Nitrogen fertilization and tree species effect on the soil microbial communities and consequences for soil carbon

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Abstract

In the face of ever increasing atmospheric CO_2 a better understanding of soil properties and processes and the effect of management practices, such as the application of nitrogen fertilizer is of importance and could potentially improve our ability to sustainably manage forestry systems. With that in mind this study was conducted in order to investigate the effects of tree species and fertilization on soil carbon and the soil microbial community. To this end, soil from fertilized and unfertilized plots at Berwick forest, under stands of *Pinus radiata* and *Sequoia sempervirens* at Hanmer and under six different tree species at Holt forest was sampled. Two glasshouse pot trials were established using soil collected from the Hanmer and Berwick forest sites and seedlings of *Pinus radiata*, *Sequoia sempervirens,* and *Eucalyptus nitens* were grown.

Soil properties were determined from both the field sites and pot trials including soil organic matter, carbon, nitrogen, and microbial biomass by chloroform fumigation extraction. Biolog ecoplates were used to determine the relative differences in diversity based on substrate utilization patterns of the soil microbial communities in soil sampled from the glasshouse pot trials.

Soil microbial biomass carbon, nitrogen and the ratio of microbial biomass carbon:nitrogen differed significantly between *Pinus radiata* and the other tree species sampled at Holt forest. Significant effects of fertilization and tree species on soil carbon and microbial biomass were observed in both pot trials. Soil carbon differed significantly between *Eucalyptus nitens* and both *Pinus radiata* and *Sequoia sempervirens* in the first pot trial and relative to both, *E. nitens* contributed significantly more carbon. No significant effect of either fertilization or tree species on the catabolic diversity of the soil microbial community in both glasshouse pot trials was observed. The results demonstrated the effects that fertilization and tree species can have. Particularly notable was the short-time period in which tree species effects became apparent coupled with the absence of any aboveground inputs to the soil.

Chapter I

Literature Review

1.1. Introduction

Soil can be seen as an immensely complex system arising from the interaction of biotic and abiotic factors, consisting of combinations of mineral and organic particles, air and solutions. It is the medium from which plant root systems acquire water and nutrients and is host to an array of macro and microorganisms. Soil contains a great diversity of organisms the vast majority of which are microbes. In terrestrial ecosystems the greatest diversity of organisms are in the soil (Wardle, 2002). A single gram of soil is estimated to contain several thousand different species of bacteria alone (Torsvik *et al*., 1996).

1.2. Soil carbon dynamics

The focus of soil research has historically been on fertility. More recently, in the context of increasing atmospheric carbon dioxide and resulting possibility for climate change, the role of soil in carbon balance has come to the fore. Though much smaller than the oceanic carbon pool, soil is the largest reservoir of carbon in the terrestrial biosphere. It has been estimated to contain four times as much carbon as is present in the biotic pool and as much as three times the atmospheric pool (Lal, 2004; Schmidt *et al*., 2011). At the global level soil organic carbon has been estimated between 1500 Pg C and 2000 Pg C which compares to 500-550 Pg C in terrestrial biomass (Bardgett *et al*., 2005; Kutsch *et al*., 2009). Forest ecosystems in particular contain an estimated 70% of all soil organic carbon (Jandl *et al*., 2007). For New Zealand, typical estimates of soil carbon range from 44 to 268 t ha^{-1} for grassland and forest sites respectively (Tate *et al*., 1995). Furthermore, carbon dioxide released to atmosphere from belowground respiration is an order of

magnitude greater than anthropogenic sources (Raich and Potter, 1995). Soil then represents an immense potential sink for anthropogenic emissions of carbon dioxide (Lal, 2004). As soil carbon accumulates in excess of fluxes of respiration and decomposition and spends a relatively long time in the soil withheld from the atmosphere it is said to be sequestered (Swift, 2001). Recently, due to increasing levels of carbon dioxide in the atmosphere and the possibility of climate change much scientific study and debate has focused on carbon sequestration in soils and the roles that management may play to increase this. This potential role for soil carbon in climate change mitigation efforts has heightened the need for greater knowledge of tree species effects on soil carbon (Jandl *et al*., 2007). Utilizing different tree species is recognized as a possible strategy for increasing soil carbon sequestration through forest management.

1.3. How plants affect the belowground environment

In terrestrial ecosystems the above and belowground components are inexorably linked and dependent upon each other. The aboveground component in which most primary production occurs is the primary source of carbon for soil organisms belowground and the belowground component is where most decomposition occurs and is therefore essential in nutrient cycling and ecosystem functioning. As such an understanding of above and belowground linkages is essential for a proper understanding of ecosystem processes. Plant species profoundly affect, and are in turn, affected by the soil environment. Plants influence the belowground environment through a variety of mechanisms. Indirectly plants are determinants of the soil decomposer communities through determining the quantity and quality of litter available to decomposer organisms. Another mechanism through which plants have an influence upon the soil environment is through their mutualistic or antagonistic relations to root-associated soil organisms, for example, mycorrhizal fungi and root pathogens (Bardgett and Wardle, 2010). The connection between trees and the soil microbial community are complex and diverse. The microbial community is not a passive bystander reacting to the influence of trees. Any direct effect of trees on soil is largely mediated through the soil microbial community and

as a consequence distinct soil microbial communities develop (Binkley and Giardina, 1998). Soil processes such as the mineralization of dead organic matter from plants into carbon dioxide and the production of nutrients available for plant and microbial uptake are mediated by the soil microbiota.

A key concept in understanding soil and soil carbon is soil organic matter. Soil organic matter represents all organic matter in soil and includes all plant and animal residues at various stages of decomposition (Bardgett *et al*., 2005). Soil organic carbon composes 50 to 60% of soil organic matter (Kutsch *et al*., 2009). The source of soil organic carbon inputs in terrestrial ecosystems is from primary production and the overwhelming majority of soil organisms are heterotrophs which rely on organic carbon fixed by plants. Major factors which affect soil organic matter decomposition include: substrate quality, in terms of the chemical composition of the decomposing matter, soil moisture and temperature which are major factors affecting microbial growth, living plants which provide the rhizospheres much favoured for microbial growth, soil pH, and the physical accessibility of soil (Bardgett and Wardle, 2010). The soil carbon pool results from the balance between carbon inputs and outputs. The inputs are those chiefly derived from primary production and the outputs from decomposition and mineralization of carbon compounds to carbon dioxide (and to a lesser extent leaching, volatilization and burning).

1.4. Species specific effects of plants on the belowground environment

It is well established that plant species have species-specific effects on soil properties, e.g., distinct effects on soil physical, chemical and biological properties such as pH, soil temperature, soil fertility and nutrient cycling (Augusto *et al*., 2002; Hobbie, 1992; Priha and Smolander, 1998), microbial community composition and activity, and even the distribution of soil metals (Mertens *et al*., 2007) have been observed. A range of mechanisms are known to be responsible for these distinct species-specific effects. The manner in which different plant species will vary in their effect on the soil environment will in large part be determined by differences in their functional traits. It has been long

known that different tree species differ in their traits for resource availability, climate, and other physicochemical factors. For instance, conifers are usually adapted to lower nutrient conditions than their angiosperm counterparts and as such usually have lower leaf nutrient concentrations and lower rates of tissue production. Tree species differ in many other traits including partitioning between below and aboveground tissues, rooting depth, rooting structure, chemical and physical characteristics of various tissues, and differences in canopy architecture, which are known to alter soil temperature and moisture (Prescott, 2002). Trees also use a range of soil resources such as nitrogen and water, for which the specific demands vary between species.

These distinct species-specific effects have revealed the influence of tree species on the soil environment and soil life with distinct differences in microbial community composition, biomass and activity, and soil carbon having been observed. Beneath different species of plants the composition of the soil microbial community commonly differs (e.g., Carney and Matson, 2006; Grayston and Campbell, 1996; Lejon *et al*., 2005; Ushio *et al*., 2008; Priha *et al*., 2001; Leckie *et al*., 2004). The observation that different plant species differ in the soil microbial communities they support may not be surprising. It is an understanding of why they differ and details of the mechanistic foundation for these species effects, which is important for understanding above and belowground linkages.

Soil organic matter inputs to soil are predominantly from plants, via such means as litter fall, rhizodeposition and root turnover. This is a major mechanism through which different species differ in their effects on soil biota. Species specific effects are largely a result of interspecific variation in these inputs to soil. The quality, quantity and rate of these inputs differ amongst tree species (e.g., Jha and Mohapatra, 2010; Russel *et al*., 2007). There is a large body of literature which shows that differences in litter quality (e.g., soluble carbon, nitrogen, lignin, and polyphenolic compounds) between different tree species strongly affects decomposition and therefore the activity of the soil microbial community. These species-specific effects will however, vary in their effect on soil properties depending on the environmental context. For example, decomposition

rates are primarily determined by moisture and temperature, however, it is soil microbes which are directly responsible for decomposition and in the main soil microbes produce the enzymes capable of degrading compounds such as the aromatic compound lignin and the polysaccharide cellulose. There is also evidence that leaf traits correlate with root traits across species so root litter decomposability will be influenced by the same traits that affect the decomposability of leaf litter (Wardle *et al*., 1998).

