BBS	181	0.8	7.2	33.1	4.95	1.7	2.5	0.05	3	Gr	Qs, Ss + Ss^b, Tg
RPC	206	0.88	6.6	23.3	7.51	1.2	1.9	0.05	2	Co	Qs, Sc + Ss, Tg^b

b Dominant geology.

** Average of the discharge measurements taken over study period (June-August 2010-2012).*

* Dominant substrate

Table 5-2: *Percent watershed land cover (Mature= mixed P. sitchensis and T. heterophylla forest; other = glacier, water, unidentified). Data provided by Klaar et al, submitted.*

Stream	% Sediment	% *D. drummond*	% *A. sinuatta*	% *P. trichocarpa*	% *P. sitchensis*	% Mature	% Other
SFC	13.2	15.5	41.9	16.8	4.6	2.4	5.1
WPC	18.3	11.3	24.8	23.1	8.0	4.9	9.6
IVS	3.8	17.2	8.13	25.7	31.6	4.0	9.6
NFS	0.6	23.9	13.2	28.2	21.6	5.8	6.7
BBS	0.2	8.26	6.4	24.1	39.9	18.4	2.6
RPC	3.4	10.1	7.9	25.0	43.4	8.1	2.1

Table 5-3: *Sub catchment drainage area (km²), average discharge (Q m³/s), range, and land cover (%). Data taken from Klaar et al, submitted.*

Stream sub-catchment	Drainage area (km²)	Average Q (m³/s)	Range of discharge	Land cover (%)					
				Sediment	*D. drummondii*	*A. sinuatta*	*P. trichocarpa*	*P. sitchensis*	Mature
SFC 1	3.14	0.41	0.39	2.6	28.3	46.2	13.9	0.9	1.7
SFC 2	0.23	0.0003	-	5.2	18.3	58.2	17.9	0	0
SFC 3	3.27	0.34	0.37	30.4	7.4	43.9	10.4	0.9	0.7
SFC 5	1.14	0.10	0.16	4.2	0	64.6	20.6	1.7	2.2
WPC 1*	29.8	2.38	0.82	18.3	11.3	24.8	23.1	8	4.9
WPC3	1.85	0.13	-	1.2	2.5	28.5	41.9	0.7	1.1
IVS 1*	11.87	0.95	0.79	9.9	21.8	12.6	26.4	13.6	6.4
IVS 2*	11.87	0.91	0.89	9.9	21.8	12.6	26.4	13.6	6.4
IVS 3	1.69	0.22	0.41	20.6	24	17	8.6	6.5	2.7
IVS 4	3.24	0.10	0.10	10.1	21.2	21	24.2	15.6	0.5
IVS-GIS-A	1.69	0.14	-	20.6	24	17	8.6	6.5	2.7
NFS 1*	16.8	0.93	0.5	0.6	23.9	13.16	28.2	21.6	27.6

* Sample taken from main channel

Stream sub-catchment	Drainage area (km^2)	Average Q ($m^3 s^{-1}$)	Range of discharge	Land cover (%)					
				Sediment	*D. drummondii*	*A. sinuatta*	*P. trichocarpa*	*P. sitchensis*	Mature
BBS 1*	13.61	0.40	0.35	0.2	11.8	14.8	28	34.4	9.2
BBS 2	0.68	0.02	0.01	0.1	1.3	2.1	12.2	45.3	38.8
BBS 3	1.36	0.004	0.01	0	0.2	1.6	16.1	42	39.8
BBS 4	3.82	0.17	0.27	0	1.2	1.8	11.5	41.9	43.4
BBS-GIS-B	1.6	0.12	-	0.1	6.9	14.1	26.5	35.6	16.7
RPC 1*	16.8	0.55	0.27	5.3	15	10.4	27.5	31.7	6.9
RPC 2	0.73	0.01	0.01	0	0	0	29.6	55.7	14.7
RPC 3	1.91	0.02	0.04	0	0.6	2.1	22.2	69.3	5.8
RPC 4*	19.68	1.08	1.3	3.4	10.1	7.9	25.1	43.4	8.1
RPC-GIS-C	1.36	0.01	-	0.1	20.3	8.1	24.6	35.7	7.5
RPC-GIS-D	2.7	0.01	-	1.3	4.6	3.9	17.7	53.3	18.1

* Sample taken from main channel

Sub catchments are those of tributaries to the main channel whilst other are taken from the main channel upstream of the stream mouth, with drainage basin and vegetation covers calculated using the remote sensing data, please note that 'mature' vegetation is a mixed of *P. sitchensis* and *T. heterophylla* forest (Klaar *et al,* in revision) (Table 5-3).

5.3 Results

Surface water samples were taken from river mouths and sampling points further up the stream system including tributaries and the main channel. The six study catchments were of different ages (Table 5-1) with different vegetation types (Table 5-2).

5.3.1 Stream outlet seasonal properties

Changes in discharge between various sampling campaigns are displayed in Table 5-4, variations between sampling campaigns is displayed in Figure 5-1.

Table 5-4: *Monthly comparison of river mouth discharges across study years. * indicates mean of two measurements taken that month; - no measurement.*

	Discharge ($m^3 s^{-1}$) comparison across sampling months and years								
	2010			2011			2012		
Stream	June	July	August	June	July	August	June	July	August
SFC (38 years)	1.39	1.2	0.3*	-	0.67	0.45	-	-	-
WPC (65 years)	3.59*	-	1.1*	2.86	-	-	-	-	4.0
IVS (141 years)	0.87	-	0.92*	1.67	-	1.1	-	-	3.79
NFS (166 years)	1.7	0.68	1.06	1.26	-	0.64	-	1.58	-
BBS (181 years)	2.89	0.4	3.4	-	1.14	0.96	-		1.17
RPC (207 years)	1.38*	0.69	0.57	-	1.2	0.53	-	2.4	-

For the first 2 sampling campaigns (2010-2011) discharges are generally highest in June with lowest flows in August. During the final campaign (2012) water samples are discharge measurements were taken only once per stream, and as shown in the table discharges were higher due to more rainfall during this summer period.

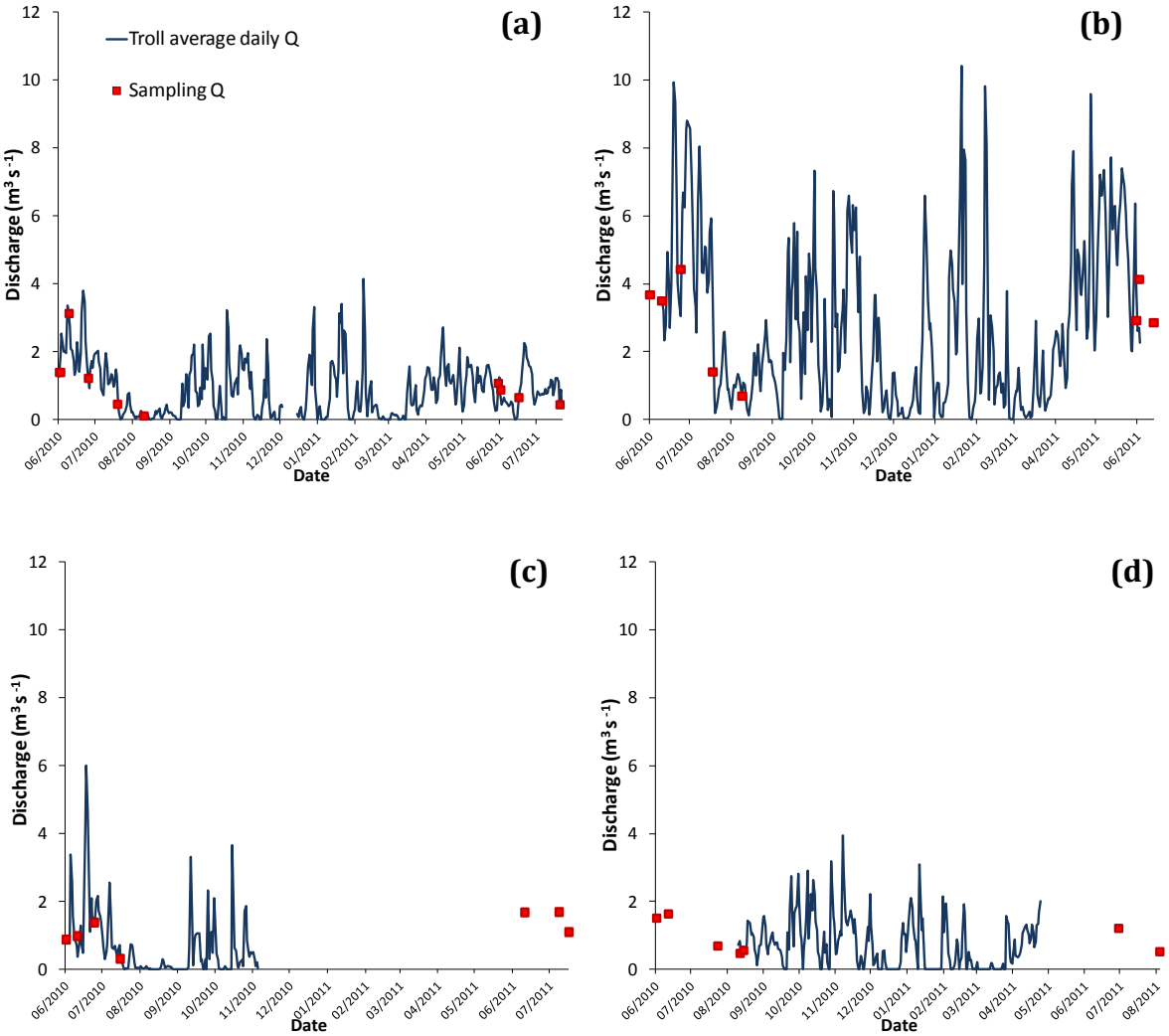

Figure 5-1: *Level Troll discharge data between 2010 – 2011 field campaigns with insitu discharge measurements. A) Stone Fly Creek; B) Wolf Point Creek; C) Ice Valley Stream; D) Rush Point Creek.*

In situ level trolls were installed at each river mouth to measure discharge throughout the year, unfortunately two trolls (BBS and NFS) were lost or recorded unusable data during this period, furthermore as shown the trolls at IVS and RPC had large sections of data that were not useable. Upon collection the trolls were covered partially or completely in sediment therefore it is assumed that this is the cause of the loss of data (Figure 5-1).

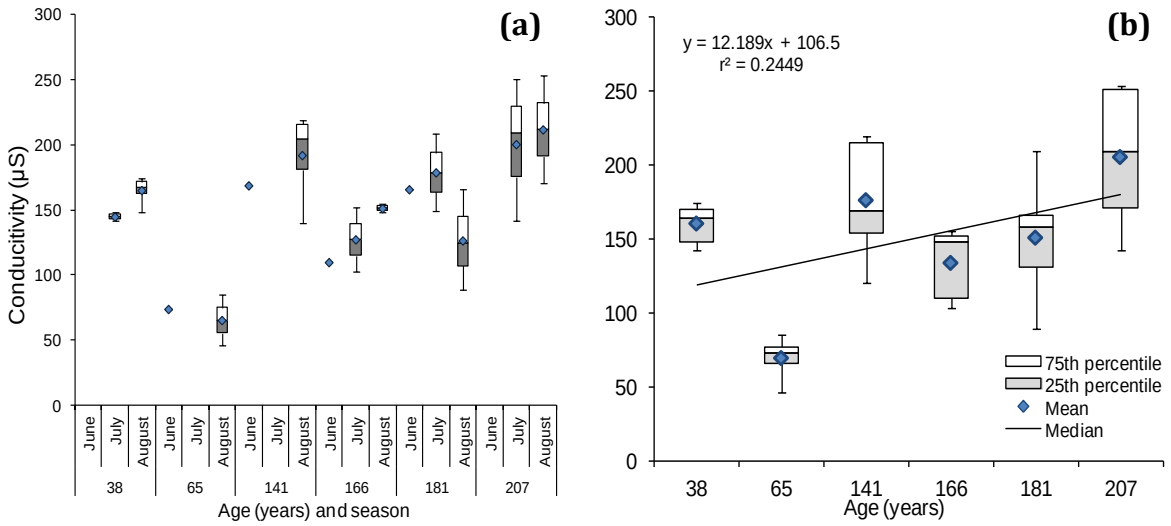

Figure 5-2: *a) Mouth monthly conductivity (µS) from June to August; b) mean values. (Whiskers indicate minimum and maximum values).*

Conductivity increased with stream age with higher levels generally observed in August (except BBS). Older streams (141-207 years) displayed higher values with more variation between sampling dates than the younger (38-65 years) streams. Early successional WPC (65 years) was significantly different to the mid to late successional streams of IVS (141 years) and RPC (207 years) (Kruskal-Wallis, df = 5, P <0.05).

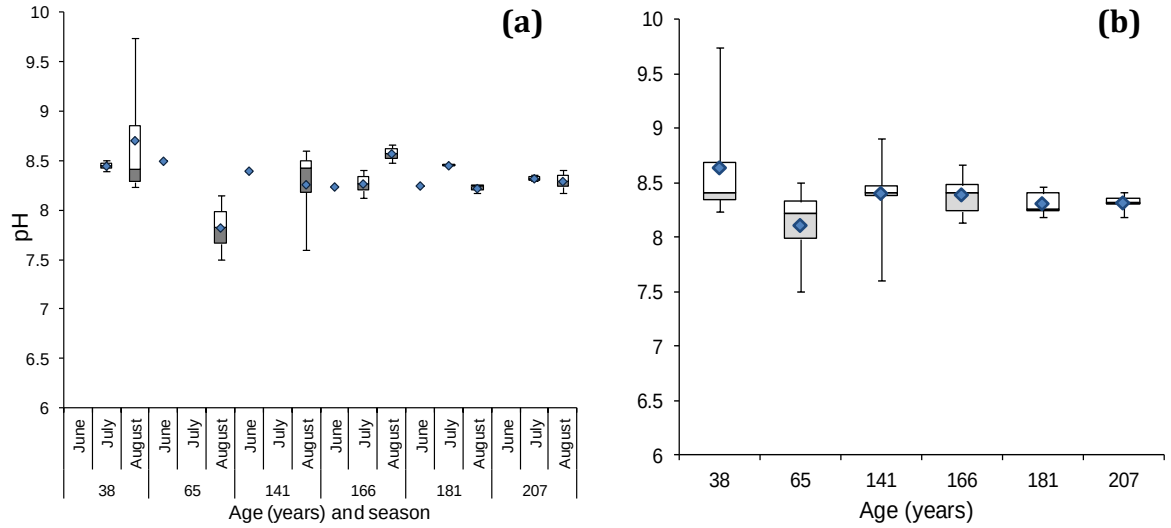

Figure 5-3: *a) Monthly pH from June to August; b) mean values. (Whiskers indicate the minimum and maximum values).*

Surface water pH values generally range around 8.5; but more variability was evident in the early to mid successional streams (38-141 year).