1.5. Tree species effects on soil carbon

The species-specific effects that different plant species exert are evident in soil carbon. As an example, Lemineh *et al*., (2004) observed that net accretion of soil organic carbon on abandoned farmland reforested with Mexican cypress (*Cupressus lusitanica*) was greater at 27.1 Mg ha^{-1} than that after reforestation with *Eucaplytus saligna* which was only 6.1 Mg ha^{-1}. The author concluded that these differences were species dependent. These species specific effects are often a result of variation between species in the quantity and quality of carbon inputs to soil. For instance, litterfall mass can vary up to 5-fold between different tree species in the same stand (Binkley and Giardina, 1998). These variations affect the availability of carbon resources to microorganisms, and hence decomposability and turnover. For example, higher plant lignin content is known to increase soil organic carbon as lignin retards decomposition due to the specificity of strongly oxidizing lignin degrading enzymes, the relatively few soil microorganisms capable of completely mineralizing lignin, and the formation of humic substances (Swift *et al*., 1979). Conifer needles contain more compounds that are difficult to decompose including lignin which results in greater accumulation of litter on the forest floor. Studies often see a negative correlation with lignin content and leaf litter decomposition that result in the slower decomposition of conifer needle litter than broadleaf litter (e.g., Prescott *et al*., 2000; Zhang *et al*., 2009). Conifer species also tend to have shallower rooting systems and so accumulate more carbon in the upper soil layers. This means that generally soil under conifer stands is relatively higher in carbon than under broadleaf stands (Schulp *et al*., 2008).This difference is often seen in studies e.g., Augusto *et al*., (2002) Russel *et al*., (2007) which all found higher soil organic carbon content under

conifers than broadleaves. A study in Denmark found higher carbon stocks under Norway Spruce (*Picea abies*), Sitka Spruce (*Picea sitchensis*), and Lodgepole pine (*Pinus contorta* Doug.) than European beech (*Fagus sylvatica*), and Oak (*Quercus robur* L.) (Vesterdal and Raulund-Rasmussen, 1998). However, contrary to expectations of higher lignin content retarding decomposition, Raich *et al*., (2006) found faster litter decomposition with higher lignin contents in tree plantations in lowland Costa Rica. The authors hypothesized that this was due to the decomposer community at the particular site or alternatively other leaf litter characteristics such as cell soluble carbon or tannins.

Hagen-Thorn *et al*., (2004) found that differences in litter quality and decomposability were probably most important for differences in soil chemistry between the six European tree species (oak *Quercus robur* L., lime – *Tilia cordata* Mill., ash *Fraxinus excelsior* L., birch *Betula pendula* Roth., beech *Fagus sylvatica* L. and spruce *Picea abies* L.) studied, but no significant difference in soil carbon content between the species was found. However, the results were confounded by the fact that soil was from different sites and interactions between species and site factors was not taken into account. The impact of a tree species on soil will vary significantly with factors such as soil type, climate, and geology and this will confound the results of any study looking at soil from multiple sites (Augusto *et al*., 2002). Similar results were found by Vesterdal *et al*., (2008) who also studied the same tree species, with the exception of sycamore maple (*Acer pseudoplatanus* L.) in place of birch. It was found that whilst forest floor C and N was strongly influenced by species this was not the case for mineral soil C and N which, because the study took in different sites, was largely influenced by the site. Therefore, it is difficult to draw conclusions about the effects of tree species on the soil without taking these factors into account or preferably conducting studies with the same soil. Ideally then a study should look at the effect of tree species on the same site or the same soil.

Root growth rates and chemistry also differ amongst species and like leaf litter the substrate quality of root litter varies from species to species (Raich *et al*., 2010). However, the contributions made by rhizodeposition and root turnover are harder to quantify than that from aboveground inputs. Root turnover is an important source of detritus in soils

with an important influence on soil organic matter dynamics it makes up an important part of the global carbon cycle. Root detritus is generally a poorer quality resource than aboveground inputs and hence represents a more recalcitrant carbon pool in soil. This is due to the generally higher chemical resistance of roots compared to shoots as they have less easily decomposable soluble compounds, and are generally more lignified (Rasse, *et al*., 2005). Most studies focus on aboveground inputs to soil. However, some studies have found that root growth contributes more to soil organic carbon stocks than do aboveground inputs (e.g., Russel *et al*., 2004; Russel *et al*., 2007). Rasse *et al*., (2005) argue that soil carbon is mostly root carbon. Russel *et al*., (2007) found that SOC differed significantly amongst several different tree species and that this was correlated with fine root growth and not aboveground detrital inputs and interestingly SOC declined with increasing fine root lignin content. Root exudates (e.g., carbohydrates, amino acids, phenolics) are also a significant carbon input to soil. Root exudates can account for 5-33% of daily photoassimilate (De Dyne *et al*., 2008). Root exudates and root turnover also contribute to carbon sequestration through interaction with soil minerals to form soil aggregates. Soil aggregation is known to increase carbon sequestration. The fundamental mechanism through which this is achieved is the exclusion of microorganisms from carbon resources within microaggregates (Blanco-Canqui and Lal, 2004).

Most of the studies mentioned above only examined total organic carbon and did not examine the different forms of soil organic carbon. SOC can broadly be divided into three different forms, labile, slow, and recalcitrant (Kutsch *et al*., 2009). These pools are defined by their differing turnover times. The labile pool represents the carbon pool which turns over most rapidly, usually cited as a few days to a few months. The slow pool has a turnover time usually cited on the order of years to decades and the recalcitrant pool centuries to millennia (Kumar, 2006). Because of its faster turnover time than total SOC labile organic carbon is considered a sensitive indicator of changes that may be occurring in soil. Microbial biomass carbon (MBC) is one such pool of labile organic carbon. Microbial biomass generally constitutes 2-4% of total soil organic carbon which equates to between 0.5 and 2 t C ha^{-1} (White, 1997). Tree species have different impacts on the dynamics of these different carbon pools. Jiang and Xu (2006) looked at the soil

labile carbon pools under Mason Pine (*Pinus massoniana* Lamb.) and Chinese Fir (*Cunninghamia lanceolata* Hook.). Soil under Mason Pine has significantly higher total organic carbon which related to greater levels of different labile organic carbon pools including easily oxidized carbon (EOC), microbial biomass carbon and water soluble organic carbon (WSOC), but when expressed as a percentage of total carbon these pools were significantly lower than those under Chinese Fir.

Much of the carbon that enters soil enters in dissolved organic matter therefore dissolved organic carbon (DOC) is an important component of soil organic carbon, and included in the formation of soil organic matter. DOC plays an important part in soil biological processes. Because an aqueous medium is required for microbial uptake mechanisms DOC is an important substrate for soil microorganisms (Bardgett, *et al*., 2005). As such, DOC can be a sensitive measure of changing soil conditions. Conditions which increase mineralization and high microbial activity tend to increase DOC concentrations. Strobel *et al*., (2001) found the concentration of DOC varied with species. However, the composition and chemical properties of DOC were not influenced by species.

Species-specific effects not only concern carbon sequestered, but carbon loss as well. Most loss of soil carbon is from respiration. This respiratory loss results from the metabolic activity of both the microbial and plant members of the soil community. The separation of these two sources is incredibly difficult because there is no easy way to distinguish the sources. Plants directly affect soil respiratory losses through their own respiration or indirectly through their influence on the heterotrophic activity (De Dyne *et al*., 2008). Faster growing species generally have higher rates of respiration than slower growing species as they are more metabolically active (Opik *et al*., 2005). The quality and quantity of plant carbon inputs to soil will indirectly affect carbon loss through soil respiration. Brechet *et al*., (2009) found that variability in soil respiration across 16 different monospecific forest tree plots was mainly explained by variability in both quantity and quality of leaf litter and tree productivity with no relationship between respiration and soil temperature, moisture or root biomass. The release of labile carbon by plants especially in the form of root exudates stimulates mineralization of not only

recent but old SOC. This effect is known as 'priming' (Bardgett and Wardle, 2010). Root exudation is thus very important in the gain and loss of soil organic carbon.

1.6. Tree species effects on soil microbial communities

Distinct species-specific effects have been revealed showing the influence of tree species on microbial community composition, biomass and activity (e.g., Carney and Matson, 2006; Grayston and Campbell, 1996; Lejon *et al*., 2005; Ushio *et al*., 2008; Priha *et al*., 2001; Leckies *et al*., 2004). These species specific effects are due to the same mechanisms already outlined, most significantly the interspecific differences in the quality and quantity of aboveground inputs to soil, and root inputs such as root turnover and rhizodeposition. Allelopathic chemicals are also known to affect the soil microbial community (Pellissier and Souto, 1999).These species specific differences have been demonstrated through physiological profiling, enzyme activities, and the use of molecular techniques. Evidence from other plants such as grasses (e.g., Grayston, *et al*., 2001, 2004) and agricultural crops (e.g., Johnson, *et al*., 2003; Wieland, *et al*., 2001) show that distinct soil microbial communities are very often associated with different plant species.

One approach for studying soil microbial communities is physiological profiling. Community level physiological profiles (CLPPs) based on sole carbon source oxidation have been used as a method to study soil microbial functional diversity as the utilization of carbon is a factor governing microbial growth in soil. This can be achieved by measuring substrate induced respiration or CO_2 following the addition of various carbon substrates or through BIOLOG™ a redox based system which utilizes microtiter plates containing various carbon substrates (Garland and Mills, 1991). Using BIOLOG™, Grayston and Campbell (1996) observed significant differences in the carbon source utilization by microbial communities from the rhizospheres of larch (*Larix eurolepsis*) and Sitka spruce (*Picea sitchensis*) suggesting that the two species differ in the root exudates they produce. Grayston (2000) found distinct differences in carbon source utilization profiles between larch, Sitka spruce and sycamore (*Acer pseudoplatanus*) in rhizoplane communities, however, no difference was found between their rhizosphere

communities. Selmants *et al*., (2005) using community level physiological profiling and enzyme assays, observed a dramatic alteration of the community-level soil microbial function with the presence of the N-fixing Red alder (*Alnus rubra*) in conifer forests. N-fixing trees such as the Red alder have been shown to increase soil carbon sequestration and rates of nutrient cycling (Resh *et al*., 2002). Although community level physiological profiling provides useful information for assessing soil microbial diversity it suffers from the same bias as any culture-dependent method. That is, that the vast majority of soil microorganisms are as yet unculturable and results are biased towards those microorganisms that grow best under assay conditions which may not be the dominant members of the microbial community. This makes the interpretation of results difficult. Furthermore, the substrates available in BIOLOG™ are not necessarily ecologically relevant (Hill *et al*., 2000). Therefore, results from such methods can only be used to describe the 'potential' functional diversity of the soil microbial community. Analysis using 16s rDNA of the microbial populations in BIOLOG™ wells has demonstrated that the culture conditions select for a very limited subset of microbial genera and so are not representative of the functional abilities of the entire soil microbial community (Ros *et al*., 2008).

Enzyme activities also reflect the functional capacity of the soil microbial community. Waldrop *et al*., (2000) showed differences in the enzyme activities in soil dominated by different species where tropical forest had been converted to pineapple plantations. For instance, β -glucosidase and sulphatase activity was lower in the pineapple plantation than the forest. β -glucosidase is an important carbon degrading enzyme in soils as it is involved in the depolymerization of cellulose into glucose. Interestingly, in this case BIOLOG™ was unable to discriminate between the forest and plantation soil communities whereas the enzyme assays, phospholipid fatty acid (PLFA) profiles and biomass determinations were able to make the distinction. Ushio *et al*., (2009) also found differences in β -glucosidase activity under different tree species.

Many studies have characterized soil microbial community structure by using PLFA profiles. More recently molecular methods based on analysis of nucleic acids extracted

from soil have been used extensively. Several techniques have been utilized including terminal restriction fragment length polymorphism (T-RFLP), ribosomal intergenic spacer analysis (RISA), and denaturing gradient gel electrophoresis (DGGE). Fluorescent *in situ* hybridization (FISH) differs in that it allows direct identification of taxonomic groups or specific microorganisms in their microhabitat. In this technique cells are fixed and fluorescently labeled with an oligonucleotide probe then viewed by scanning confocal laser microscopy (Hill *et al*., 2000). Due to the inherent bias problems of culture-based methods culture-independent methods, which do not suffer from culturability bias, have become a mainstay in the study of soil microbial community diversity (Hill, *et al*., 2000).

Hackl *et al*. (2004), using T-RFLP and sequence analysis of 16S rRNA genes, compared the diversity and composition of soil bacterial communities in six Austrian forests. It was found that pine forests were related with high-GC content Gram-positive bacteria whereas the oak-hornbeam and spruce-fir-beech forests were mainly related with *Holophaga/Acidobacterium* group. These studies were carried out in different forest ecosystems consequently they confound the tree species effect and the influence of physicochemical variations of the soil on the microbial community. To draw conclusions about the effect of tree species on microbial communities, studies need to be carried out on the same soil. Lejon *et al*., (2005) studied the long term effects of tree species substitution on indigenous soil microbial communities using monospecific tree plantations on the same soil type in Morvan, France. Using DNA fingerprinting, differences in the soil community profiles under different tree species (Douglas fir, Norway spruce, oak, and mixed oak and beech) were observed. Only small differences in microbial biomass were observed between the tree species. Other studies including soil microbial biomass have shown little variation between tree species (e.g., Ayers *et al*., 2009) whereas other studies have shown significant differences between tree species (e.g., Priha and Smolander 1999; Grayston and Prescott, 2005; Iovieno, *et al*., 2010).

Mirroring the results of studies on soil carbon, Ushio *et al*. (2008), found that microbial community composition, as judged by lipid profiles, differed between conifers and

broadleaved species alongside significant differences in total carbon. The relative abundance of bacteria and fungi has also been found to differ amongst tree species. In contrast, Ayers *et al*. (2009), whilst studying adjacent near-monotypic stands of Trembling aspen (*Populus tremuloides*), lodgepole pine, and Engelmann spruce (*Picea engelmannii*), found no species effect on soil carbon or microbial biomass carbon, but microbial community composition differed as measured by T-RFLP analysis.

Assessing microbial diversity by molecular methods has its limitations. Unlike physiological methods, molecular methods provide relatively little information about microbial activities in soil and there is bias associated with DNA extraction and PCR amplification (Leckie, 2005). To reveal a more complete picture of a microbial community and to overcome the shortcomings of various individual techniques the use of a combination of different methods is desirable. For instance, a study by Kourtev *et al*., (2002) showed that the microbial community under three different grass species differed in both structure via PLFA composition and function via enzyme activities and substrate induced respiration. It was found that the bulk and rhizosphere soil communities were distinct but both were different from the initial soil when the plants were not present demonstrating that the species effect in this instance extends through the soil. The relationship between the structure and the function of the soil microbial community, such as is shown in the study by Waldrop *et al*. (2000), mentioned in the preceding section, and Kourtev *et al*. (2002), is rarely explored. Such studies show that changes in microbial community composition are accompanied by changes in their functionality and could provide links between the degradation of certain carbon compounds and community characteristics.

The effect of tree species on the soil environment is also mediated by associated, e.g., symbiotic and commensal, microorganisms. Tree species form close associations with mycorrhizal fungi. Tyler (1992) studied the affinity of 137 species of ectomycorrhizal and decomposer fungi for Beech (*Fagus sylvatica* L.), Oak (*Quercus robur* L.) and Hornbeam (*Carpinus betulus* L.). A high level of affinity was observed with half of the species occurring mainly or exclusively in one of the forest types. Philips and Fahey

(2006) found that variability in the magnitude of the rhizosphere effect between different tree species could be explained by the different mycorrhizal associations they formed. These being, arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM). The rhizosphere effect of ECM tree species was larger than that of AM tree species with microbial biomass, N mineralization and phosphatase enzyme activity being greater.

Not only have the effects on soil microbial communities of different tree species been observed but there is even an effect of within species variation. Schweitzer *et al*., (2004) showed that variations in condensed leaf tannin concentrations amongst Poplar (*Populus angustifolia*) hybrids affected litter decomposition and soil nitrogen mineralisation rates. In another study using PLFA profiles it was shown that different genotypes of the same Poplar hybrid significantly influenced microbial biomass and community composition (Schweitzer *et al*., 2008).

The soil microbial biomass is the living fraction of soil organic matter and represents a fraction of organic and inorganic nutrient pools in ecosystems. It excludes plant roots and macrofauna. It is often expressed in terms of the microbial biomass carbon (MBC) or microbial biomass nitrogen (MBN). Because of its relatively fast turnover time it is an important indicator of changes in soil organic matter (Powlson, *et al*., 1987). Measurements of microbial activity, such as carbon usage through the aforementioned BIOLOG™ provide a more sensitive measure of changes in soil.

Studies have shown that it is not always the most productive plant species that support the greatest biomass of soil organisms. For instance, in a study involving plant functional groups in serpentine grassland soils the most productive monocultures did not necessarily support the highest levels of soil microbial biomass (Hooper and Vitousek, 1997, 1998). Many studies however, show that species which produce high quality rapidly decomposing litter support a greater biomass of soil organisms (e.g., Hooper and Vitousek, 1997).

Plant species also differ in the resources they release through the rhizosphere. It is well established that different plant species can differ greatly in their rhizosphere communities and that there is some level of specificity between plants and their associated mycorrhizal fungi, with an estimated 90% of plant species commonly mycorrhizal, ranging from narrow to broadly receptive of mycorrhizal symbiosis (Allen, 1992). Plants also affect decomposer organisms through the release of recently fixed carbon from their root system. It has long been known to be an important process in fast growing herbaceous plant species. From tree girdling (Hogberg, *et al*., 2001) and ^{13}C labeling experiments (Pollierer, *et al*., 2007; Hogberg, *et al*., 2008) it has recently been shown that tree species also release substantial amounts of recently fixed carbon through rhizodeposition and that this exerts a major influence on soil microbial communities (Hogberg *et al*., 2008). For instance, Hogberg, *et al*., (2001) observed that within 1-2 months of girdling in a boreal Scots pine (*Pinus sylvestris* L.) forest soil respiration was reduced by approximately 54% relative to controls.

1.7. Soil nitrogen and carbon relations

The terrestrial carbon and nitrogen cycles are closely linked. Nitrogen is cycled through different process in the soil system involving fixation, mineralization, nitrification and denitrification, volatilization and immobilization. In soil, nitrogen exists in three major reservoirs, the majority of which is in the form of organic N associated with humus, plant material and soil organisms which are not available for plant uptake; ammonium fixed with clay minerals and lastly, the smallest pool of N, mineral N or inorganic N (e.g., nitrate, nitrite and ammonium) available to plants. Because it is predominately in the organic form it needs to be broken down by soil microorganisms to produce the mineral forms available for plant uptake (Bardgett and Wardle, 2010).

Nitrogen inputs into soil affect soil carbon through effects on plant growth, litter production and decomposition and stabilization of soil organic matter. Nitrogen fertilization is an increasingly common forestry management and agricultural practice.

Such practices and other anthropogenic nitrogen sources, including atmospheric or wet deposition of fixed nitrogen (Goulding *et al*., 1998) have significantly increased nitrogen deposition globally. It has been estimated that human driven processes fix an estimated 140 Tg N per annum (Galloway *et al*., 1995) which is thought to be more than is fixed naturally. This affects both the nitrogen and the carbon cycles. As soil is a major source and sink of atmospheric carbon and is the principal storehouse of energy for the nutrient recycling activity of heterotrophic microorganisms an understanding of the effects of nitrogen supply on soil carbon is therefore of importance.

The effect increased nitrogen availability has on soil carbon is uncertain, but is generally expected to be positive because nitrogen fertilization of forests is expected to lead to carbon accumulation in soil due both to increased primary productivity and stabilization of carbon (Townsend *et al*., 1996). However, results from studies on the effects of nitrogen inputs to soil, through a range of N-fertilization experiments, investigations of the biogeochemistry of N-fixing plants and simulated N deposition, have been highly variable. For example, Nef *et al*. (2002), found that in dry meadow community soils nitrogen addition significantly accelerated the decomposition of labile soil carbon and in contrast contributed to the stabilization of recalcitrant soil carbon with no significant change in total soil organic carbon being observed. In an attempt to answer the question of whether N fertilized forest soils are sinks or sources of carbon Miegroet and Jandl (2007) developed a conceptual model that suggested that N fertilization could lead to a depletion of the soil C pool because the microbial transformation of excess N necessitates increased microbial biomass which is accompanied by increased respiratory losses of C as labile soil C is mineralized. They concluded that N fertilization would reap the greatest benefits in terms of C sequestration at high productivity sites, but would be a poor strategy at marginal sites.

A study by Waldrop *et al*. (2004), involving oak dominated ecosystems with low quality litter and sugar maple (*Acer saccharum*) dominated ecosystems with high quality litter demonstrated the importance of litter quality deriving from different tree species. As a result of N fertilization a significant loss of soil carbon was observed in the oak

dominated ecosystems and a gain in the sugar maple dominated ecosystem. This demonstrates that the effects of N fertilization on soil C are not capable of being generalised across sites and are mediated by local factors including, and importantly, tree species.