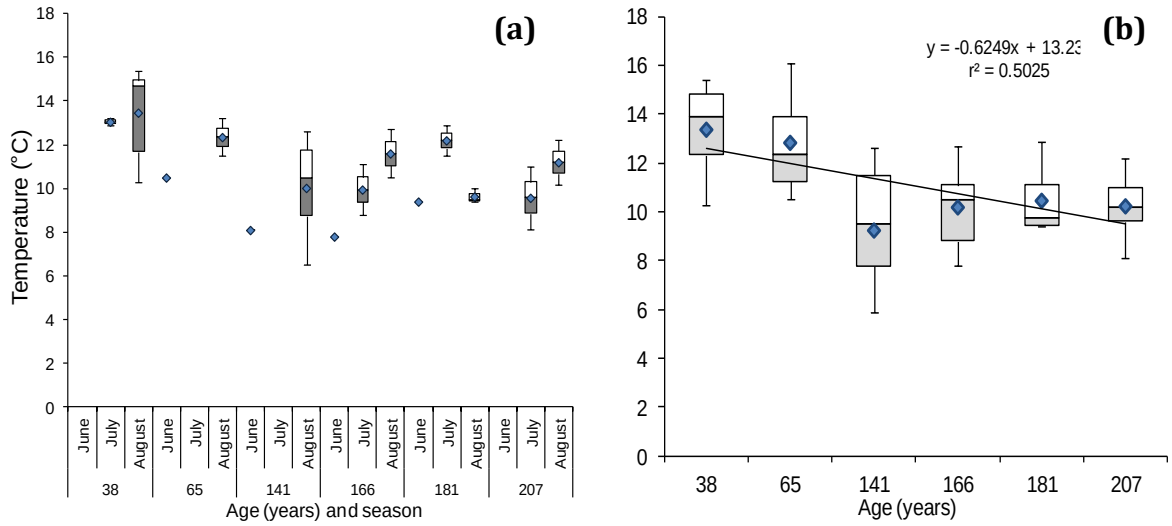

Figure 5-4: *a) Monthly temperature (°C) from June to August; b) mean values. (Whiskers indicate the minimum and maximum values).*

Water temperature (°C) decreased from the early successional sites to the mid and late successional streams, although this trend of changing water temperature is not statistically significant.

5.3.2 **Outlet nutrients**

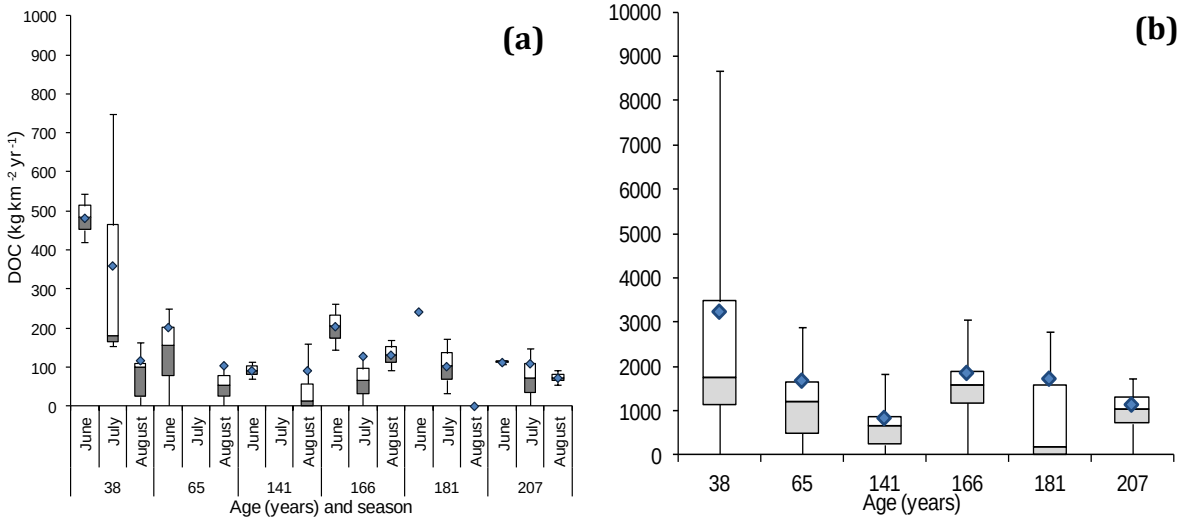

Figure 5-5: *a) Monthly DOC from June to August; b) mean values. (Whiskers indicate the minimum and maximum values).*

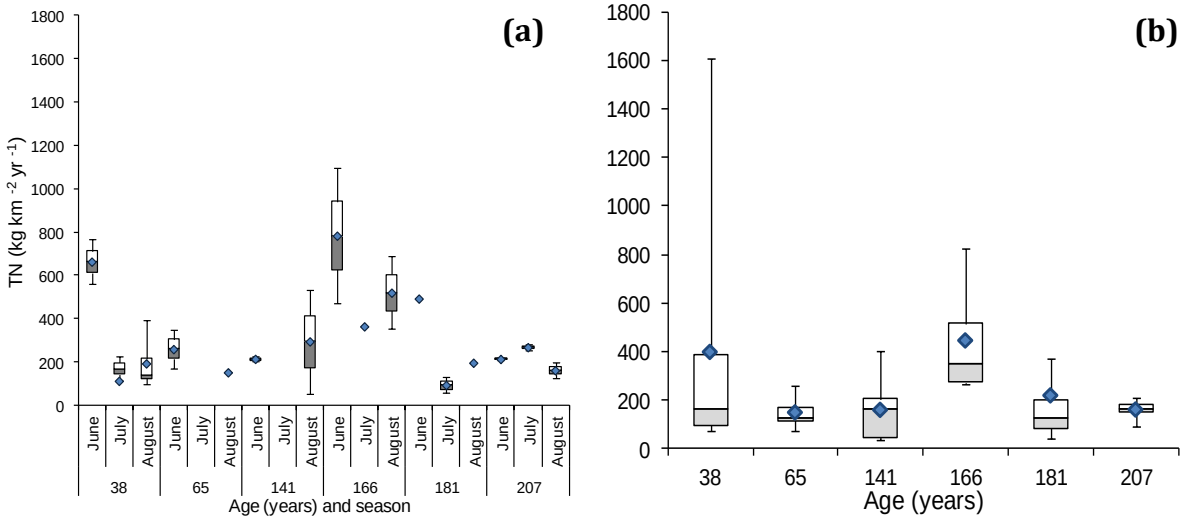

Figure 5-6: *a) Monthly TN from June to August; b) mean values. (Whiskers indicate the minimum and maximum values).*

DOC was variable across the chronosequence with the highest daily load in the youngest stream SFC (Figure 5-6). TN was also highly variable showing peaks in mean load at SFC (35 years) and NFS (166 years), corresponding with the greatest cover of early successional vegetation.

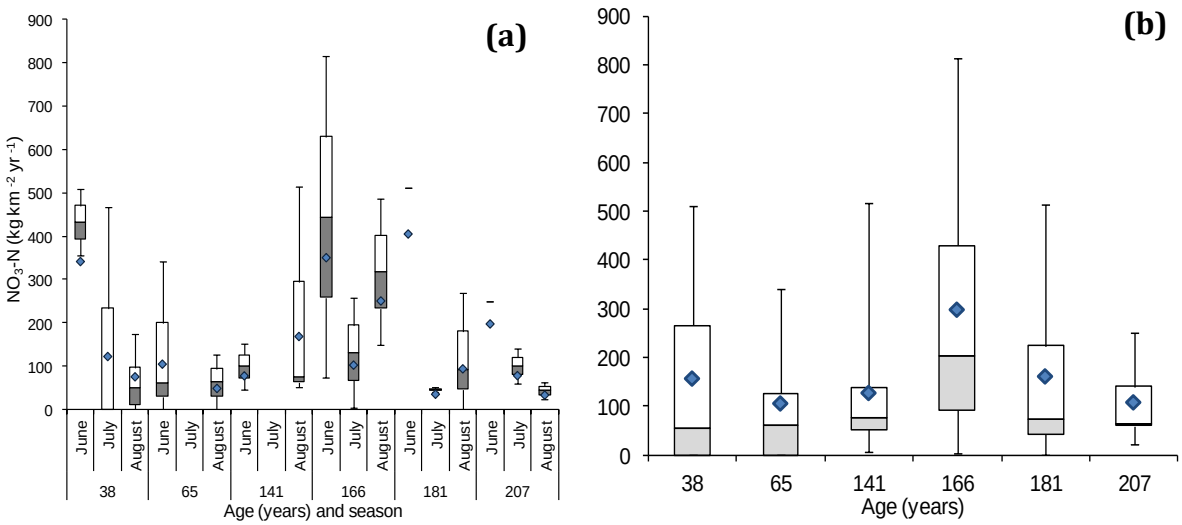

Figure 5-7: *a) Monthly NO_3-N from June to August; b) mean values. (Whiskers indicate the minimum and maximum values).*

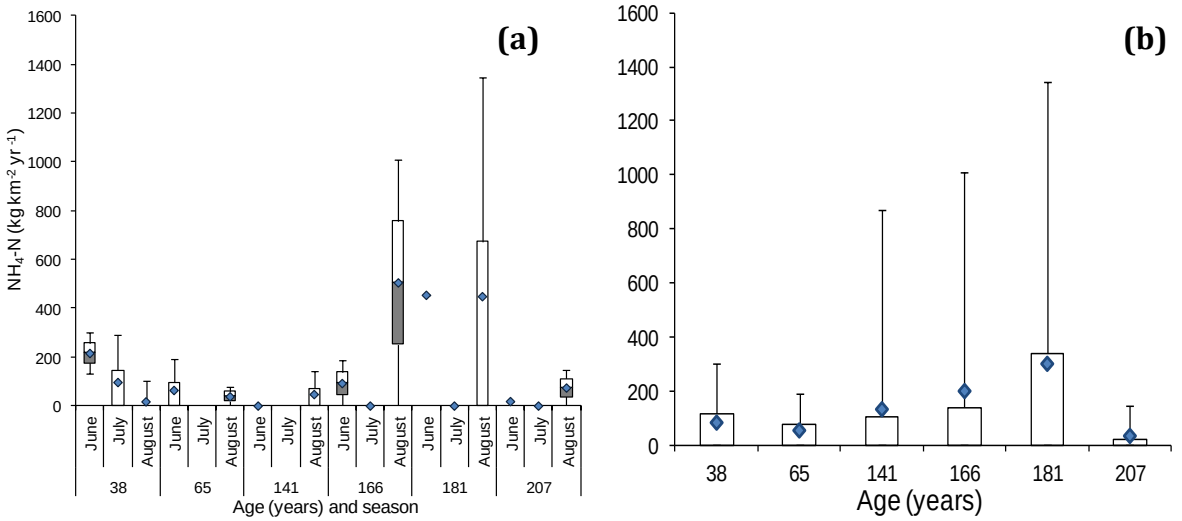

Figure 5-8: *a) Monthly NH_4-N from June to August; b) mean values. (Whiskers indicate the minimum and maximum values).*

Surface water NO_3^--N was highly variable across the chronosequence and seasonally, peaking in mid successional streams (Figure 5-7). Surface water NH_4^+ were generally below detection limits for most samples, but high peaks were observed in August in the older streams. Statistical analysis of these data revealed no significant difference between inorganic N collected during different months.

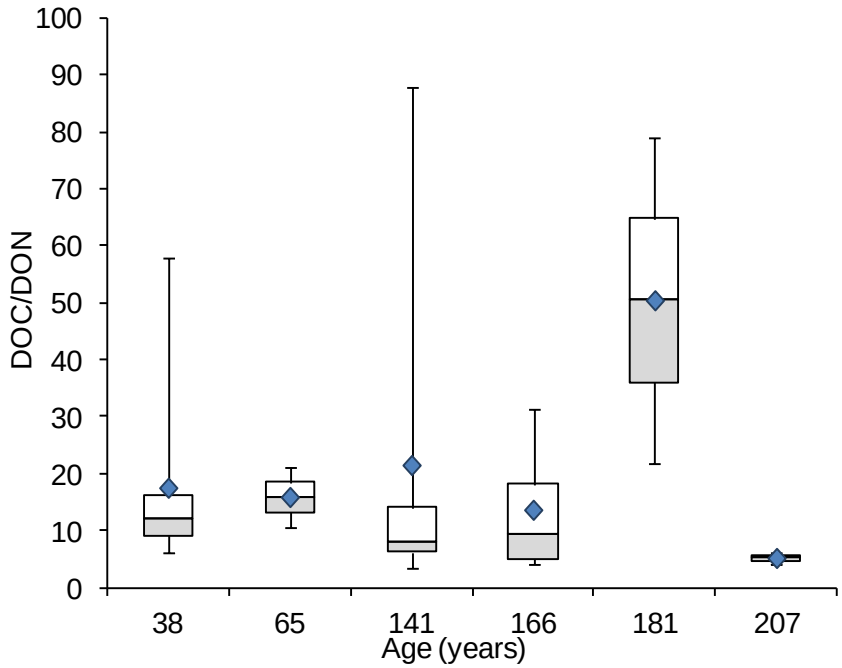

Figure 5-9: *Mouth surface water DOC/DON ratio. Diamond is the mean, solid line the median, clear box is the 75th and shaded box is the 25th percentile and whiskers indicate the minimum and maximum values.*

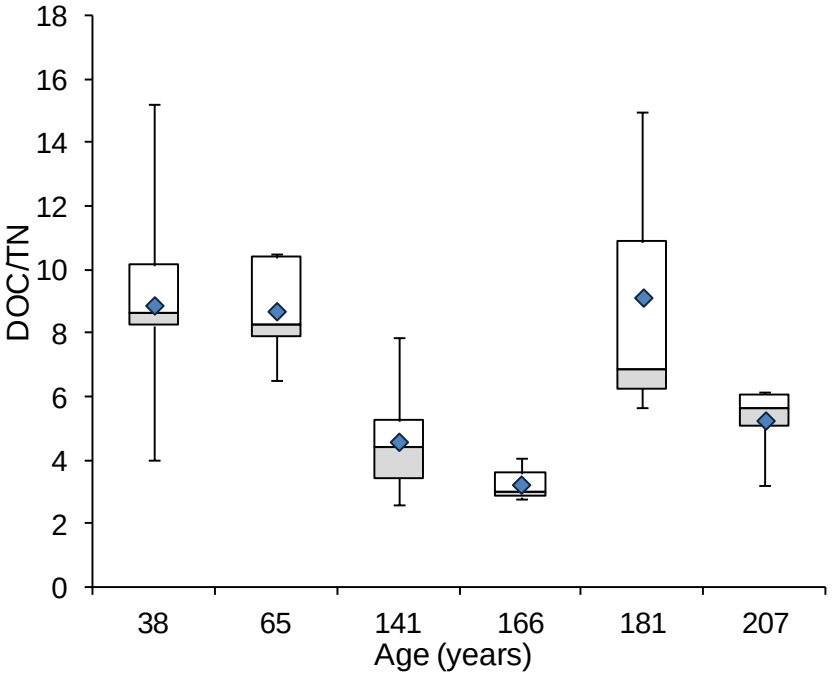

Figure 5-10: *Mouth surface water DOC/TN ratio. Diamond is the mean, solid line the median, clear box is the 75th and shaded box is the 25th percentile and whiskers indicate the minimum and maximum values.*

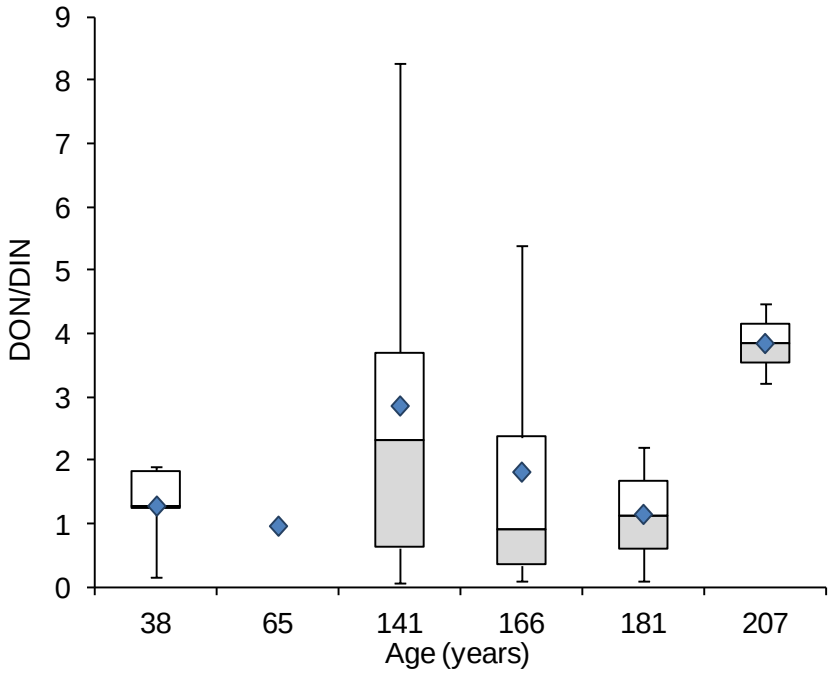

Figure 5-11: *Mouth surface water DON/DIN ratio. Diamond is the mean, solid line the median, clear box is the 75th and shaded box is the 25th percentile and whiskers indicate the minimum and maximum values.*

The ratio of DOC/DON across the chronosequence is highly variable at the stream outlets (Figure 6-9), although TN does show a general trend of decreasing over time relative to DOC (Figure 6-10) with an increasing proportion of this being in the inorganic (DIN) form particularly at the mid successional stream ages, as shown by the increase in mean and variability (Figure 5-11).

5.3.3 **Sub-catchment vegetation cover and water chemistry**

Data in this section is from samples collected from tributaries and main channels upstream of the stream outlet for each watershed; each had different vegetation land cover depending on successional stage (Table 5-3). All months were averaged according to the percent watershed vegetation cover.

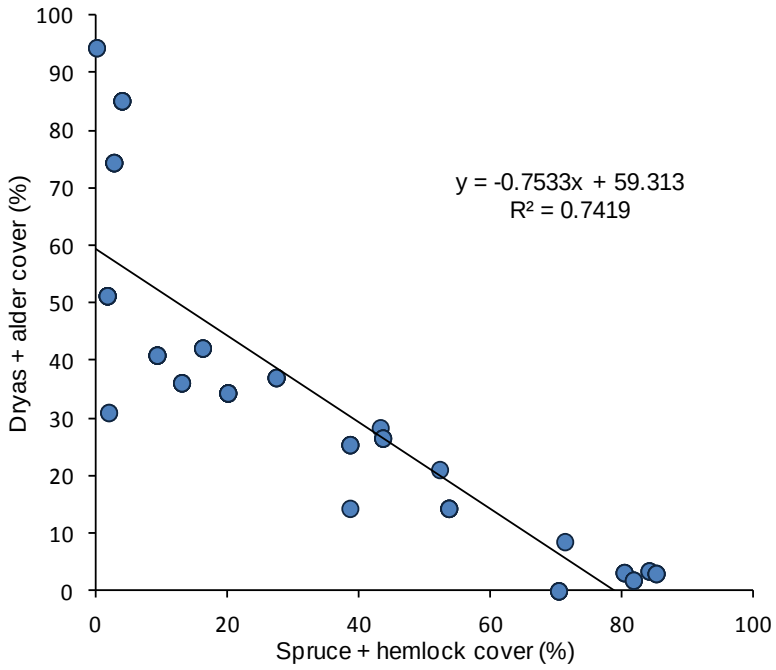

Figure 5-12: *Sub catchment regression of early successional versus later successional vegetation cover (%).*

Because of the strong relationship between early (*D. drummondii* and *A. sinuatta*) and later successional vegetation (*P. sitchensis* and *T. heterophylla*) can be used (Figure 5-12), either of these groupings as a proxy for the other. Hence only percent cover of later successional vegetation was used.

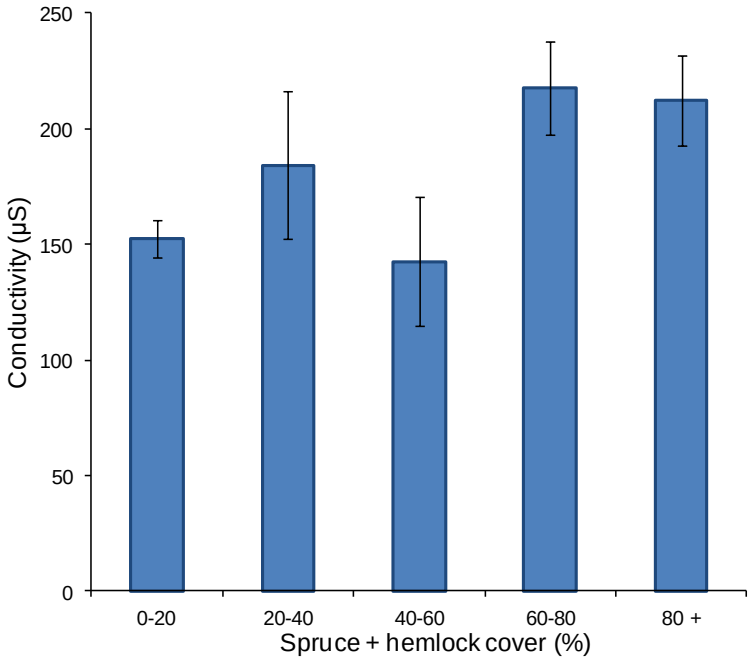

Figure 5-13: *Sub catchment mean surface water conductivity (µS) (±1 S.E) against vegetation (P. sitchensis + T. heterophylla) cover (%).*

Streams were separated by age and vegetation cover, with early, mid and later successional sites in three groups. Later successional sub catchments with greater percent cover of mature vegetation generally exhibiting higher conductivity up to 319 µS (Figure 5-13).

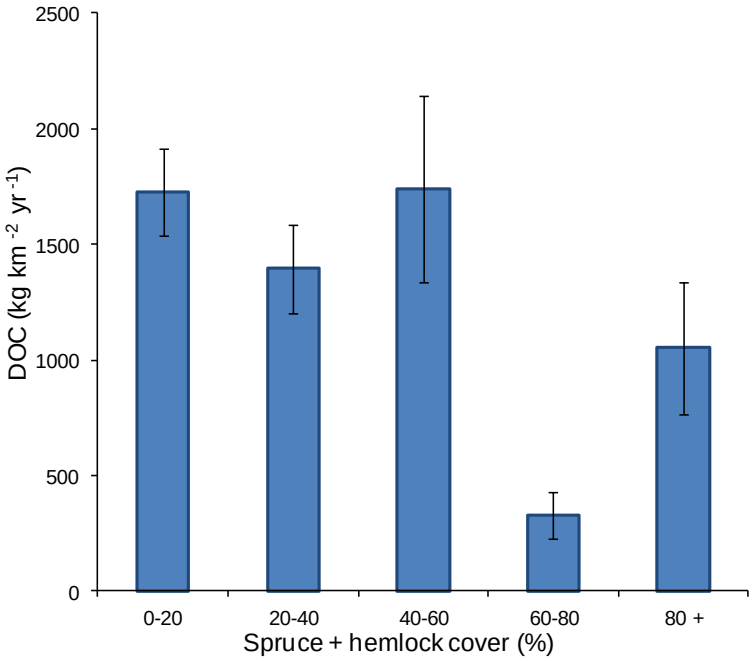

Figure 5-14: *Sub catchment mean surface water DOC (± S.E) against vegetation (P. sitchensis + T. heterophylla) cover (%).*

Sub catchments within the younger watersheds had the lowest percent *P. sitchensis* + *T. heterophylla* cover and showed the highest fluxes of DOC, particularly SFC (161 mg s km^2) which had high early vegetation cover (74.5 %). Within the mid to late successional streams (141-207 years) a clear trend of decreasing DOC flux with increasing forest cover was evident (Figure 5-14).

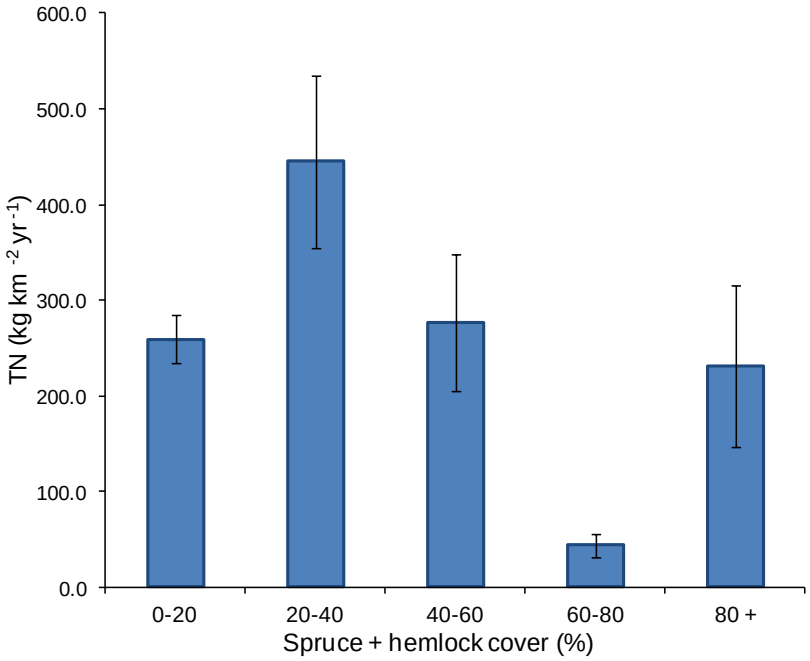

Figure 5-15: *Sub catchment mean surface water TN (± S.E) against vegetation (P. sitchensis + T. heterophylla) cover (%).*

TN concentration showed no relationship with sub catchment vegetation cover being highly variable across age and vegetation cover types.

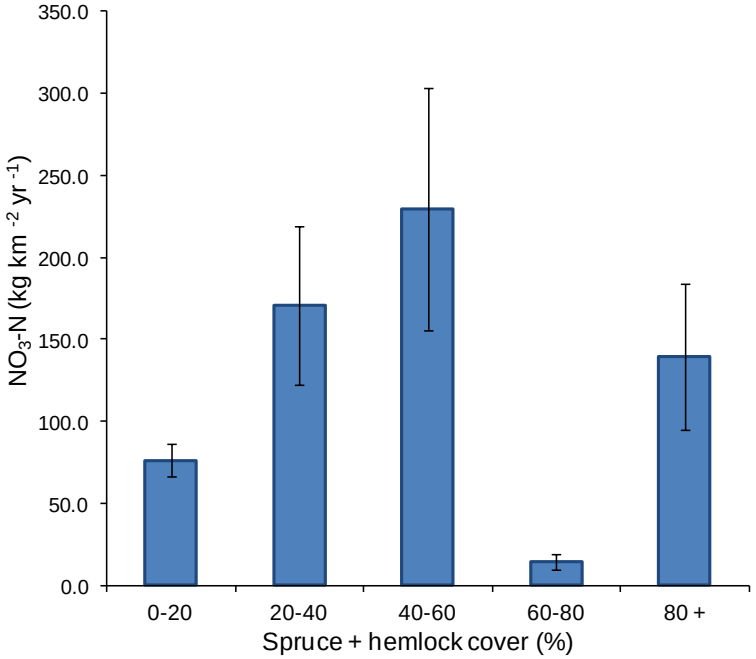

Figure 5-16: *Sub catchment NO_3^--N concentration against vegetation type (%).*

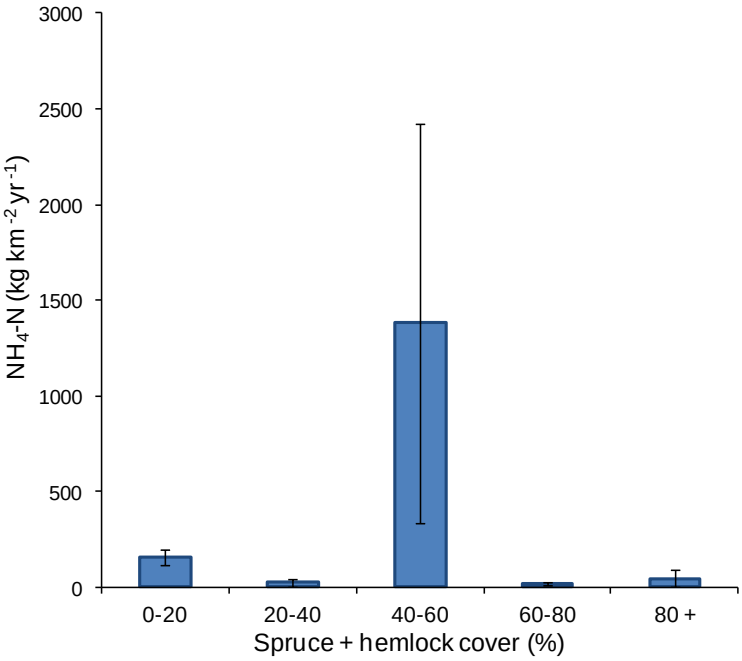

Figure 5-17: *Sub catchment NH_4^+-N concentration against vegetation type (%).*

Both forms of inorganic nitrogen are extremely variable across the sub catchments with NO_3^--N peaking in catchments of ca 40% late successional vegetation cover and NH_4^+-N in sub catchments with ca 20%, although the majority of samples had NH_4^+-N concentrations below detection limits (Figure 5-17).