Species specific effects of trees on soil nitrogen have been found in a number of studies. For example in a study of nitrogen availability under stands of douglas fir (*Pseudotsuga menziesii*), lodgepole pine, and paper birch (*Betula papyrifera*) it was found that the highest N concentrations were under douglas fir which correlated to N mineralization rates, but not to N concentrations of the litter with douglas fir litter having the lowest N concentration of the three species (Thomas and Prescott, 2000). In contrast, Ayres *et al*. (2009), in studying the influence of tree species traits on soil found that, all things being equal, total soil N did not differ between stands of the three tree species studied, trembling aspen, lodgepole pine, and engelmann spruce, despite large differences in the N concentrations of the litter, e.g., aspen litter N concentrations were twice as high as the pine and spruce, although nitrate concentrations were highest in aspen stands. Priha and Smolander (1998) found differences in microbial biomass nitrogen, net ammonification and nitrification, denitrification potential, total N and C to N ratio between stands of Scots pine (*Pinus sylvestris*), European spruce, and birch which were correlated to microbiological characteristics.

The results of short term studies of the effects of nitrogen fertilization on microbial biomass have given varying results. Longer term studies however, have shown decreases in microbial biomass. A meta-analysis of ecosystem studies determined that microbial biomass declines on average 15.4% due to N fertilization (Treseder, 2008). However, when fungi and bacteria are examined separately no significant changes in either group are shown. Results of such studies are confounded by a lack of consideration of turnover rates. The standing biomass of microbes will not necessarily reflect turnover rates. This aspect is rarely studied in soil under N fertilization. Using thymidine incorporation to determine bacterial growth rates, Stapleton *et al*. (2005), observed positive effects of N fertilization without concomitant changes in standing biomass. In contrast Demoling *et al*,

(2008) saw a decline in bacterial turnover rates measured by the same method and no change for fungal rates when determined by acetate incorporation. Results from a study of regenerating conifer forests with and without N fertilization indicated that it was not the direct effects of fertilization which were the influencing factor on the microbial communities, but rather differences in plant growth rates (Leckie *et al*., 2004).

The effect of increased N availability on soil microbial biodiversity has also been studied. For instance, in an Alaskan boreal forest N addition reduced fungal biodiversity (Allison *et al*., 2007). A study by Bardgett *et al*., (1999) in upland grasslands found that N additions had no consistent effect on soil microbial biomass or activity, however, the microbial community structure was significantly altered with an increased proportion of fungi to bacteria as measured by fungal:bacterial PLFA. In contrast, a study of chronic nitrogen enrichment in pine and hardwood stands found a reduced fungal:bacterial biomass ratio along with a decrease in ectomycorrhizal diversity (Frey, *et al*., 2004).

1.8. Summary

It is clear from the evidence in the literature of numerous past studies that plants, and in particular trees, substantially affect the belowground environment and are in turn affected by it in an intimate relationship including biotic interactions with soil microbial communities which are fundamental components and drivers of global processes, including the carbon and nitrogen cycles. Soil microbial community composition, biomass and activity have all been shown to be influenced by tree species present. Plant inputs to soil from such sources as leaf and root litter and rhizodeposition are important mechanisms through which the composition and activity of the soil microbial community is influenced and consequently soil carbon is affected. Species specific effects of trees to soil carbon are evidenced in the literature and as the largest reservoir of carbon in the terrestrial biosphere (Kutsch *et al*., 2009) this has significant implications for global processes. Nitrogen fertilization, an increasingly important management practice in forestry and agriculture, likewise influences soil carbon and soil microbial communities

and interacts, via plant growth, with the way in which plants impact upon them. This in consequence has effects on the carbon cycle.

1.9. Objectives and research rationale

The main objectives of my research were to investigate tree species effect on soil carbon and the microbial community and in conjunction the effects of nitrogen fertilizer addition on these. Field sites were selected from which soil was sampled and analysed from under stands of different tree species. To further investigate this, two pot trials were established with soil collected from the same field sites, including soil from a long term productivity study, the pots were planted individually with trees of different species and plant free controls were included. Nitrogen fertilizer was applied to a selection of them to be compared to those without such addition. The research rationale was that this would provide a controlled study of the tree species effect on soil carbon and the microbial community in conjunction with information provided by field studies. The following four main hypotheses were tested:

1. There would be a significant tree species effect on soil carbon.
2. The application of N fertilization would have a significant effect upon soil carbon.
3. There would be a significant tree species effect on the soil microbial community.
4. The application of N fertilization would have a significant effect on the soil microbial community.

To test these hypotheses, using laboratory analysis, soil characteristics including, total carbon and nitrogen, microbial biomass, and community level physiological profiling, which to my knowledge had not been carried out in a pot trial scenario such as that used in this study before, were investigated.

This study sought to further our understanding of tree-soil interactions and how tree species affect soils by providing data on the effect of chosen tree species and the effect of nitrogen addition to soil. This study will also consider the wider implications for the

environment and forestry management of the results gained and the previous work done in this field.

Chapter II

Methods

2.1. Site Descriptions

2.1.1. Berwick Forest

The first site for sampling was Berwick Forest (coordinates 45º57'37.60" S 170º04'57.79" E see Fig 3) near Mosgiel, approximately 45 km southwest of Dunedin, New Zealand. It has an area of 13,141 hectares. The site is part of a long term soil productivity study (LTSP) part of which involved the application of fertilizer to plots to an amount of 950 kg ha^{-1} whilst others were left unfertilized. The plots which are of Radiata pine (*Pinus radiata*) were planted in 1990. The plots are also divided into those that had whole tree harvest or log only harvest and weed control or no weed control. For a map of the plots see Fig 1.

2.1.2. Hanmer

Two further sites were sampled near Hanmer, New Zealand. The first was a Redwood (*Sequoia sempervirens*) plantation on Department of Conservation land (coordinates 42º31'04.80" S 172º50'17.16" E see Fig 2). The second site, was an adjacent Radiata pine plantation. The pine plot was a 48 by 56 m plot. There was very little forest floor cover at both sites. The little forest floor cover in the Radiata pine plantation consisted of blackberry (*Rubus armenciacus*), and Douglas fir (*Pseudotsuga menziesii*) seedlings all less than 1 m in height.

2.1.3. Holt Forest

Holt Forest (coordinates 39º14'43.74" S 176º48"23.25" E see Fig 4) is a 15 hectare arboretum north of Napier, New Zealand. Established in 1933 and maintained by the Holt

Forest Trust. It contains both native and introduced tree species including Douglas fir (*Pseudotsuga menziesii*), Scots pine (*Pinus sylvestris*), white cedar (*Thuja occidentalis*), kauri (*Agathis australis*)**,** rimu (*Dacrydium cupressinu*), kahikatea (*Dacrycarpus dacrydioides*), coast redwood (*Sequoia sempervirens*), tōtara (*Podocarpus totara*), and radiata pine (*Pinus radiata*).

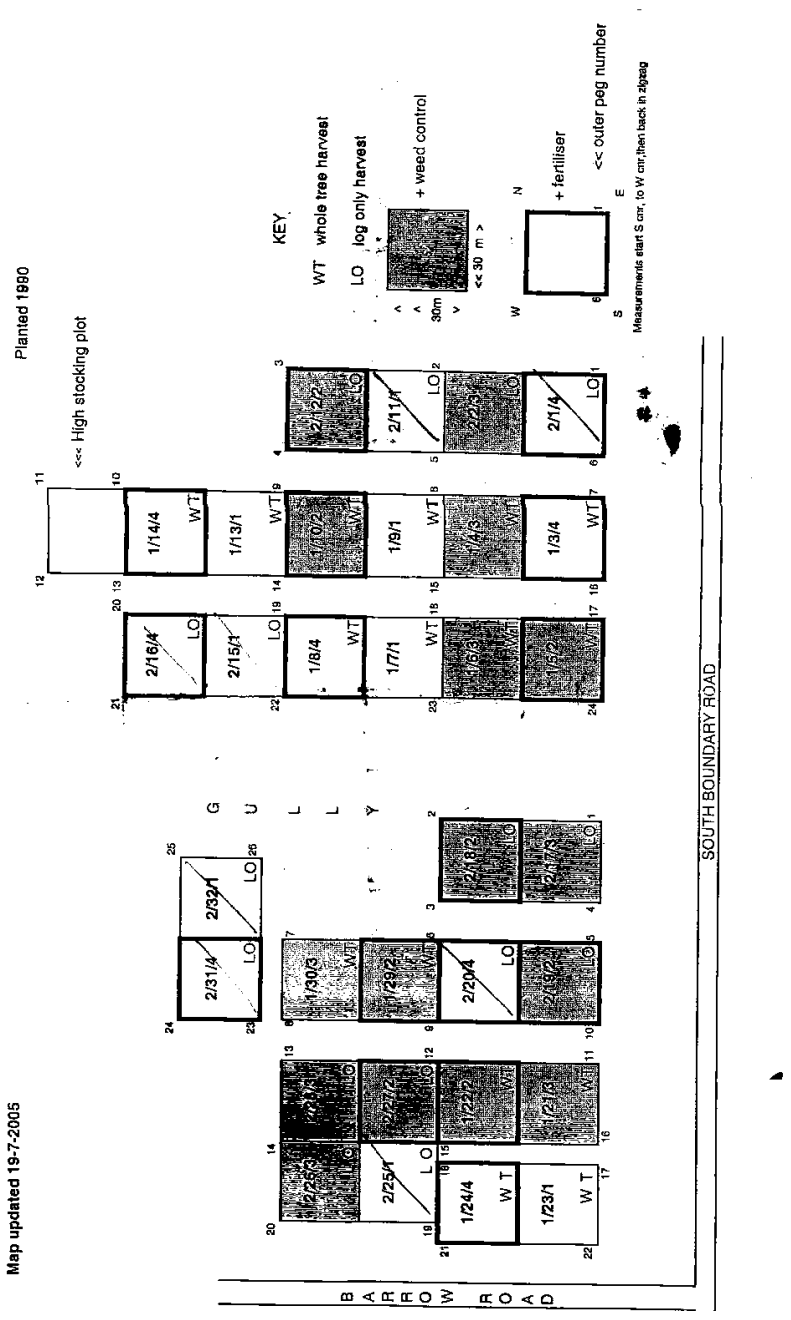

Figure 1: *FR127 Sustainable Forestry Trial Berwick CPT 79*

Figure 2: *Hanmer sampling site satellite image.*

Figure 3: *Berwick forest sampling site satellite image.*

Figure 4: *Holt forest satellite image.*

2.2. Field Methods

To gather samples for analysis and collect soil for use in two pot trials (see section 2.3.1) soil was sampled over two days from eight fertilized and unfertilized plots at a long-term soil productivity from Berwick Forest from plots which had received weed control and log only harvest. The litter layer was removed by hand and the soil was sampled to a depth of 5cm. Soil was also collected from both the redwood and radiata pine Hanmer sites over the course of one day. The litter layer was removed and the soil was collected to a depth of 5 cm.