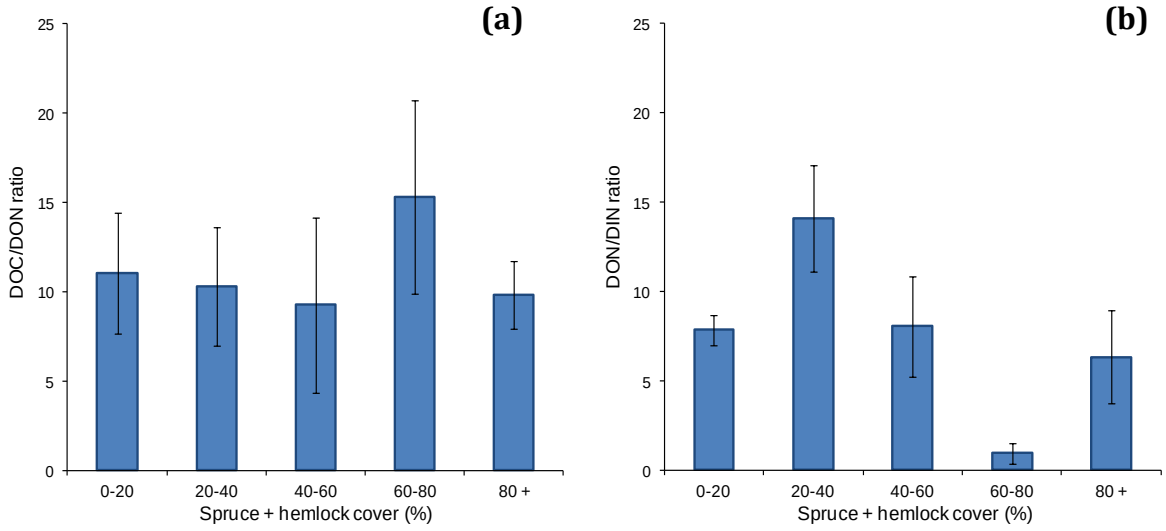

Figure 5-18: ***a)*** *Sub catchment DOC/DON ratio and vegetation cover (%);* ***b)*** *DON/DIN ration and vegetation cover (%).*

DOC/DON ratios tended to increase with later successional vegetation cover as the DON/DIN ratio decreased, suggesting an increasing proportion of DIN with the TN (Figure 5-17).

The age of the parent watershed significantly (Kruskal-Wallis, df = 5, $P < 0.05$) affected sub catchment water conductivity, pH and DOC flux between the youngest and oldest sites (Table 5-5). However there were few additional significant trends associated with age. Therefore sub catchments were divided into 5 groups according to mature (*P. sitchensis* + *T. heterophylla*) vegetation cover (0-100%). From these data it is clear that a number of variables are significantly influenced by the percent cover of mature vegetation, with significantly lower DOC, TN, NO_3^- and DON/DIN ratio associated with a high coverage (60-80%) than sites with less mature vegetation cover (Table 5-6).

Table 5-5: *Sub catchment water chemistry by watershed age. Results are given as means (± SE), values with the same suffix letter are significantly different to the P<0.05 level (by Kruskal-Wallis with multiple comparisons).*

	Watershed age (years)					
	38	**65**	**141**	**166**	**181**	**207**
Conductivity (µS)	138 (9)[a]	133 (36)[b]	171 (9)	119 (13)[c]	175 (19)	230 (17)[abc]
pH	8.6 (.11)[ab]	8.2 (.06)[a]	8.4 (.04)	8.3 (.12)	8.4 (.05)	8.3 (.09)[b]
Temperature (°C)	11 (1.4)	12.9 (1.2)	9.9 (.61)	10 (1.1)	10.2 (.77)	10.6 (.75)
DOC (kg km^{-2} yr^{-1})	3233 (834)[a]	1675 (383)	833 (187)	1850 (411)	1720 (498)	1135 (211)[a]
TN (kg km^{-2} yr^{-1})	398 (148)	149 (32)	159 (43)	445 (104)[ab]	219 (71)[a]	161 (16)[b]
NO_3-N (kg km^{-2} yr^{-1})	156 (59)	105 (63)	127 (51)	298 (124)	161 (80)	107 (40)
NH_4-N (kg km^{-2} yr^{-1})	83 (36)	54 (32)	132 (88)	199 (165)	300 (222)	33 (24)
DON/DIN ratio	11 (1.6)	7.3 (1)	5.6 (.95)	13.8 (1.6)	6.6 (2.3)	7.7 (2.8)
DOC/DON ratio	16.6 (5.5)	8.6 (2.7)	13.2 (3.7)	8.5 (4.2)	13.3 (2.8)	13.4 (3.4)

Table 5-6: *Sub catchment water chemistry by P. sitchensis + T. heterophylla vegetation cover (%). Results are given as means (±SE), values with the same suffix letter are significantly different to the P<0.05 level (by Kruskal-Wallis with multiple comparisons).*

	Grouping based on *P. sitchensis* + *T. heterophylla* vegetation cover (%)				
	0-20	**20-40**	**40-60**	**60-80**	**80 +**
Conductivity (µS)	152 (8)[a]	184 (32)	143 (28)	218 (20)[a]	212 (19.6)
pH	8.5 (.05)	8.4 (.18)	8.3 (.06)	8.3 (.05)	8.3 (.07)
Temperature (°C)	10.9 (.61)	10.7 (.94)	10.9 (.9)	10.6 (.8)	9 (1.2)
DOC (kg km^{-2} yr^{-1})	1738 (188)[a]	1395 (190)[b]	1742 (404)[c]	329 (98)[abc]	1051 (283)
TN (kg km^{-2} yr^{-1})	259 (24.5)[a]	445 (90)[b]	276 (71.4)[c]	44 (12.1)[abc]	231 (84)
NO_3-N (kg km^{-2} yr^{-1})	76 (9.9)	170 (48)[a]	229 (74)[b]	14 (4.7)[abc]	139 (44)[c]
NH_4-N (kg km^{-2} yr^{-1})	156 (41)	22 (21)	1379 (1045)	16.2 (9.2)	43.8 (43.8)
DON/DIN ratio	7.8 (.86)[a]	14 (3)[b]	8 (2.8)	0.9 (.6)[ab]	6.3 (2.6)
DOC/DON ratio	14.7 (3.4)	9 (3.3)	13 (4.9)	17.2 (5.4)	11.8 (1.9)

5.3.4 **Hyporheic water**

Hyporheic water sampling was performed between 10/07/2010 – 18/08/2010 across all study streams. Samples were collected from within the stream channels along with a surface water sample for comparison (except at SFC).

Table 5-7: *Stream bed sediment characteristics (Klaar, 2011)*

Stream	Minimum stable particle size (mm)	D_{50} (mm)
SFC		
WPC	261	80
IVS	235	80
NFS	227	90
BBS	241	39
RPC	312	50

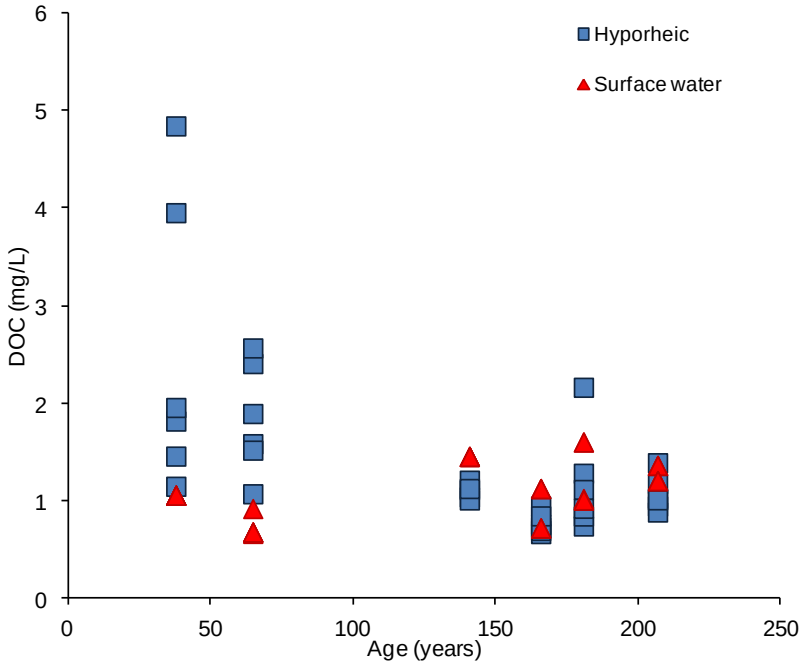

Figure 5-19: *Hyporheic and surface water DOC (mg/L), across chronosequence.*

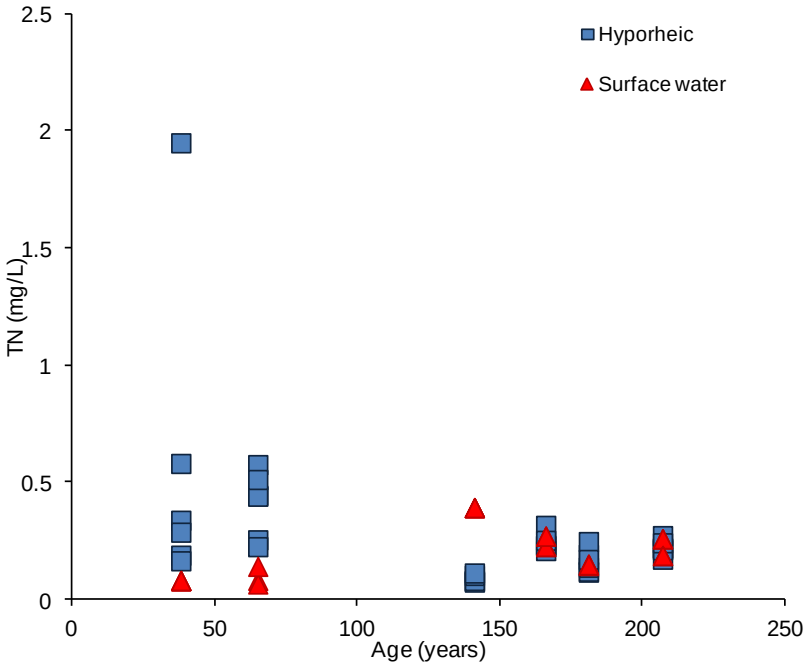

Figure 5-20: *Hyporheic and surface water TN (mg/L), across chronosequence.*

DOC and TN samples show higher more variable concentrations in the two youngest streams compared to the older stream sites, which are closer to each other and surface water data.

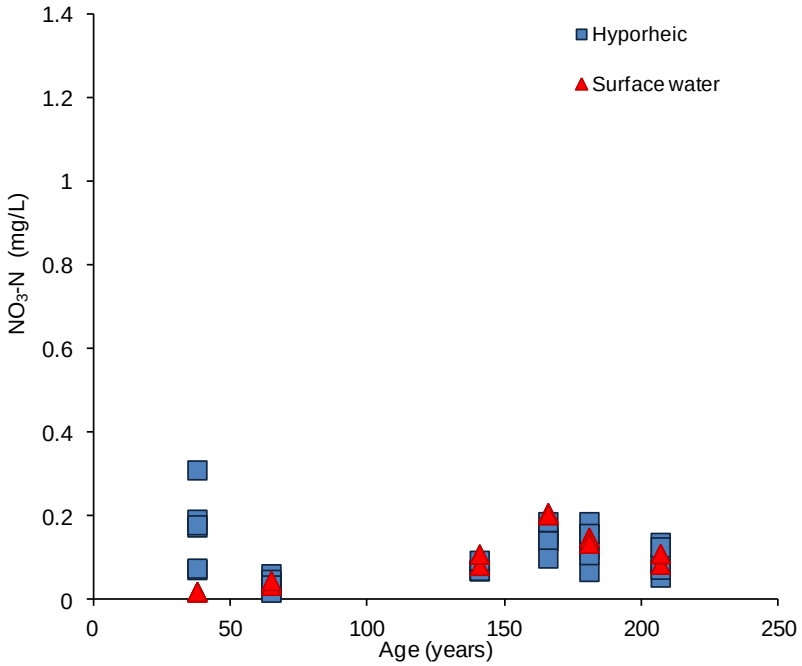

Figure 5-21: *Hyporheic and surface water NO_3^--N (mg/L), across chronosequence.*

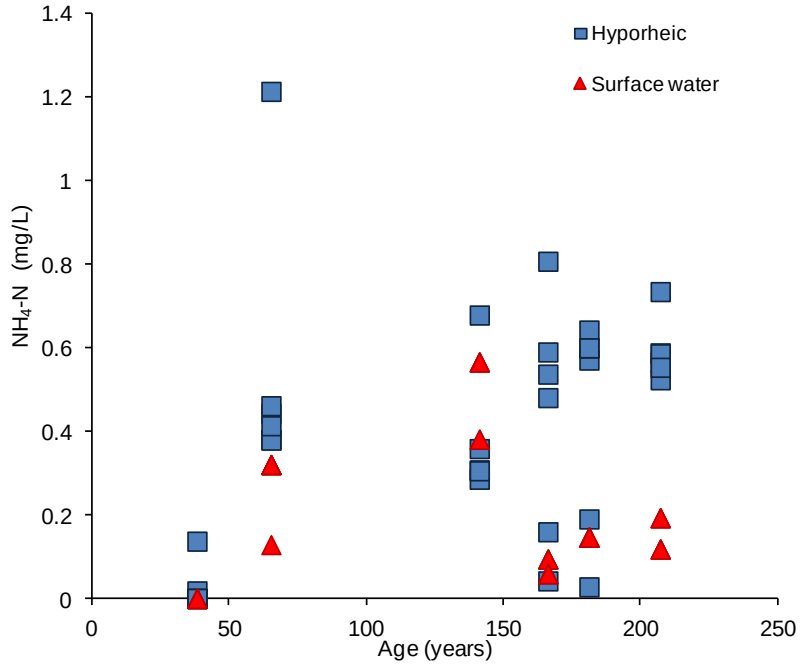

Figure 5-22: *Hyporheic and surface water NH_4^+-N (mg/L), across chronosequence.*

Inorganic N was composed predominantly of NH_4^+-N with lower levels of NO_3^--N, with surface water NO_3^- closely matching hyporheic concentrations. Hyporheic NH_4^+ concentrations were higher than surface water concentrations, particularly in the older streams (Figure 5-21). Within both sets of water samples the surface water chemistry matched hyporheic water closely (with the exception of NH_4^+-N concentrations in 141-207 years) indicating a close connection between these water bodies.