The soil collected was broken up by hand and plant roots removed before it was sieved to 2mm. It was then spread out to air dry for a about a week and a half over which period samples were weighed regularly to a constant weight before being bulked together. Subsamples from each plot were set aside and stored. The soil was then bulked together by hand and soil weighed.

The soil from Holt Forest was sampled over the course of two days. Eight sites were chosen one for each of the following species Douglas fir (*Pseudotsuga menziesii*), white cedar (*Thuja occidentalis*) rimu (*Dacrydium cupressinu*), kauri (*Agathis australis*), eastern white pine (*Pinus strobus*), Radiata pine (*Pinus radiata*), and coast redwood (*Sequoia sempervirens*). 4 sampling sites within each of those sites were randomly selected and four soil cores were taken with a soil corer to a depth 10 cm within each.

2.3. Experimental Design

2.3.1. Pot Trial

Two glasshouse pot trials were carried out using soil from the Hanmer and Berwick forest sampling sites.

For use in the pot trials *Pinus radiata, Sequioa sempervirens,* and *Eucalyptus nitens* seeds were obtained from a seed supplier. The *Pinus radiata,* and *Sequioa sempervirens* seeds were imbibed in water on seed germination paper and stratified for three weeks. The fungicide captan (ethanethiol) was added to prevent the growth of fungi.

40 mm diameter tubes were used and ~95 g of the sieved and air-dried soil collected from the field (see section 2.2) was added to a depth of 90mm and brought to a water content of ~ 40% with distilled water. 40 mm of Oasis® planting material was placed on the end of each tube and the setup placed within another container (see fig 2).

14 replicates of each of the *Pinus radiata, Sequioa sempervirens,* and *Eucalyptus nitens* and 10 plant free controls in the Berwick soil in both sets of soil, fertilized and not fertilized, as part of the LTSP were seeded giving a total of 104 pots in the first trial.

For the second pot trial the soil collected from the Hanmer sampling sites was used. 28 *Sequioa sempervirens* seedlings were planted, as above, in the soil collected from under the *Sequioa sempervirens* stands and 28 *Pinus radiata* seedlings were planted in the soil

collected from under the *Pinus radiata* stand. With this 20 plant free control pots for each soil were prepared giving a total of 96 pots in the second trial. Half of the pots received fertilization with urea (CH_4N_2O) dissolved in distilled water and half did not. A total 0.12 g of urea, equivalent to 955 kg ha^{-1}, was applied over the course of the trial divided between 6 separate applications.

The plants and plant-free controls for both trials were kept in the same glasshouse conditions in the University of Canterbury, School of Biological Sciences glasshouses. They were watered regularly with distilled water. To prevent their location within the glasshouse becoming a confounding factor in the trial the pots were assigned numbers and randomly rearranged on a monthly basis, via the use of a random number generator to generate new positions.

Figure 5: *Left: Eucalyptus nitens plant in pot trial. Right: Image showing a portion of the pot trial.*

2.4. Laboratory Methods

2.4.1. Sub-sample Preparation

The samples collected from the field were stored in labeled polyethylene bags overnight at 4ºC. Large soil aggregates were broken up before sieving with a 2mm sieve to remove stones, roots and other debris. The sieve was thoroughly cleaned with a brush between the sieving of each sample to avoid contamination. Sieving of the soil is necessary to allow for proper penetration of the fumigant for microbial biomass determination, for total carbon and nitrogen analysis and to obtain representative samples.

To determine whether or not carbonates were present in the soil a test was performed by adding a few drops of HCl to a sample of the soil and observing any effervescence. There was none.

2.4.2. Soil Water Content

Soil water content was determined by the gravimetric method as described in Carter and Gregorich (2008). Briefly, replicates of approximately 5g of soil from each sample were weighed into pre-weighed crucibles. Then they were placed in an oven at 105ºC for 24 hrs. After which they were cooled in a desiccator before being re-weighed. The following calculation was used to determine the gravimetric water content (equation 1):

Gravimetric water content:

$$= \frac{\text{(mass of moist soil + container) – (mass of container)}}{\text{(mass of oven dry soil + container) – (mass of container)}}$$

(Carter and Gregorich, 2008).

2.4.3. Soil pH

The pH of the soil was determined in suspension (Carter and Gregorich, 2008). Deionised water was added to a 5 g soil sample in a 1:2 soil:water ratio. This was mixed thoroughly by vigorous shaking. Following which the slurry was allowed to settle for 5 minutes. The pH of the suspension was then measured with a pH meter at room temperature by placing the electrode into the solution and the level recorded when a stable reading was reached.

2.4.4. Soil Organic Matter Content

Soil organic matter was determined by using the weight loss on ignition method. This method is based on measuring the weight lost from an oven dry soil sample when exposed to high temperatures.

Soil samples were first oven-dried in pre-weighed porcelain crucibles, as for the determination of soil water content. Following which, soil and crucibles were re-weighed and then placed in a muffle furnace at 450ºC for 12 hours. After removal from the muffle furnace they were allowed to cool in a desiccator before being re-weighed to determine the weight lost on ignition. The calculation to determine the organic matter content was as follows (equation 2):

% Organic Matter =

$$\frac{\text{(weight remaining)}}{\text{(original weight)}} \text{X100}$$

2.4.5. Soil Microbial Biomass

The fumigation extraction method (Brookes *et al*., 1985) was used to determine microbial biomass carbon and microbial biomass nitrogen.

Soil Preparation

Plant roots were removed by handpicking. 10 g portions, on an oven dry basis, of fresh soil were weighed out into 125 mL glass flasks.

Fumigation treatment

The fumigation was carried out in large desiccators. The desiccators were lined with freshly moistened paper towels and the glass flasks containing the soil samples were placed into the desiccators together with a 100 mL glass beaker containing $CHCl_3$ and boiling chips. Using a water aspirator pump the desiccators were sealed and evacuated until the $CHCl_3$ boiled vigorously. The desiccators were sealed under a vacuum and left at room temperature for 48 hrs.

After 48 hrs of fumigation the vacuum was released and the beaker of $CHCl_3$ was removed. Residual $CHCl_3$ vapor was removed by repeated evacuations using a water aspirator pump.

Extraction of Microbial Biomass Carbon and Nitrogen

0.5 *M* K_2SO_4 was added to the sample bottles containing the fumigated and unfumigated control samples. The flasks were then placed on an orbital shaker for 1 h. After shaking, the K_2SO_4 soil suspension was filtered through Whatman GFA filter paper. The filtrate was frozen before analysis.

Microbial biomass nitrogen was determined by the micro-kjeldahl digestion method followed by steam distillation as described in Carter and Gregorich (2008). To 0.5 L distillation flasks 5 g of soil was added. To each distillation flask 3.5 g of K_2SO_4:$CuSO_4$, one hangar granule and 10 mL of concentrated H_2SO_4. The distillation flasks were then placed on a digestion block and the temperature raised and maintained until digestion was complete. The flasks were then connected to the steam distillation apparatus, under which

a 100 mL beaker containing 5 mL of H_3BO_3 was placed with the tip of the condenser immersed in the H_3BO_3, and secured with a clamp. The steam supply was opened. Slowly an excess of 10 M NaOH was added through the distillation head. The steam was then directed into the distillation flask until approximately 40 mL of distillate was collected. The distillate was then titrated with 0.01 M H_2SO_4.

Microbial biomass nitrogen was determined by the use of flow injection analysis in a persulphate digest. In this method, dissolved organic N and NH_4+ are oxidized to NO_3- by persulphate.

Microbial biomass carbon and nitrogen was measured using an elemental analyser.

Microbial biomass carbon (MBC) in the soil was calculated as follows (Carter and Gregorich 2008):

$$\text{MBC } (\mu g\ g^{-1} \text{ soil}) = (C_F - C_{UF})/k_{EC} \quad \text{(equation 3)}$$

where k_{EC} is the assumed value for the efficiency with which microbial biomass carbon is extracted, assumed here to be 0.35 (Joergensen, 1996), and where C_F (total weight of extractable C in the fumigated soil) C_{UF} (total weight of extractable C in the unfumigated soil) is calculated as follows:

$$C_F, C_{UF}\ (\mu g\ g^{-1} \text{ soil}) = \text{organic C } (\mu g/mL) \times [\text{VS (ml)}/\text{MS (g)}] \quad \text{(equation 4)}$$

where MS is the weight of the soil sample (oven-dry equivalent) used and VS is total volume of solution in extracted soil (mL) calculated as:

$$\text{VS (mL)} = [(\text{soil wet weight (g)} - \text{soil dry weight (g)})/1 g\ mL^{-1}] + \text{extractant volume (mL)} \quad \text{(equation 5)}$$

Microbial biomass nitrogen (MBN) in the soil was calculated as follows:

$$MBN\ (\mu g\ g^{-1}\ soil) = (N_F - N_{UF})/k_{EN}\ \text{(equation 6)}$$

where k_{EN} is the assumed value for the efficiency with which microbial biomass nitrogen is extracted, assumed here to be 0.5 (Joergensen and Mueller, 1996), and where N_F (total weight of extractable N in the fumigated soil) N_{UF} (total weight of extractable N in the unfumigated soil) is calculated as:

$$N_F, N_{UF}\ (\mu g\ g^{-1}\ soil) = \text{organic N } (\mu g/mL) \times [VS\ (ml)/MS\ (g)]\ \text{(equation 7)}$$

where MS and VS are calculated the same as for MBC (equation 5).

2.4.6. Total Carbon and Nitrogen

Total organic carbon (TOC) and nitrogen was measured by use of an elemental analyser. New Zealand soils generally have an insignificant free carbonate content in consequence of soil pH levels generally below 7. Furthermore, testing of the soil for carbonates with HCl showed no effervescence hence it was assumed that the total carbon measurements received from the elemental analyser represented the total organic carbon content of the soil.