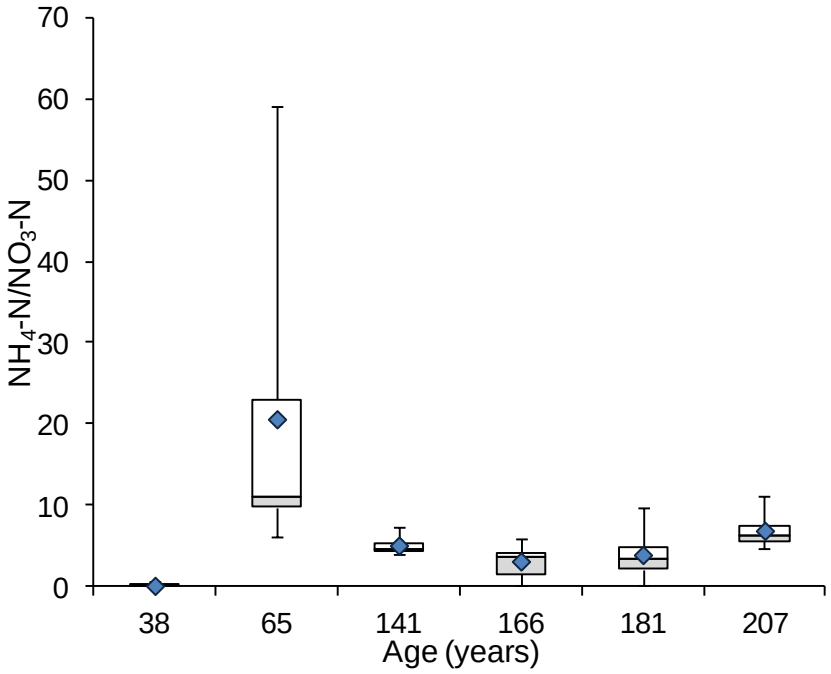

Figure 5-23: *Hyporheic ammonia/nitrate ratio.*

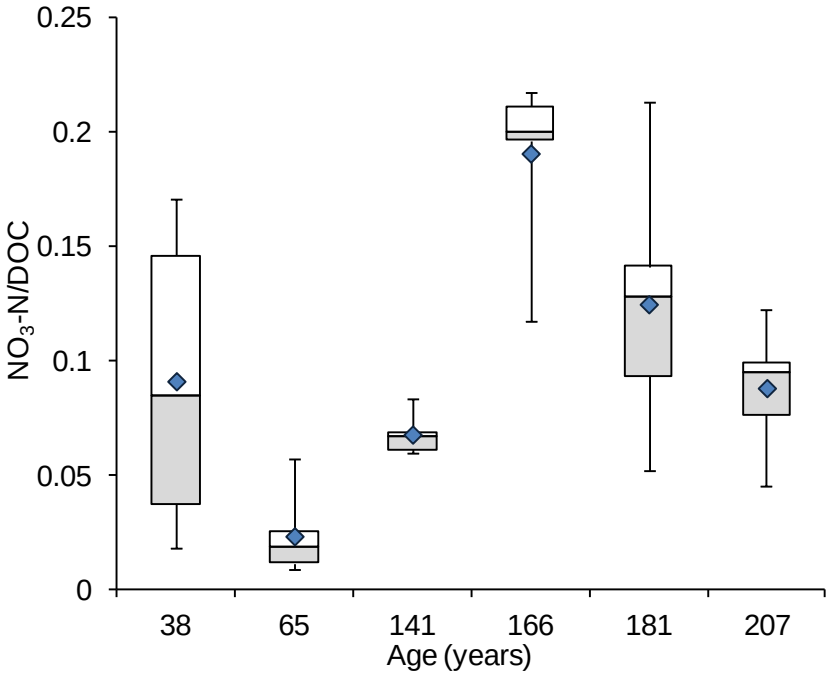

Figure 5-24: *Hyporheic nitrate/DOC ratio.*

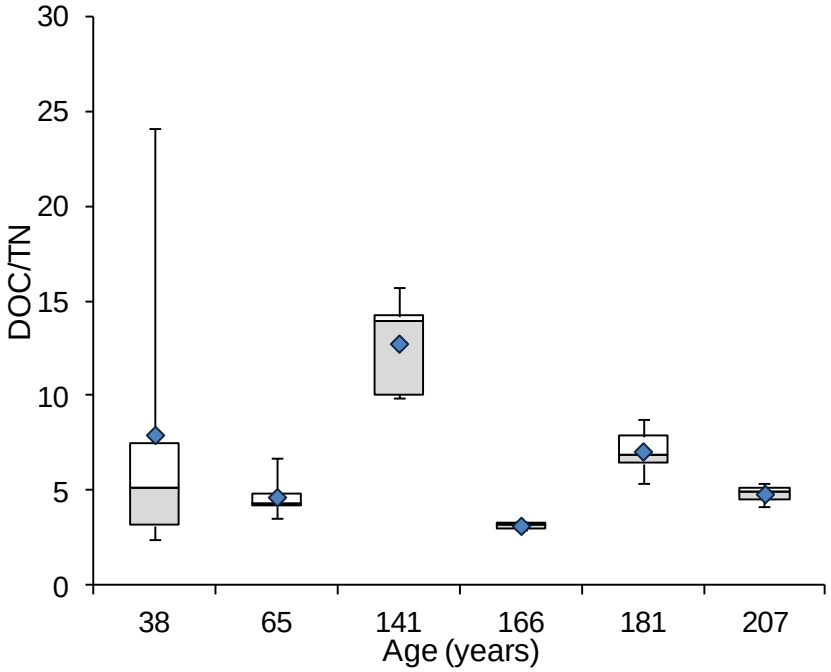

Figure 5-25: *Hyporheic DOC/TN ratio.*

Hyporheic NH_4^+/NO_3^- ratio remained low over chronosequence with some high values at WPC (Figure 5-22). NO_3-N/DOC and DOC/TN ratios show similar trends to each other,

with highly variable values at youngest stream, then peak values at mid successional streams which then decrease in the older sites. NO_3^--N/DOC rations are more variable however at most sites (Figure 5-23).

5.4 Discussion

5.4.1 Surface water chemistry

Surface water conductivity increased significantly over time between the early (38-65 years) and late (181-207 years) successional sites. Conductivity was used as an indication of total dissolved solids (TDS) in this section due to their very close linear relationship between these two variables. This is consistent with watershed soil development and the accumulation of dissolvable material sources over time which would then able available for transport to the river system. Water temperature shows a counterintuitive decrease over time, I believe this was due to a combination of bare sediment and the low growing vegetation of early succession are replaced with tall later successional forests that provide more shade to the river channels.

At stream outlets TN and DOC showed peak, highly variable estimated daily loads at the youngest stream during June and July, with decreasing variability with increasing stream age. The youngest stream had high percent cover of early successional vegetation and bare sediment and still contained remnant ice. Active DOC cycling has been shown to occur under glacial ice influencing its bioavailability to heterotrophic organisms (Barker, 2006). The ice remnant may be acting as a similar source here causing fluxes of DOC during melting periods. Also the lakes in this system could also be acting as sources or even storage pool for the DOC derived from the ice remnant. The presence of lakes in river systems has been shown to change water chemistry by removing N and increasing DOC load, whilst reducing its bioavailability to downstream areas (Goodman, 2011). The early successional streams of SFC, WPC still have lakes attached to river system, and IVS has a lake for a source. The high DOC yields of SFC could be a result of the numerous lake systems still present in this stream system. Lakes can also provide a 'nitrogen buffer' to downstream areas limiting the amount of DIN to

downstream areas (Goodman, 2011) particularly at WPC where the lake is large and close to the sampling sites and river mouth. Examination of the sub catchment water data revealed the highly variable nature of these tributaries over time, with high loads at tributary outlets from lake and remnant ice during different months. Also these sub catchment loads did not influence the watershed outlet water chemistry, with samples taken during the same day at these points not reflecting each other. DOC loads were much lower in the remaining watersheds across the chronosequence which indicates that bare sediment cover must influence this variable. Interestingly most of the N exported from the watersheds was organic, with variability caused by changing inorganic N content. TN increased at the mid successional streams, corresponding with increasing inorganic N. Inorganic N was low across most sites increasing at mid successional streams, particularly for NH_4^+-N which showed two high peaks in two of the older stream sites during August. These high values may be a consequence of salmon runs during these sampling periods, with spawning and mortality providing increased organic matter for decomposition to NH_4^+-N. The lack of LWD in the younger streams would cause less salmon carcass retention and could be the reason these August peaks did not appear there, however its absence from the oldest stream is not explained by this. An interesting finding of this study is that the bioavailability of watershed outlet DOM remains similar during early to mid successional ages then became very different in the oldest sites. The ratio of DOC/DON is a standard measure of the bioavailability of DOM because this ratio shows a strong inverse correlation with DOM lability (Hood, 2008).

Sub catchment water chemistry showed no significant differences over the chronosequence. Only when sub catchments were grouped into broader vegetation cover categories did differences emerge in the data. There was a clear decrease in nutrients (TN, inorganic N and DOC) as *P. sitchensis* and *T. heterophylla* forest cover increased, though this trend was not found in the highest *P. sitchensis* and *T. heterophylla* forest grouping (80%+). DOM bioavailability remained similar over the difference vegetation covers showing no significant differences. It is clear across both

watershed and sub catchment scales that vegetation cover had a significant influence on important surface water chemistry.

5.4.2 **Hyporheic**

Hyporheic DOC and TN showed peak concentrations and highest variability in the youngest stream at the youngest site, mirroring surface water samples. The main form of inorganic N was NH_4^+ with low concentrations NO_3^- indicating mainly anaerobic environment with some aerobic micro-sites. Over time NH_4^+ concentration increased with site age. Elevated levels of NH_4^+ in hyporheic samples are indicative of salmon spawning streams. However as spawning occurs immediately prior to scouring floods and at the end of the growing season retention of N is unknown (O'keefe and Edwards, 2003). As all streams in this study have salmon runs the increased concentrations with age could be a consequence of LWD input to the stream system as vegetation changes to larger woody tree species. As a consequence LWD trapped in channel sediments could promote mixing between surface and hyporheic water, as well as storage of DOM over a long period of time influencing stream productivity (Fellman, 2008).

From these data it is clear that watershed development over time with increasing watershed age did not have the expected influences on stream water chemistry as no significant differences emerged. However grouping sub-catchments by percent vegetation covers revealed some interesting changes in water chemistry. Of course other variables could be influencing these data; rainfall events will influence surface water DOC through mobilisation of different terrestrial DOC deposits than during base flow events. As a consequence DOM character and amount from watersheds will change between these conditions (Fellman, 2008; Wiegner, 2009), however this change is not measured in this study. Sampling in a temperate rainforest with a spatially extensive and time intensive sampling regime, these factors are difficult to avoid and need to be considered. However our aim was only to measure the 'footprint' of each catchment, to get a general idea of the influence that vegetation cover and change can have on water chemistry which has been accomplished.

5.5 Conclusions

Nutrient fluxes from post glacial watersheds undergoing primary succession decrease and become less variable over time (c.a. 200 years). This study highlights the influence of different catchment vegetation cover on water chemistry, as cover changes towards immature *P. sitchensis*/*T. heterophylla* forests surface water DOC, N and bioavailability of DOM also change. In addition to changes in nutrient sources with changing vegetation and soil development over time, there are parallel affects such as salmon carcass retention within stream and riparian zones caused by LWD. These processes are indicated within the data by the high NH_4^+ concentration in surface water samples during August and its increase in hyporheic water samples with increasing stream age.

These processes could be made clearer with higher resolution monitoring and performing fluorescence analysis to identify DOC source and how this may change with frequency of rainfall, vegetation cover, seasonally and with salmon spawning. Unfortunately fluorescence analysis was impossible due to the time between sampling and analysis. This is unfortunate as would provide information on changing organic matter bioavailability and subsidies to near shore food webs over time.

5.6 References

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6: Summary

6.1 Overview

The work included in this thesis concerns the study of N cycling in recently deglaciated watersheds. Specifically the objectives of the research were:

- Using space for time comparison show the time frame for riparian sediment development from an abiotic to biotic controlled environment.

- Examine the change over time of riparian sediment properties, such as physical characteristics, nutrient retention and microbial activity.

- Quantify watershed soil nutrient content (N and C) and microbial community size and activity across the GBNP chronosequence.

- Determine the driving variables behind any differences at these watershed sites (age, slope, forest cover).

- Use satellite imagery data to scale up these processes to the catchment scale for the present (2009) and past environments (22 years), and calculate the rate of change for these watersheds over this time frame.

- Assess the change in surface water chemistry of six streams of different ages in GBNP.

- Determine the effect of watershed and sub catchment vegetation cover on surface and hyporheic water chemistry.

These objectives were achieved through sampling of riparian and wider catchment soils within GBNP over a series of watersheds of different ages. Soil samples were analysed to determine physical (grain size, bulk density, soil moisture), chemical (pH and N, C concentrations and isotopes) and microbial (population size, net and potential activity)

properties using a range of insitu and laboratory experiments. These data were then linked to vegetation cover data derived from satellite imagery to estimate the current and past soil nutrient status and microbial activity within these areas. Furthermore surface and hyporheic water were determined over the stream chronosequence to determine how this changed over time with vegetation succession at the watershed and sub catchment scale.

6.2 Discussion

The following conceptual diagrams and tables provide a summary of the findings of this thesis, building on the work of Milner *et al,* 2007.