2.4.7. Carbon and Nitrogen Microbial Quotient

The carbon microbial quotient (C_{mic}/C_{org}) and nitrogen microbial quotient (N_{mic}/N_{org}) represent the proportion of soil total carbon (C_{org}) and nitrogen (N_{org}) present as microbial biomass carbon (C_{mic}) and microbial biomass nitrogen (N_{mic}) respectively. The carbon microbial quotient is determined using the following calculation:

Carbon microbial quotient (%) =

$$\frac{C_{mic}\ (mg\ C\ g^{-1}\ soil)}{C_{org}\ (mg\ C\ g^{-1}\ soil)} \times 100 \qquad \text{(equation 8)}$$

The nitrogen microbial quotient is determined using the following calculation:

Nitrogen microbial quotient (%) =

$$\frac{N_{mic}\ (mg\ N\ g^{-1}\ soil)}{N_{org}\ (mg\ N\ g^{-1}\ soil)} \times 100 \qquad \text{(equation 9)}$$

2.4.8. Direct Plate Count

Direct plate counting was carried out using the pour plate method where a microbial suspension is mixed with molten agar in a petri dish to determine the number of culturable cells as colony forming units (CFUs) (Aneja, 2003). For both bacterial and fungal counts thousand fold dilution (10^{-3}) soil solutions were prepared using sterile autoclaved water by serially diluting soil samples from each pot in sterile universal containers. 0.3% yeast extract agar with a pH adjusted to 7 was autoclaved and held molten at 50°C before pouring. 1 mL of the soil dilution was added to each sterile Petri dish containing the molten agar. For the estimation of fungi in the soil samples, lactic acid was diluted to 2.5% and sterilized. 0.5 mL of the 2.5% lactic acid was added to those plates from which fungi were to be estimated. 15 mL of molten yeast extract agar was then added to each of the sterile petri dilutions and mixed thoroughly. The plates were placed in an incubator at 30ºC in the dark for 3 days after which the number of colonies were counted on a Quebec colony counter. The number of colony forming units was calculated as follows (Aneja, 2003):

Number of CFUs mL^{-1} =

$$\frac{\text{number of colonies}}{\text{amount plated} \times \text{dilution}} \qquad \text{(equation 10)}$$

2.4.9. Community level substrate utilization analysis

To compare the potential metabolic diversity of the soil microbial communities in both pot trials, community level substrate utilization analysis was carried out using the Biolog microplate system (Biolog Inc.). First developed by Garland and Mills (1991) the analysis is based on the ability of micro-organisms to utilize a sole carbon source. The 96 well microtitre ecoplate was used. This contains 31 different carbon sources (carboxylic acids, carbohydrates, amines, amino acids, polymers, and phenolic compounds) and a negative control (water) in triplicate (see Appendix). Combined with the carbon source in the wells is the redox dye tetrazolium violet. If the micro-organisms utilize the carbon source and start to respire the tetrazolium dye is reduced to formazan producing a purple colouration. The level of this colour development is spectrophotometrically measured to determine the substrate utilization of the microbial suspensions.

Following procedure modified from Epelde *et al*. (2008), 5 g of rhizosphere soil was serially diluted a thousand fold (10^{-3}). This dilution was chosen to reduce the effect of any soil particles and carbon sources on colour development. The 5 g soil sample was placed in a glass flask containing 45 mL of sterile autoclaved water and then placed on an orbital shaker. This was further diluted by pipetting 200 µl into a sterile universal vial containing 9.8 mL of sterile autoclaved water resulting in a final dilution of 10^{-3}. Each well of the Biolog™ eco plates was then directly inoculated in a laminar flow cabinet at room temperature with 250 µl of the diluted suspensions. An initial absorbance reading at 595 nm was automatically recorded at time zero using a plate reader and then the plates were incubated in the dark at 25ºC for 72 h after which the intensity of colour development was read at 595 nm.

Substrate diversity (*H*) was calculated using Shannon's diversity index (Zak *et al*., 1994).

$$H = -\Sigma\, p_i\, (\ln p_i) \qquad \text{(equation 11)}$$

where p_i is the ratio of activity on a particular substrate to the sum of activity on all substrates (Zak *et al*., 94).

Substrate evenness (*E*) was calculated using the following calculation (Zak *et al*., 1994):

$$E = H/H_{max} = H/\log S \qquad \text{(equation 12)}$$

where *H* is substrate diversity and *S* is substrate richness (the number of utilized substrates).

Table 1: *The substrates present in Biolog ecoplates and the class to which they belong based on the nature of the molecules.*

Carbon source	Class
Water	Control
β-methyl-D-glucose	Carbohydrate
D-galactonic acid γ–lactone	Carbohydrate
D-xylose	Carbohydrate
i-erythritol	Carbohydrate
D-mannitol	Carbohydrate
N-acetyl-D-glucosamine	Carbohydrate
D-cellobiose	Carbohydrate
Glucose-1-phosphate	Carbohydrate
α-D-lactose	Carbohydrate
D,l-α-glycerol phosphate	Carbohydrate
Pyruvic acid methyl ester	Carboxylic acid
D-galacturonic acid	Carboxylic acid
γ –hydroxybutyric acid	Carboxylic acid
D-glucosaminic acid	Carboxylic acid
Itaconic acid	Carboxylic acid
α-ketobutyric acid	Carboxylic acid
D-malic acid	Carboxylic acid
L-arginine	Amino acid
L-asparagine	Amino acid
L-phenylalaine	Amino acid
L-serine	Amino acid
L-threonine	Amino acid
Glycyl-L-glutamic acid	Amino acid
Tween 40	Polymer
Tween 80	Polymer
α-cyclodextrin	Polymer
glycogen	Polymer
Phenylethylamine	Amine
Putrescine	Amine
2-hydroxy benzoic acid	Phenolic compound
4-hydroxy benzoic acid	Phenolic compound

2.4.10. Plant Biomass

Plant biomass was measured on a dry weight basis. Entire plants were removed from the soil and loose soil removed. The plants were then frozen before analysis. After defrosting the plants were weighed in pre-weighed containers then placed in a drying oven at 105ºC overnight. Once removed the plants were cooled in desiccators before re-weighing.

Ash content in plant biomass was determined by the following method. Approximately 1.0 g of each dried plant specimen was weighed to the nearest 0.1 mg into tared aluminium crucibles and the weight (container plus sample minus weight of container) as the initial weight. The crucibles were then placed in a muffle furnace at 550ºC. The crucible was removed and allowed to cool to room temperature in a desiccator before the weight was recorded to the nearest 0.1 mg. The following calculation was used to determine the percentage of ash:

Ash, % = (*W1/W2*) x 100 (equation 13)

where: *W1* = weight of ash, and
W2 = initial weight of 105^{o}C dried sample.

2.4.11. Shoot:Root biomass ratio

To determine the shoot:root biomass ratio following oven drying and re-weighing for the determination of plant dry weight biomass, plants were cut at the soil line to separate roots from shoots then weighed separately to determine the weight of the shoot and root of each plant. The shoot:root biomass ratio was then calculated as follows:

Shoot:Root biomass ratio =

$$\frac{\text{Dry weight for shoot}}{\text{Total weight of plant}}$$

(equation 14)

2.5. Statistical Analysis

The statistical package 'R' was used to perform statistical analysis on the data. Data from the pot trials was compared by means of two-way analysis of variance (ANOVA) with fertilization and tree species as fixed factors. Holt forest, Berwick forest and Hanmer data was compared by means of one-way ANOVA. Post-hoc analysis was carried by means of multiple comparison analyses (Tukey's) where appropriate with a confidence level of α=0.05 unless otherwise stated.

For Biolog data raw absorbance values were normalized by subtracting the initial reading values from the final readings taken at 72 h and subtracting the absorbance of the negative control from the absorbance readings of all the other wells to correct for background absorbance. All negative absorbance values were then set to zero. The resultant substrate utilization patterns were analysed to characterize the microbial communities. The two main statistical analyses performed on the data were the multivariate statistical analysis technique principal component analysis (PCA) and ANOVA. PCA was carried out to characterize the microbial communities based on the variability in the utilization of the 31 substrates present in Biolog ecoplates. To determine the statistical significance of the PCA output multiple comparison analysis (Tukey's) was carried out. The number of substrates utilized (S), Shannon's diversity index (H), and substrate evenness (E), were compared using two-way ANOVA with fertilization and tree species as fixed factors. Average well colour development (AWCD) of four different carbon substrate groups (carbohydrates, carboxylic acids, amino acids, and polymers) was also analysed using two-way ANOVA. AWCD was calculated as the mean of the

absorbance values for all wells. Post-hoc analysis was carried by means of multiple comparison analyses (Tukey's) where this was appropriate.

Chapter III

Results

3.1. Introduction

This chapter presents results obtained from laboratory experiments on soil collected from Hanmer, Berwick and Holt forests, and the results from the glasshouse pot trial. Measurements were made of moist and air dried soil samples, including total carbon and nitrogen, microbial biomass, and community level substrate utilization and relationships between these variables are explored. Full data sets are provided in Appendices A through R (pages 104-123).

3.2. Holt Forest

3.2.1. Carbon and Nitrogen

Soil from below six different tree species was sampled at Holt forest; *Agathis australis, P. strobus, Sequioa sempervirens, P. radiata, Thuja occidentalis,* and *Dacrydium cupressinum.* Soil samples were air dried and analysed for total nitrogen the results of which are given in Table 2. ANOVA indicated a significant species effect ($P=2.8\times 10^{-5}$). Comparison of means showed that total N in the soil sampled under *P. radiata* differed from all other samples and that under *Agathis australis* differed from all other samples with the exception of *Dacrydium cupressinum*. Soil under *Agathis australis* had the highest mean total N at 5.78 mg N per g soil and soil under *P. radiata* had the lowest mean total N at 0.5 mg N per g soil.

Soil samples from Holt forest were air dried and analysed for total organic carbon the results of which are given in Table 2. Mean total carbon differed significantly (P=0.000269) in soil samples under the different tree species sampled. Soil under *Agathis*

australis had the highest total C at 158.57 mg C per g soil and that under *P. radiata* the lowest at 11.45 mg C per g soil.