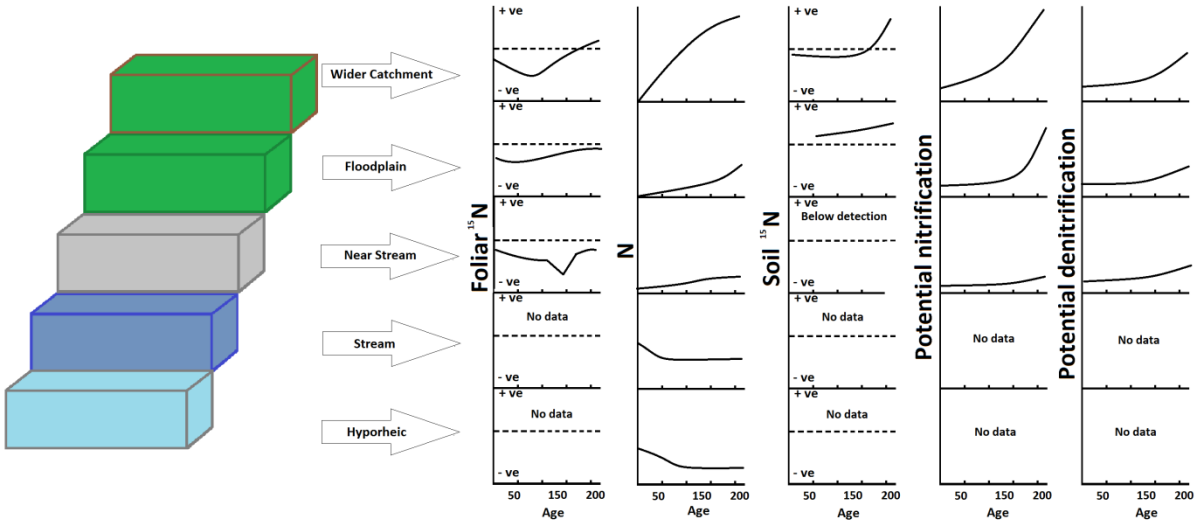

Figure 6-1: *Conceptual diagram of changing watershed and riparian N over time.*

Table 6-1: *Summary of chronological change in nitrogen.*

5 to 50 years	50 to 150 years	150+ years
	Vegetation	
Depleted $\delta^{15}N$ in non-N fixing species.	$\delta^{15}N$ becomes more depleted across all sites.	Increasing $\delta^{15}N$ in non-N fixing species. Positive enrichment of *P. sitchensis* in wider catchment, and increase of floodplain and near stream vegetation. *EF* of foliar samples becomes less negative over time across all non N fixing species.
	Soil nutrients	
Across all sites low soil N (%) and low C:N ratio. Slightly depleted $\delta^{15}N$ (0—1 $‰$) in wider catchment and below detection in riparian zone sites.	Increasing soil N (%), depleted $\delta^{15}N$ in wider catchment, floodplain soils have highly variable values. .	$\delta^{15}N$ enrichment over time under all vegetation types. Soil % N and C:N ratio increase under *P. sitchensis* vegetation. Floodplain soils $\delta^{15}N$ are enriched but highly variable.
	Soil microbial activity	
Low potential nitrification and denitrification activity.	Increase in potential nitrification and denitrification activity.	Continued increase in both activities with potential nitrification considerably greater than denitrification. Low soil NO_3^- concentrations indicate efficient removal of NO_3^- from soil by plants and heterotrophic microbes.
	Surface water	
High TN. Highly variable data.	Decrease TN. Decrease in inorganic N.	N remains low.
	Hyporheic water	
High organic N, low inorganic N.	Decreasing organic N. Increasing levels of NH_4^+.	Increased NH_4^+-N concentrations with watershed age.

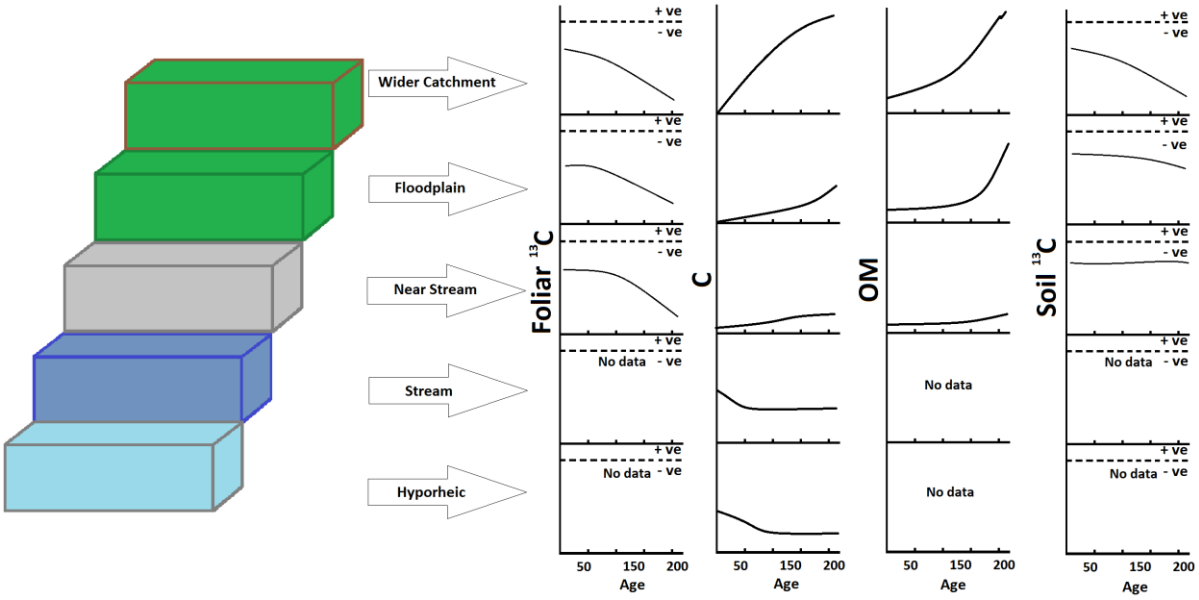

Figure 6-2: *Conceptual diagram of changing watershed and riparian C over time.*

Table 6-2: *Summary of chronological change in carbon.*

5 to 50 years	50 to 150 years	150+ years
	Vegetation	
$\delta^{13}C$ -28 to -30 across all species in wider catchment. Less depleted values in floodplain and near stream.	Decreasing $\delta^{13}C$ with watershed age in wider catchment and floodplain.	Continued decrease in foliar $\delta^{13}C$ over time, across all sites.
	Soil nutrients	
Low C (%) and C:N ratio, Low soil OM (%) in all soils.	Depleted soil $\delta^{13}C$, increase in C (%) and OM in wider catchment soils, not within floodplain and near stream.	Increased soil % C, C:N ratio and peak OM under *P. sitchensis* vegetation in wider catchment. Continued decrease in soil $\delta^{13}C$ with watershed age. Floodplain soils begin to show increases in C and OM, and $\delta^{13}C$ begins to decrease reflecting vegetation. Near stream remains similar to earlier sites.
	Surface water	
High DOC concentrations. High variability.	Decrease in DOC.	DOC remains low.
	Hyporheic water	
High DOC	Decrease in DOC	DOC remains low. Increase in NH_4^+ concentration.

6.2.1 **Riparian development**

The natural time frame for the formation and development of riparian land-water interfaces and the consequences on nitrogen cycling and retention have been determined. Floodplain soil variables became statistically different from near stream and younger floodplain soils after 207 years of development. These data provide a time frame for development and support the hypothesis that floodplains would shift from physically to biologically driven environments over time.

Early successional riparian sites (0-50 years) supported soils with low percent OM, N, C, and high C:N ratios, characteristic of a physically driven abiotic environment. Microbial N cycling activity was low as indicated by the low rates of net mineralisation and nitrification, as well as potential nitrification and denitrification. In watersheds of mid successional age (50-150 years) floodplains developed to a more biologically driven environment as soil percent OM and moisture increased as bulk density decreased. Net microbial transformations increased, particularly mineralisation. What is evident throughout this age range is the increasing difference between near stream and floodplain areas. In the oldest successional stages in GBNP (150+ years) the floodplains supported higher amounts of organic matter, percent C, N, inorganic N concentrations and lower C:N ratios compared to younger sites. These streams showed an interesting shift to negative net mineralisation, indicating rates of organic matter decomposition are reduced in these areas due to the change in dominant vegetation type in the surrounding watershed to *P. sitchensis*/*T. heterophylla* forest and *P. sitchensis* encroachment into the riparian zone. Also potential rates of nitrification increase in these areas suggesting of a large microbial community. Potential nitrification rates were considerably higher than those of potential denitrification, with ratios in the older streams ranging from 30-50 times greater than younger systems. Insitu soil NO_3^- was low across the chronosequence, with NH_4^+ the dominant form of inorganic N. These variables indicate that N availability increases with age, but also that it is subject to efficient uptake by plants and microbes in these older soil systems.

6.2.2 **Wider watershed soil development**

Distinct patterns of soil development were observed over time within watersheds, not influenced by stream dynamics. Dominant vegetation type influenced soil characteristics significantly over the chronosequence. These data confirmed the hypothesis that dominant vegetation type would be the driving influence on soil nutrients, microbial activity.

Soil under the later successional vegetation types (*P. sitchensis* and *P. sitchensis* + *T. heterophylla* forest) contained higher amounts of N, C and OM and supported larger

microbial activity, compared to early successional vegetation types (*A. sinuatta*, *D. drummondii*). Changing *EF* between soil and leaf samples demonstrates a recycling mechanism of N within the forest system, becoming increasingly different to the atmospheric ratio. Over time there is enrichment of N in the soil-vegetation system as vegetative inputs alter soil properties which are then exacerbated with vegetative organic matter feedback and loss of lighter N isotopes from the soil system by volatilisation, denitrification and immobilisation. Potential nitrification exceeds potential denitrification in watershed soils across GBNP, increasing with watershed age to up to c.a. 40 x greater N consumption rates. As the predominant form of inorganic N in soils is NH_4^+ which increases with age, and low ambient levels of NO_3^-. This indicates loss of NO_3^- from the soil system via microbial and plant uptake as well as DEA and volatilisation.

Due to its dominant influence on soil characteristics vegetation type was used as a basis for estimating watershed scale processes over time. Using past and present land satellite imagery of GBNP to determine vegetation type of the study catchments (%) in order to estimate the change over a 22 year period of soil nutrient status and microbial activities. The rate of change was quantified within recently deglaciated areas over c.a. 200 years, with regressions showing a significant linear increase in soil N, C and OM (%) over time. Particularly rapid rates of accumulation were predicted during early successional stages, as bare sediment is colonised by vegetation leading to the input and decomposition of organic matter. The deviation from this trend in mid to late successional ages was caused by the low values found in the samples from these stream ages, particularly in BBS, within the dominant *P. sitchensis* compared to the other ages. Interestingly NO_3^- availability over time shown by the ratio between potential nitrification and denitrification in these mid- late successional sites fitted closely with the linear increase displayed by the other sites, suggesting that NO_3^- availability is similar despite the differences in soil N and C (%). Potential nitrite oxidation and denitrification showed a rapid increase during early successional *A. sinuatta* development, stabilising to a plateau in the older streams. However denitrification enzyme activity (with and without C_2H_2) showed lower values than nitrification and is

more variable throughout the later successional periods, caused by the sporadic nature of this process.

It is clear from this research that dominant tree species is an important variable influencing N cycling of forest soils. This suggests that N cycling processes are vulnerable to changes in forest species composition, possible causes of which being climate change, disease and forest management practices.

In addition continued glacial recession in sub arctic regions caused by global warming will also substantially alter N and C cycling in these watersheds. Modelling of how forest N cycling will respond to such changes should account for changes in forest species composition that profoundly alter N cycling and dynamics of forest ecosystems, influencing emissions of gasses, and N retention.

6.2.3 **Stream water chemistry**

This research highlights the link between post glacial recession and the associated land cover change caused by vegetation succession in coastal watersheds, and fluxes of organic and inorganic nutrients between the terrestrial and marine environments. Changes to fluxes with vegetation change will have the most pronounced impacts on near-shore marine ecosystems, particularly those with limited mixing with the open ocean, such as those found along fjord and archipelago coastlines in south eastern Alaska, New Zealand and southern Chile.

Using space for time substitution this study compared surface and hyporheic water chemistry over a chronosequence of six watersheds to determine their change over time with primary succession. The influence of changing watershed vegetation cover was considered along with sub catchment vegetation cover, determined using satellite imagery. This research clearly shows that sub catchment vegetation cover altered surface water chemistry, confirming this hypothesis.

TN and DOC showed peak, highly variable concentrations in the youngest stream during June and July, with decreasing variability as stream age increased. The predominant form of TN exported from the watersheds was organic, with variability caused by changing concentrations of inorganic N in the older watersheds. Inorganic N was low across most sites increasing at mid successional streams, particularly for NH_4-N which showed two high peaks in two of the older stream sites during August. These high values may be a consequence of salmon runs during these sampling periods, with spawning and mortality providing increased organic matter for decomposition to NH_4-N. The lack of LWD in the younger streams would cause less salmon carcass retention than in the older sites where it was present, and could be the reason these August peaks did not appear there, however its absence from the oldest stream is not explained by this. An interesting finding of this study is that the bioavailability of DOM remains similar during early to mid successional ages then became significantly different in the oldest sites.

Sub catchment water chemistry showed no significant differences over the stream chronosequence. Only when sub catchments were grouped into broader vegetation cover categories did differences emerge. There was a clear decrease in nutrients (TN, inorganic N and DOC) as *P. sitchensis* and *T. heterophylla* forest cover increased within sub catchments, though this trend was not significant in the highest *P. sitchensis* and *T. heterophylla* forest grouping (80%+). DOM bioavailability remained similar over the difference vegetation covers.

Hyporheic DOC and TN showed peak concentrations and highest variability in the youngest stream, similar to surface water. The main form of inorganic N was NH_4^+ with small concentrations NO_3^- indicating mainly anaerobic environment with some aerobic micro-sites. Over time NH_4^+ concentration increased with site age. Elevated levels of NH_4^+ in hyporheic samples are indicative of salmon spawning streams. However as spawning occurs immediately prior to scouring floods and at the end of the growing season retention of N is unknown. As all streams in this study support salmon runs the increase over age could be a consequence of LWD input to the stream system with changing successional vegetation. LWD trapped in channel sediments could promote

mixing between surface and hyporheic water, as well as storage of DOM over a long period of time influencing stream productivity.

6.3 Future study

This study highlighted the increase in NH_4^+ in hyporheic and surface water with increasing age stream age. A possible explanation for this could be terrestrial-aquatic linkage with surface water chemistry influenced by increasing soil ammonium concentrations observed within the study watersheds. Another possible explanation is the influence of LWD entrained in and around stream channels facilitating the mixing of salmon derived nutrients; however this conclusion is tentative as there was no definite link.