C:N ratios for Holt forest soil samples are given in Table 2. There was a significant species effect (P=0.0008874) on C:N ratios. *Agathis australis* differed significantly from *P. strobus, Thuja occidentalis,* and *Dacrydium cupressinum.* Soil under *Agathis australis* had the highest C:N ratio at 26.47 and soil under *Thuja occidentalis* the lowest at 17.88.

Table 2: *Mean total carbon and nitrogen of Holt forest soil samples on mg N g^{-1} soil basis across species. S.E.M values are given in parentheses.*

Soil (0-10 cm)	**Total C** (mg C per g soil)	**Total N** (mg N per g soil)	**C/N**
Agathis australis	158.57 (35.56)	5.78 (0.91)	26.47 (2.77)
Pinus strobus	64.40 (7.54)	3.48 (0.46)	18.65 (0.87)
Sequioa sempervirens	80.90 (10.50)	3.60 (0.38)	22.37 (0.92)
Pinus radiata	11.45 (1.61)	0.5 (0.07)	22.92 (0.25)
Thuja occidentalis	65.88 (10.84)	3.65 (0.45)	17.88 (0.71)
Dacrydium cupressinum	72.93 (4.99)	3.98 (0.21)	18.33 (0.38)

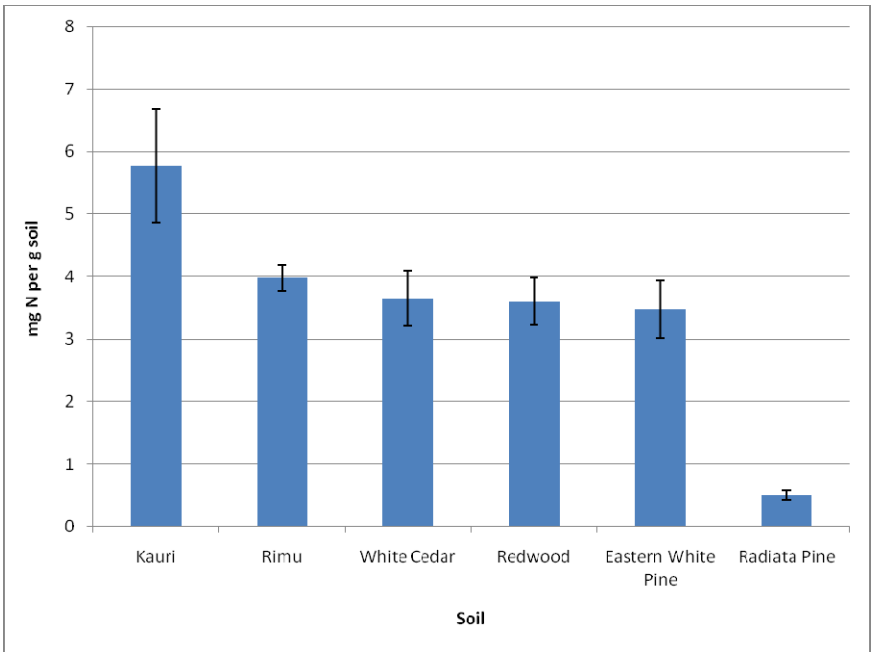

Figure 6: *Total soil nitrogen from Holt forest soils. Values are means and error bars indicate the standard error of the mean.*

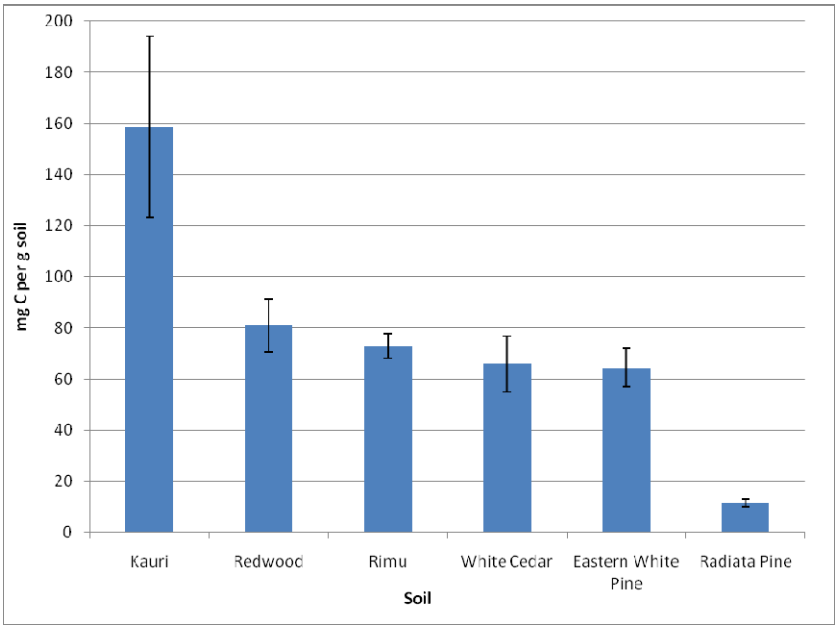

Figure 7: *Total soil carbon from Holt forest soil samples. Values are means and error bars indicate the standard error of the mean.*

3.2.2. Microbial Biomass Carbon and Nitrogen

Microbial biomass carbon, nitrogen and carbon:nitrogen results for Holt forest soil samples are given in Table 3. Mean microbial biomass carbon was greatest under *Agathis australis* (2.47 mg C per g soil) followed by that under *Dacrydium cupressinum* (2.19 mg C per g soil). Microbial biomass carbon under *P. radiata* was the lowest with a mean of 0.99 mg C per g soil. There was a significant species effect on microbial biomass C (P=0.001) with *P. radiata* differing significantly from all other species sampled. Differences amongst the other species were not significant

Microbial biomass nitrogen was greatest under *Thuja occidentalis* with a mean of 0.18 mg N per g soil and lowest under *P. radiata* with a mean of 0.04 mg N per g soil. There was a significant species effect on microbial biomass C (P=0.001735) with *P. radiata* differing significantly from all other species sampled. Differences amongst the other species were not significant.

The highest ratio of microbial biomass carbon to nitrogen at the Holt forest site was under *P. radiata* with a mean of 27.69 the next highest was that under *S. sempervirens* with a mean of 17.46. The lowest ratio of microbial biomass carbon to nitrogen was from that under *Thuja occidentalis* with a mean of 12.21. The only statistically significant difference was between *Pinus strobus, Thuja occidentalis* and *P. radiata*.

Table 3: *Mean microbial biomass carbon (MBC) in mg C g^{-1} soil, microbial biomass nitrogen (MBN) in mg N g^{-1} soil and mean microbial biomass carbon:nitrogen ratios of Holt Forest soil samples across species. S.E.M values are given in parentheses.*

Soil (0-10 cm)	**Microbial Biomass Nitrogen** (mg N per g soil)	**Microbial Biomass Carbon** (mg C per g soil)	**MBC:MBN**
Agathis australis	0.15 (0.02)	2.47 (0.27)	16.65 (1.62)
Pinus strobus	0.17 (0.02)	2.05 (0.22)	12.56 (1.65)
Sequioa sempervirens	0.17 (0.04)	1.97 (0.11)	17.46 (2.80)
Pinus radiata	0.04 (0.01)	0.99 (0.21)	27.69 (6.48)
Thuja occidentalis	0.18 (0.02)	2.16 (0.18)	12.21 (1.65)
Dacrydium cupressinum	0.15 (0.02)	2.19 (0.16)	15.64 (1.95)

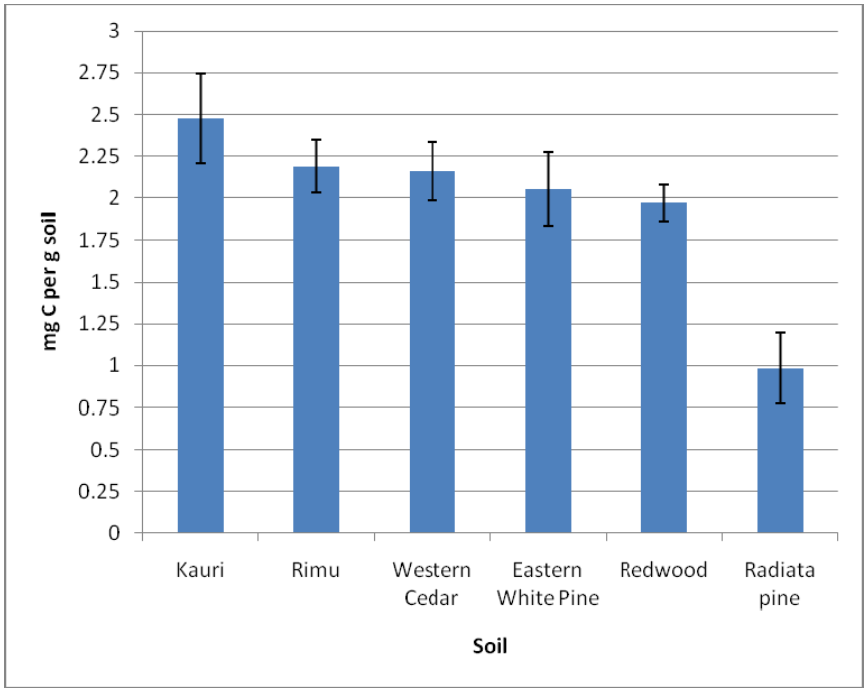

Figure 8: *Microbial biomass carbon in mg C per g soil across species from Holt forest soil samples. Values are means and error bars indicate the standard error of the mean.*