Therefore further investigation of possible sources of nutrients is required to decipher theses links. Hyporheic sampling and tracing experiments around LWD features could quantify the effect that they have on in stream N cycling and nutrient transfer between surface-subsurface water. Also fluorescence analysis of DOC from these hyporheic and surface water samples would be a useful tool to determine the changing sources of DOC over time, either from changing soil conditions or salmon carcass retention in river with LWD accumulation.

Other further avenues for future research include the use of isotope tracers for the measurement of insitu rates of N cycling. These types of experiments could provide definitive quantification of N cycling within successional vegetation. This could also build upon the changes in ratio between nitrification and denitrification with age as highlighted in this study.

7: Appendices

7.1 Appendix A

7.1.1 Site GPS locations

Table 7-1: *Riparian sample site locations*

Stream name (age)	Site I.D	Co-ordinates	
		N°	W°
SFC (38 years)	A	58° 58' 04.4''	136° 20' 57.7''
	B	58° 57' 37.6''	136° 21' 21.6''
	C	58 57' 32.0''	136° 21' 20.8''
WPC (65 years)	A	58° 48' 09.5''	136° 09' 41.5''
	B	58° 48' 12.8''	136° 09' 50.8''
	C	58° 48' 09.8''	136° 09' 50.8''
IVS (141 years)	A	58° 59' 48''	136° 10' 18.4''
	B	58° 59' 48.0''	136° 10' 16.9''
	C	58° 59' 48.4''	136° 10' 10.6''
NFS (165 years)	A	58° 34' 33.9''	136° 13' 03.6''
	B	58° 34' 30.7''	136° 13' 12.9''
	C	58° 34 '28.8	136° 13' 16.3
BBS (181 years)	A	58° 29' 56.1''	136° 13' 42.2''
	B	58° 29' 52.1''	136° 13' 33.3''
	C	58° 29' 48.4''	136° 13' 29.4''
RPC (207 years)	A	58° 28' 20.9''	136° 06' 40.5''
	B	58° 28' 12.0''	136° 06' 15.1''
	C	58° 28' 21.3''	136° 06' 44.0''

Table 7-2: *Catchment sample site co-ordinates, vegetation and slope data. (low slope: 0-10°; Medium: 10-35°; High >35°)*

Stream name	Site I.D-GIS:	Site description		Co-ordinates	
		Vegetation	Slope	N°	W°
SFC	E	Sediment	Low	58 58' 03.5''	136 20' 41.5''
	F	*A. sinuatta*	Low	58 57' 46.3''	136 21' 07.01''
	B	*A. sinuatta*	Medium		
	G	*A. sinuatta*	High		
WPC	C	*P. trichocarpa*	Low	58 59' 53.0''	136 10' 00.6''
	B	*P. trichocarpa*	Medium	58 59' 52.3''	136 10' 05.3''
	F	*A. sinuatta*	Low		
	SHA	*A. sinuatta*	High	58 59' 48''	136 10' 18.4''
	SHB	*A. sinuatta*	High	58 59' 48''	136 10' 17.5''
IVS	E	*P. sitchensis*	Low	58 49' 05.39''	136 10' 41.56''
	C	*P. trichocarpa*	Medium	58 48' 28.4''	136 10' 24.6''
	D	*P. sitchensis*	Low	58 48' 40.2''	136 10' 26.7''
	SH	*P. sitchensis*	High		
	SL	*P. sitchensis*	Low	58 48' 14.3''	136 09' 41.4''
NFS	H	*P. sitchensis*	High	58 34' 35.9''	136 12' 59.8''
	B	*P. trichocarpa*	Medium	58 59' 48.0''	136 10' 16.9''
	SH	*P. sitchensis*	High	58 34' 37.8''	136 12' 58.9''
BBS	C	Mature	High	58 28' 13.3''	136 15' 09.0''
	B	Mature	Medium	58 28' 14.7''	136 15' 10.5''
	D	Mature	Low	58 28' 14.1''	136 15' 12.7''
	E	Open	Low	58 28' 27.7''	136 15' 03.7''
	F	*P. trichocarpa*	Medium	58 28' 13.3''	136 15' 09.0''
	CL	*P. trichocarpa*	Low	58 30' 21.8	136 14' 09.8''
	SH	*P. sitchensis*	High	58 29' 53''	136 13' 33.2''
RPC	A	*P. sitchensis*	Medium	58 57' 27.86''	136 21' 21.97''
	B	*P. sitchensis*	Low	58 57' 48.9''	136 21' 05.5''
	C	*P. sitchensis*	High	58 28' 30.5''	136 07' 37.8''
	D	*P. trichocarpa*	Low	58 28' 33.6''	136 07' 45.5''

Table 7-3: *Water sample site co-ordinates*

Stream name (age)	Site I.D	Co-ordinates	
		N°	W°
SFC	1	58 58' 03.5''	136 20' 41.5''
	2	58 58' 04.3''	136 20' 56.5''
	3	58 58' 02.0''	136 21' 16.8''
	4	58 57' 46.3''	136 21' 07.01''
	5		
	6	58 57' 33.2''	136 21' 20.6''
	7	58 57' 29.4''	136 21' 23.0''
WPC	A	58 59' 41.9''	136 11' 33.6''
	B	58 59' 48.4''	136 10' 10.6''
	C	58 59' 48.4''	136 10' 10.6''
	GIS-B	58 59 50.9	136 10 00.8
IVS	1	58 49' 15.9''	136 10' 52.6''
	2	58 48' 57.3''	136 10' 43''
	3		
	4	58 48' 28.4''	136 10' 24.6''
	5	58 48' 12.8''	136 09' 37.5''
	GIS-A	58 48 52 .5	136 11 0.8.4
NFS	1		
	2	58 34' 33.9''	136 13' 03.6''
BBS	1	58 29' 05.5''	136 14' 15.4''
	2	58 29' 07.1''	136 14' 11.4''
	3	58 29' 28.3''	136 13' 35.1''
	4	58 29' 23.1''	136 13' 26.2''
	5	58 29' 36.4''	136 13' 14.8''
	6	58 30' 20.5''	136 14' 10.0''
RPC	1	58 28' 37''	136 07' 44.6''
	2	58 28' 30.9''	136 07' 44.3''
	3	58 28' 29.0''	136 07' 13.7''
	4	58 28' 22.2''	136 06' 41.5''
	5		

7.2 Appendix B

7.2.1 Average daily soil temperature

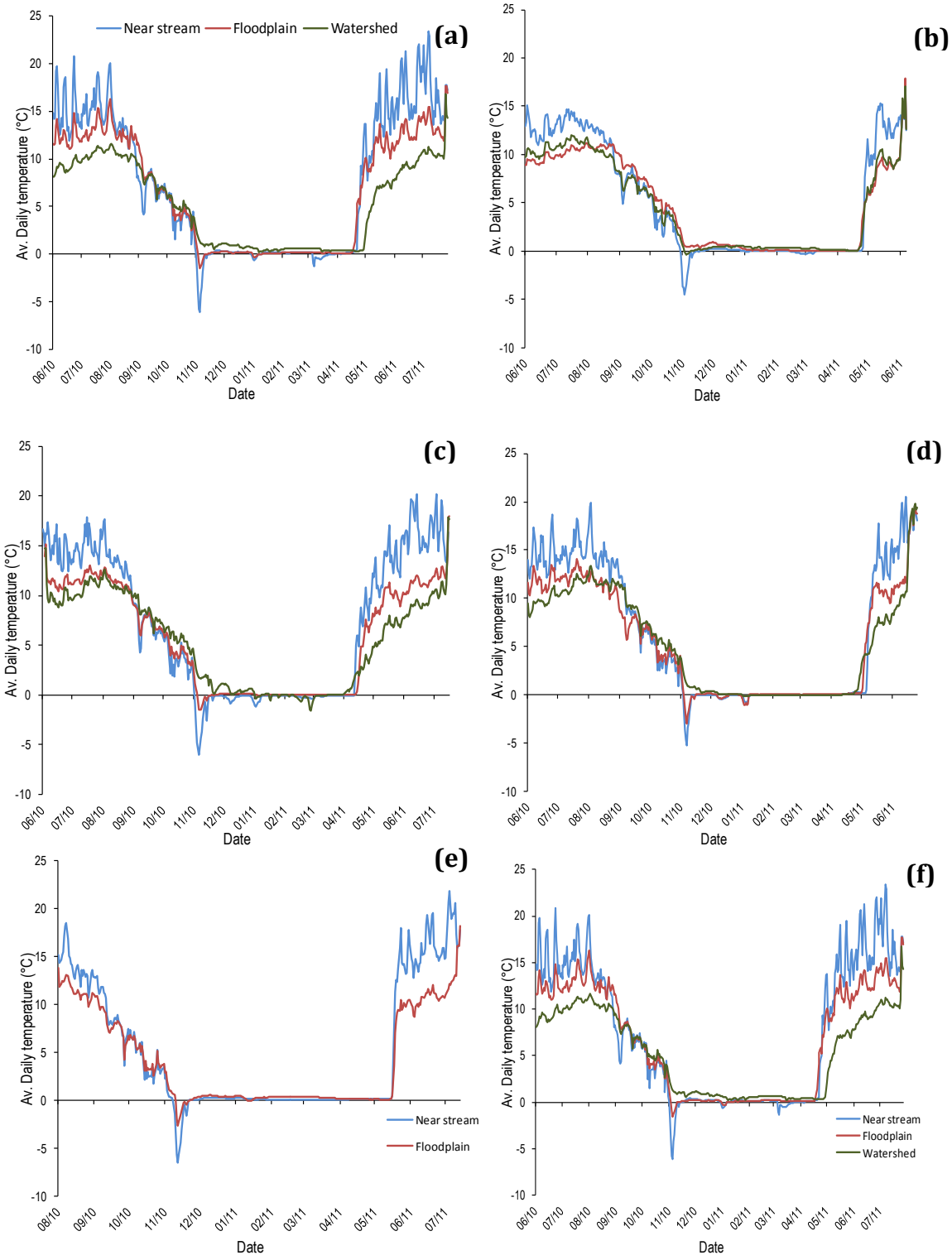

Figure 7-1*: Average daily soil temperature (°C) (Tiny tag temp. logger to c.a. 15cm depth) at riparian and wider catchment sites. a) SFC, b) WPC, c) IVS, d) NFS, e) BBS, f) RPC.*

7.3 Appendix D

7.3.1 Water chemistry

***Table 7-4**: All surface water data*

Stream	Site	Date	Q (m^3/s)	Cond (µS)	Temp (°C)	pH	DOC (mg/L)	TN (mg/L)	NO_3-N (µg/ml)	NH_4-N (µg/ml)
RPC	1	28.7.10	0.54	218	9.1	8.3	1.18	0.85	0.1	
RPC	2	28.7.10	0.00	251	10.1	8.3	1.19	0.22	0.2	
RPC	3	28.7.10	0.01	319	14.1	8.2	1.23	0.09	0.1	
RPC	4	28.7.10	0.66	240	11.2	8.3	1.23	0.16	0.1	
RPC	5	28.7.10	0.70	251	11.0	8.3	0.86	0.27	0.1	
BBS	1	29.7.10	0.27	120	11.1	8.4	1.22	0.18	0.1	
BBS	2	29.7.10	0.02	227	8.2	8.2	0.86	0.15	0.2	
BBS	3	29.7.10	0.00	209	10.1	8.3	0.88	0.15	0.2	
BBS	4	29.7.10	0.32	190	8.3	8.3	0.87	0.27	0.1	
BBS	5	29.7.10	0.40	149	11.5	8.5	1.00	0.15	0.1	
NFS	1	30.7.10	0.77	145	10.1	8.3	0.86	0.26	0.2	
NFS	2	30.7.10	0.68	152	11.1	8.4	1.17	0.29	0.2	
IVS	1	02.8.10	0.60	217	8.5	8.2	0.53	0.08	0.1	0.0
IVS	2	02.8.10	0.59	178	11.7	8.3	0.39	0.07	0.1	0.1
IVS	3	02.8.10	0.05	115	11.5	8.3	0.48	0.08	0.2	0.1
IVS	4	02.8.10	0.05	154	11.6	8.4	0.51	0.10	0.2	
IVS	5	02.8.10	0.31	219	11.5	8.4	0.54	0.11	0.1	
SFC	1	03.8.10	0.58	145	17.5	8.5	0.88	0.07	0.0	0.0
SFC	3	03.8.10	0.52	157	6.8	8.4	0.71	0.11	0.0	0.0
SFC	4	03.8.10		130	14.2	9.1	0.68	0.08	0.1	0.4
SFC	5	03.8.10	0.19	176	14.2	8.8	0.61	0.08	0.0	0.0
SFC	6	03.8.10	0.49	169	14.8	8.3	0.78	0.01	0.0	0.0
SFC	7	03.8.10	0.46	166	15.0		0.86	0.08	0.0	0.0
SFC	2	08.7.10					1.02	0.09		
SFC	3	08.7.10					0.98	0.30		
WPC	1	04.8.10		75	14.5	8.1	0.66	0.08	0.0	0.3
WPC	2	04.8.10	1.41	72	16.1	8.3	0.68	0.06	0.0	
WPC	3	04.8.10		269	15.9	8.2	0.92	0.14	0.0	0.1
RPC	1	19.8.10	0.49	224	8.3	8.1	0.30	0.17	0.1	0.0
RPC	2	19.8.10	0.01	256	9.3	8.0	1.21	0.25	0.1	0.0
RPC	3	19.8.10	0.02	-	-	-	1.27	0.10	0.1	0.0
RPC	4	19.8.10	1.77	222	8.1	8.1	1.20	0.19	0.1	4.0
RPC	5	19.8.10	0.57	171	10.2	8.2	1.36	0.26	0.1	0.2
RPC	1	5.06.10					0.67	0.15	0.1	0.0
RPC	4	5.06.10					0.60	0.11	0.2	0.1
RPC	5	5.06.10	1.52				0.66	0.11	0.1	0.0
BBS	1	09.6.10					1.35	0.37	0.1	0.1
BBS	4	09.6.10					0.96	0.15	0.1	0.0
BBS	6	09.6.10	2.89				1.02	0.18	0.2	0.2
BBS	6	23.8.10	3.43	89	10.0	8.2	6.20	0.42	0.1	0.4
NFS	1	14.6.10					0.58	0.21	0.2	0.1
NFS	2	14.6.10	1.67				0.97	0.35	0.3	0.1
NFS	1	27.8.10		89	11.2	8.5	1.31	0.31	1.1	12.4
NFS	2	27.8.10		155	10.5	8.5	0.99	0.34	0.2	0.5
IVS	2	19.6.10					0.38	0.11	0.1	0.0
IVS	3	19.6.10					0.45	0.10	0.1	0.0
IVS	5	19.6.10	0.87				0.57	0.14	0.1	0.0
IVS	1	24.8.10		198	7.7	8.2	0.36	0.08	0.1	0.0
IVS	2	24.8.10		225	12.9	8.3	0.70	0.22	0.1	0.2

IVS	3	24.8.10		192	9.1	8.3	0.39	0.08	0.0	0.2
IVS	4	24.8.10		217	9.6	8.9	0.49	0.15	0.1	0.5
IVS	5	24.8.10		215	9.5	8.5	0.74	0.21	0.0	0.1
WPC	1	18.6.10					1.04	0.11	0.1	0.3
WPC	2	18.6.10	3.68				0.74	0.09	0.1	0.0
WPC	1	26.8.10		82	12.2	8.0	0.60	0.18	0.0	0.0
WPC	2	26.8.10		85	13.2	8.2	0.56	0.07	0.1	0.0
SFC	1	17.6.10					1.13	0.10	0.1	0.2
SFC	3	17.6.10					0.72	0.14	0.1	0.0
SFC	5	17.6.10					0.73	0.10	0.1	0.1
SFC	6	17.6.10					1.43	0.17	0.1	0.1
SFC	7	17.6.10	1.40				1.10	0.13	0.1	0.0
SFC	1	8.7.10					1.71	0.35	0.1	0.2
SFC	2	8.7.10					1.02	0.09	0.1	0.1
SFC	3	8.7.10					0.98	0.30	0.0	0.1
SFC	4	8.7.10					0.68	0.14	0.1	0.1
SFC	5	8.7.10					0.76	0.18	0.1	0.1
SFC	6	8.7.10	1.22				2.25	0.56	0.1	0.1
SFC	1	25.8.10		89	13.3	8.5	1.21	0.14	0.1	0.0
SFC	3	25.8.10		83	2.8	8.4	1.20	0.24	0.1	0.0
SFC	4	25.8.10		167	11.3	8.8	0.89	0.16	0.1	0.0
SFC	5	25.8.10		170	10.5	8.3	0.92	0.22	0.1	0.0
SFC	6	25.8.10		173	10.7	8.4	0.80	0.17	0.1	0.0
SFC	7	25.8.10		148	10.3	8.2	0.78	0.09	0.0	0.0
SFC	1	01.7.11	0.45	147	14.2	8.4	1.04	0.06	0.0	
SFC	2	01.7.11	0.00	106	10.1	8.5	1.05	0.06	0.0	
SFC	3	01.7.11	0.25	85	3.3	8.5	0.67	0.07	0.0	
SFC	4	01.7.11		160	14.0	9.4	0.65	0.05	0.0	
SFC	5	01.7.11	0.07	160	13.4	9.1	0.74	0.05	0.0	
SFC	6	01.7.11		142	13.2	8.5	1.01	0.11	0.0	
SFC	7	01.7.11	0.66	148	12.9	8.4	0.85	0.06	0.0	
SFC	1	04.8.11	0.19	156	17.1	8.8			0.0	
SFC	3	04.8.11	0.25	164	8.4	8.8	1.75	0.17	0.0	
SFC	4	04.8.11	0.03	166	15.0	9.5	0.95	0.11	0.0	
SFC	5	04.8.11		157	16.2	9.7	1.08	0.12	0.0	
SFC	6	04.8.11		162	15.4	9.7	0.78	0.07	0.0	
SFC	7	04.8.11	0.45	174	14.6	8.9	16.11	0.15	0.0	
WPC	1	30.6.11	2.79	73	10.7	8.4	0.71	0.05	0.0	
WPC	2	30.6.11	2.86	74	10.5	8.5	0.59	0.06	0.0	
WPC	3	30.6.11	0.13	222	8.5	8.1	0.93	0.11	0.0	
WPC	1	05.2.11	1.96	78	15.9	8.3	2.21	0.15	0.0	
WPC	2	05.2.11	1.66	76	16.4	8.5	0.69	0.10	0.0	
IVS	1	28.6.11	1.39	188	6.6	8.4	0.64	0.10	0.0	
IVS	2	28.6.11	1.48	189	7.6	8.5	0.41	0.07	0.0	
IVS	3	28.6.11	0.46	103	6.7	8.4	0.39	0.04	0.0	
IVS	4	28.6.11	0.15	120	5.9	8.4	0.37	0.05	0.0	
IVS	5	28.6.11	1.67	169	8.1	8.4	0.48	0.08	0.0	
IVS	1	03.8.11	0.85	187	12.3	8.3	0.52	0.07	0.0	
IVS	2	03.8.11	0.66	198	12.3	8.5	0.41	0.07	0.0	
IVS	3	03.8.11	0.14	133	13.0	8.6	0.56	0.11	0.1	
IVS	4	03.8.11		155	7.8	8.5	0.44	0.17	0.0	
IVS	5	03.8.11	1.10	195	12.6	8.6				
NFS	1	29.6.11	1.26	102	7.2	8.4	0.66	0.19	0.1	
NFS	2	29.6.11	1.26	110	7.8	8.2	0.71	0.20	0.0	
NFS	1	10.8.11	0.76	141	12.2	8.6	0.86	0.29	0.1	
NFS	2	10.8.11	0.64	148	12.7	8.7	0.89	0.30	0.1	
BBS	1	02.7.11	0.62	128	14.3	8.7	2.38	0.14	0.6	
BBS	2	02.7.11	0.02	279	10.8	8.5	1.00	0.14	0.1	
BBS	3	02.7.11	0.01	233	13.3	8.6	1.54	0.16	0.1	
BBS	4	02.7.11	0.14	197	14.1	8.7	0.80	0.17	0.1	
BBS	5	02.7.11	1.14	209	12.9	8.5	1.81	0.12	0.0	
BBS	1	09.8.11	0.29	78	8.6	8.4	1.14	0.18	0.7	

BBS	2	09.8.11	0.01	242	6.7	8.2	0.84	0.17	0.1
BBS	3	09.8.11	0.00	206	8.3	8.1	1.09	0.16	0.0
BBS	4	09.8.11	0.04	140	6.0	8.4	0.70	0.25	0.2
BBS	5	09.8.11	0.96	166	9.4	8.3	23.00	0.21	0.1
RPC	1	04.7.11	0.71				0.84	0.13	
RPC	2	04.7.11	0.00				1.14	0.22	
RPC	3	04.7.11	0.05				1.62	0.15	
RPC	4	04.7.11	1.40				1.20	0.19	
RPC	5	04.7.11	1.22	209	8.1	8.3	1.05	0.17	
RPC	1	08.8.11	0.44	226	12.6	8.5	1.26	0.25	0.1
RPC	2	08.8.11	0.01		13.3	8.3	1.21	0.28	0.1
RPC	3	08.8.11	0.01	306	15.2	9.4	1.19	0.10	0.0
RPC	4	08.8.11	0.47	244	12.3	8.2	0.83	0.16	0.1
RPC	5	08.8.11	0.53	253	12.2	8.4	0.89	0.18	0.0
SFC		26.6/12		50	7.2	8.2			
WPC		1.8.12		46	11.5	7.5			0.0
IVS		2.8.12		140	6.5	7.6			0.1
NFS		28.7.12		103	8.8	8.1			0.0
BBS		27.8.12		125	9.5	8.2			0.0
RPC		26.7.12		142	9.6	8.4			0.0

Table 7-5: *Hyporheic water data*

Stream	Site	Water Temp. (°C)	Cond. (µS)	pH	NO_3-N (mg/l)	NH_4-N (mg/l)	TN	DOC (mg/l)
RPC	1	10	287	8.2	0.1	0.5	0.2	0.9
(17/08/10)	2	10.6	273	8.2	0.1	0.6	0.2	0.9
	3	10.6	268	7.7	0.1	0.6	0.2	1.2
	4	9.4	333	8.1	0.1	0.7	0.3	1.4
	5	8.9	247	8.1	0.1	0.6	0.2	1.0
	6	8.8	283	8.1	0.1	0.6	0.2	1.0
BBS	2	10.7	180	8.1	0.1	0.6	0.2	1.3
(29/7/10)	1	7	347	7.9	0.2	0.6	0.1	0.7
	3	9.4	325	7.7	0.1	0.6	0.1	0.8
	4	12.9	149	8.3	0.2	0.6	0.2	2.2
	5	12.1	253	7.6	0.2	0.0	0.1	1.1
	6	10.9	214	7.9	0.1	0.2	0.2	0.9
NFS	1	8	145	8.4	0.2	0.2	0.2	0.7
(21/08/10)	2	8.6	146	8.3	0.1	0.0	0.2	0.8
	3	8.5	152	8.3	0.1	0.5	0.2	0.7
	4	8	149	8.4	0.1	0.5	0.2	0.7
	5	8.4	150	8.4	0.2	0.8	0.3	0.9
	6	8.34	157	8.7	0.1	0.6	0.3	0.8
IVS	1	8	157	8.4	0.1	0.3	0.1	1.2
(12/07/10)	2	8.2	202	8.3	0.1	0.3	0.1	1.0
	3	8.8	201	8.5	0.1	0.3	0.1	1.1
	4	8.3	202	8.4	0.1	0.4	0.1	1.0
	5	8.4	213	8.4	0.1	0.7	0.1	1.1
WPC	A1	10.7	125	8.0	0.0	1.2	0.6	2.4
(11/7/10)	B1				0.1	0.4	0.3	1.1

	B2				0.0	0.4	0.4	1.6
	C1				0.0	0.4	0.2	1.5
	C2	9.2	86	8.3	0.0	0.5	0.4	1.9
	C3	9.2	241	8.3	0.0	0.4	0.5	2.6
SFC	A				0.3	0.0	0.6	1.8
(10/7/10)	B				0.2	0.0	0.2	1.5
	C1				0.2	0.0		
	C2				0.2	0.1	1.9	4.8
	D		148	8.3	0.1	0.0	0.2	3.9
	E	14.1	137	8.3	0.1	0.0	0.3	2.0

7.4 Appendix D

7.4.1 Nitrosation data

***Table 7-6**: Nitrosation (NH_4^+-N consumption g h^{-1}) for Rush Point Creek soils.*

Stream	Site	Transect	Replicate	NH_4^+-N (µg/g) before	NH_4^+-N (µg/g) after	Nitrosation (µg g h^{-1})	NO_2-N (µg/g)
	A	**1**	**i**	30.0	2.0	1.1664	0.0000
RPC							
			ii	29.3	5.6	0.9889	0.0000
			iii	30.3	4.7	1.0674	0.0649
		2	**i**	43.4	7.7		
			ii	41.2	7.4	1.4867	0.0000
			iii	42.3	4.3	1.4062	0.0000
		3	**i**	89.2	14.6	1.5830	0.0000
			ii	85.9	43.7		
			iii	84.2	36.3	3.1093	0.0000

***Table 7-7:** Nitrosation (NH_4^+-N consumption g h^{-1}) for Berg Bay South soils.*

Stream	Site	Transect	Replicate	NH_4-N (µg/g) before	NH_4-N (µg/g) after	Nitrosation (µg g h^{-1})	NO_2-N (µg/g)
	A	**1**	**i**	32.0		32.0	
BBS							
			ii	32.1	23.0	32.1	23.0
			iii	31.9		31.9	
		2	**i**	30.5		30.5	
			ii	30.6		30.6	
			iii	30.5	28.2	30.5	28.2

	3	i	35.1		35.1	
		ii	35.1		35.1	
		iii	35.1	41.2	35.1	41.2
B	1	i	29.6	3.2	29.6	3.2
		ii	29.7	1.6	29.7	1.6
		iii	29.6		29.6	
	2	i	33.0		33.0	
		ii	33.1	3.0	33.1	3.0
		iii	33.1	3.6	33.1	3.6
	3	i	44.0		44.0	
		ii	44.1	44.1	44.1	44.1
		iii	44.1	45.8	44.1	45.8
C	1	i	29.5		29.5	
		ii	29.5		29.5	
		iii	29.4	17.6	29.4	17.6
	2	i	39.5	32.6	39.5	32.6
		ii	39.4	32.5	39.4	32.5
		iii	39.2	37.1	39.2	37.1
	3	i	32.0	26.0	32.0	26.0
		ii	31.9	27.0	31.9	27.0
		iii	32.1	36.0	32.1	36.0

***Table 7-8**: Nitrosation (NH_4^+-N consumption g h^{-1}) for North Fingers Stream soils.*

Stream	Site	Transect	Replicate	NH_4-N (µg/g) before	NH_4-N (µg/g) after	Nitrosation (µg g h^{-1})	NO_2-N (µg/g)
NFS	A	1	i	28.6	5.6	1.0	
			ii	25.8	3.1	0.9	0.0
			iii	25.1	7.3	0.7	
		2	i	25.5	5.7	0.8	
			ii	27.4	5.8	0.9	0.0
			iii	25.9	6.8	0.8	0.1
		3	i	55.4	23.6	1.3	
			ii	55.1	19.7	1.5	0.0
			iii	53.5	18.5	1.5	0.0
	B	1	i	26.9	0.0	1.1	0.0
			ii	28.9	5.9	1.0	0.1
			iii	39.6	5.4	1.4	0.0
		2	i	29.2	4.6	1.0	
			ii	29.8	3.9	1.1	0.0
			iii	32.0	4.4	1.2	
		3	i	61.6	41.1	0.9	0.0
			ii	65.1	45.8	0.8	0.0
			iii	63.5	0.0	2.6	0.0
	C	1	i	29.0	2.2	1.1	0.0
			ii	30.6	2.3	1.2	0.0
			iii	29.7	2.4	1.1	0.0

		2	i	30.7	3.1	1.1	
			ii	31.1	3.5	1.2	0.0
			iii	29.8	3.0	1.1	0.0
		3	i	35.2	12.8	0.9	
			ii	38.0	25.2	0.5	0.0
			iii	36.1	16.1	0.8	0.0

Table 7-9: *Nitrosation (NH_4^+-N consumption g h^{-1}) for Stone Fly Creek soils.*

Stream	Site	Transect	Replicate	NH_4-N (µg/g) before	NH_4-N (µg/g) after	Nitrosation (µg g h^{-1})	NO_2-N (µg/g)
SFC	A	1	i	26.7	0.4	1.1	
			ii	27.0	0.3	1.1	
			iii	27.2	1.1	1.1	0.0
		2	i	28.7	2.1	1.1	
			ii	26.1	0.6	1.1	
			iii	26.7	5.0	0.9	0.3
		3	i	25.8	0.3	1.1	
			ii	26.7	0.6	1.1	0.0
			iii	25.7	0.8	1.0	0.0