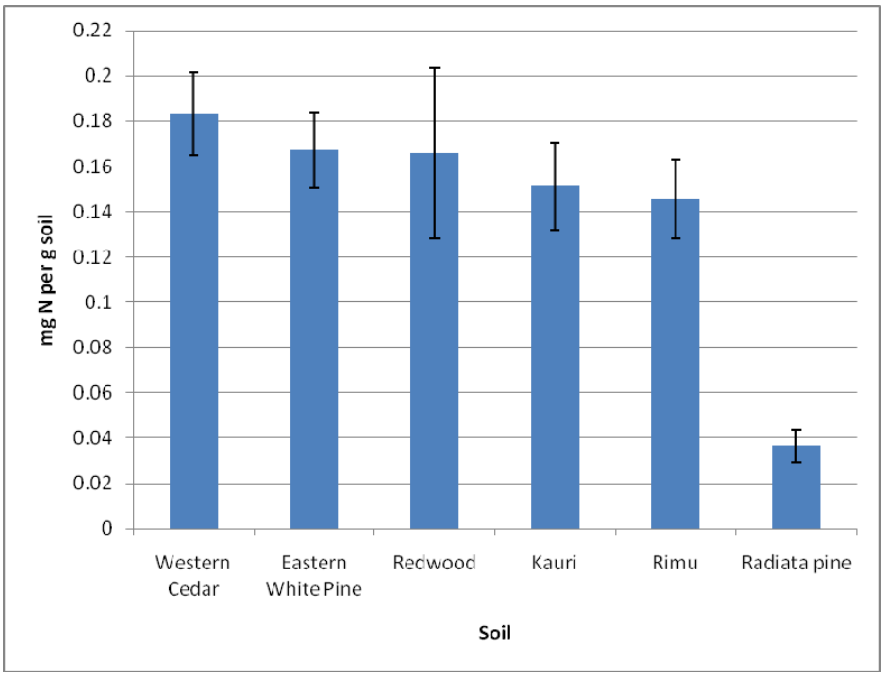

Figure 9: *Microbial biomass nitrogen in mg N per g soil across species from Holt forest soil samples. Values are means and error bars indicate the standard error of the mean.*

3.2.3. Microbial Biomass Carbon and Nitrogen Quotient

Microbial biomass carbon (C_{mic}/C_{org}) and nitrogen (N_{mic}/N_{org}) quotients for Holt forest soil samples are given in Table 4. The proportions of total carbon present as microbial carbon varied from 2.02% under *Agathis australis* to 8.52% under *P. radiata* and the proportions of total nitrogen present as microbial nitrogen varied from 2.78% under *Agathis australis* to 7.72% under *P. radiata.* The microbial biomass carbon quotient of *P. radiata* was significantly different from all other species. The microbial biomass nitrogen quotient of *P. radiata* was significantly different from *Agathis australis* and *Dacrydium cupressinum.*

Table 4: *Holt soil mean microbial biomass carbon (C_{mic}/C_{org}) and nitrogen (N_{mic}/N_{org}) quotients on a percentage basis across species. S.E.M values are given in parentheses.*

Soil (0-10 cm)	**Microbial Quotient** (C_{mic}/C_{org}) (%)	**Microbial Quotient** (N_{mic}/N_{org}) (%)
Agathis australis	2.02 (0.74)	2.78 (0.47)
Pinus strobus	3.26 (0.38)	4.99 (0.65)
Sequioa sempervirens	2.54 (0.30)	4.44 (0.59)
Pinus radiata	8.52 (1.50)	7.72 (1.70)
Thuja occidentalis	3.52 (0.57)	5.07 (0.30)
Dacrydium cupressinum	3.01 (0.14)	3.63 (0.30)

3.2.4. Organic Matter

Soil organic matter for Holt forest was determined by use of the loss on ignition method. The results are presented in table 8. Soil under *S. sempervirens* had the highest mean percentage of organic matter at 24.41%. The lowest was that under *P. radiata* at 5.31%. The only statistically significant difference was between *P. radiata* and all other species sampled.

Table 5: *Loss on ignition organic matter content (%) of Holt soil samples across species. S.E.M values are given in parentheses.*

Soil (0-10 cm)	**Organic matter** (%)
Agathis australis	19.12 (2.07)
Pinus strobus	20.31 (3.34)
Sequioa sempervirens	24.41 (3.54)
Pinus radiata	5.31 (0.96)
Thuja occidentalis	19.70 (3.54)
Dacrydium cupressinum	17.85 (1.82)

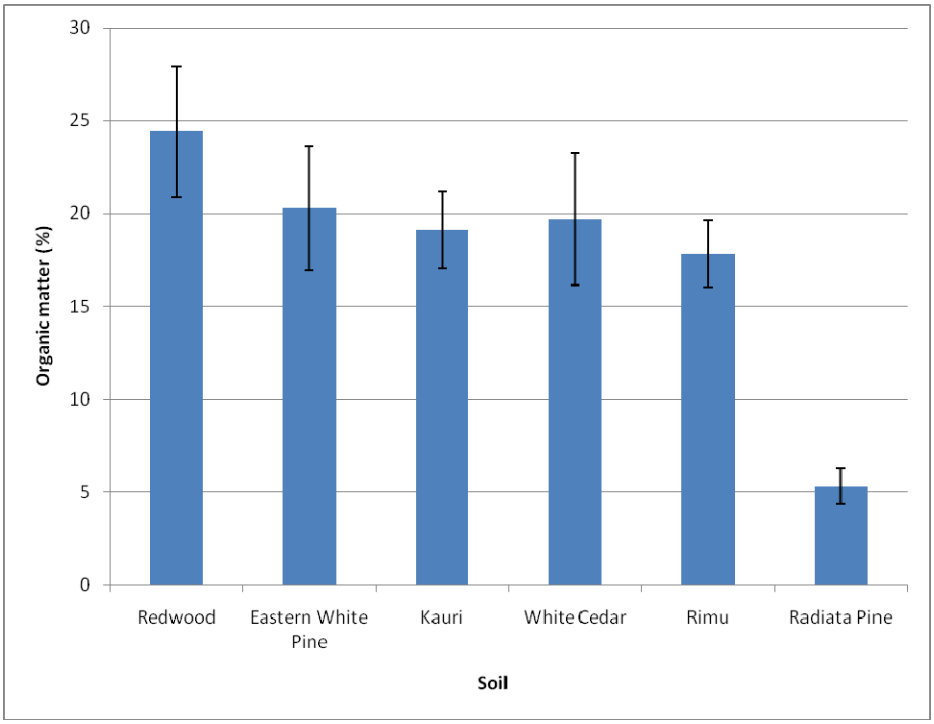

Figure 10: *Loss on ignition organic matter content on a percentage basis from Holt forest soil samples. Values are means and error bars indicate the standard error of the mean.*

3.3. Hanmer and Berwick Forest Samples

3.3.1. Soil pH

Soil pH for Berwick Forest and Hanmer soil samples are presented in Table 6. The mean pH ranged from a mean of 5.7 for fertilized Berwick plots to 6 for unfertilized Berwick plots. Soil pH for *P. radiata* and *S. sempervirens* at Hanmer were similar with a mean of 6.4 and 6.5 respectively.

Table 6: *Mean soil pH of Hanmer and Berwick forest soil samples. S.E.M values are given in parentheses.*

Soil (0-5 cm)	**pH**
Berwick fertilized	5.7 (0.29)
Berwick unfertilized	6.0 (0.07)
Hanmer Pine	6.4 (0.04)
Hanmer Redwood	6.5 (0.25)

3.3.3. Organic Matter

Soil organic matter results determined using the loss on ignition method (LOI) for Berwick forest and Hanmer sites soil samples are presented in Table 7. The LOI organic matter content differed significantly between the fertilized and unfertilized Berwick forest sites sampled (P=0.00046). The mean LOI organic matter content was higher under the fertilized sites at 11.73% and lowest under the unfertilized sites with a mean of 10.76%. LOI soil organic matter content did not differ significantly (P=0.4113) between Pine and Redwood at the Hanmer sites.

Table 7: *Loss on ignition organic matter content of Hanmer and Berwick forest soil samples. S.E.M values are given in parentheses.*

Soil (0-5 cm)	**Organic matter** (%)
Berwick fertilized	11.73 (0.13)
Berwick unfertilized	10.76 (0.06)
Hanmer Pine	13.48 (0.38)
Hanmer Redwood	14.27 (0.81)

3.3.4. Total Carbon and Nitrogen

Total soil carbon and nitrogen results for Hanmer and Berwick soil samples are given in table 8. Mean total carbon was greater in the fertilized plots than in the unfertilized plots at Berwick forest however, this difference was not statistically significant (P=0.3280) at either a 95 or 90% confidence level. Total nitrogen between the fertilized and unfertilized plots at Berwick forest was not significantly different at a 95% confidence level (P=0.05047), but was at a 90% confidence level. Mean C:N ratios differed significantly between fertilized and unfertilized plots (P=0.04519). The mean C:N ratio of the unfertilized plots was higher at 17.1 than that of the fertilized plots at 16.1.

Mean total C between *P. radiata* and *S. sempervirens* at the Hanmer sampling sites was significantly different (P=0.03631). Mean total C was greater under *P. radiata* than under *S. sempervirens*. Mean total N between the two species did not differ significantly (P=0.4973). The mean C:N ratio did not differ significantly between the two species (P=0.6914).

Table 8: *Total carbon, nitrogen and carbon:nitrogen ratios of Berwick and Hanmer soil samples. S.E.M values are given in parentheses.*

Soil (0-5 cm)	**Total N** (mg N per g soil)	**Total C** (mg C per g soil)	**C/N**
Berwick fertilized	2.64 (0.20)	42.6 (2.88)	16.1 (0.73)
Berwick unfertilized	2.33 (0.17)	39.9 (3.71)	17.1 (0.79)
Hanmer Pine	3.80 (0.94)	51.53 (4.40)	14.2 (4.53)
Hanmer Redwood	3.03 (0.22)	54.78 (3.74)	18.1 (0.63)

3.3.5. Microbial Biomass

Microbial biomass nitrogen results for Hanmer and Berwick forest soil samples are given in table 9. Mean microbial biomass nitrogen between the fertilized and unfertilized plots at Berwick forest did not differ significantly (P=0.2215). Microbial biomass nitrogen between the *S. sempervirens* and *P. radiata* stands at the Hanmer were significantly different (P=0.0041). Mean microbial biomass nitrogen was greater under the *P. radiata* stand (0.718 mg N per g soil) than under the *S. sempervirens* stand (0.333 mg N per g soil).

Table 9: *Mean microbial biomass in mg N g^{-1} soil and μg N g^{-1} soil basis for Berwick and Hanmer soil samples. S.E.M values are given in parentheses.*

Soil (0-5 cm)	**Microbial Biomass** (mg N per g soil)
Berwick fertilized	0.242 (0.05)
Berwick unfertilized	0.112 (0.01)
Hanmer Pine	0.718 (0.01)
Hanmer Redwood	0.333 (0.01)

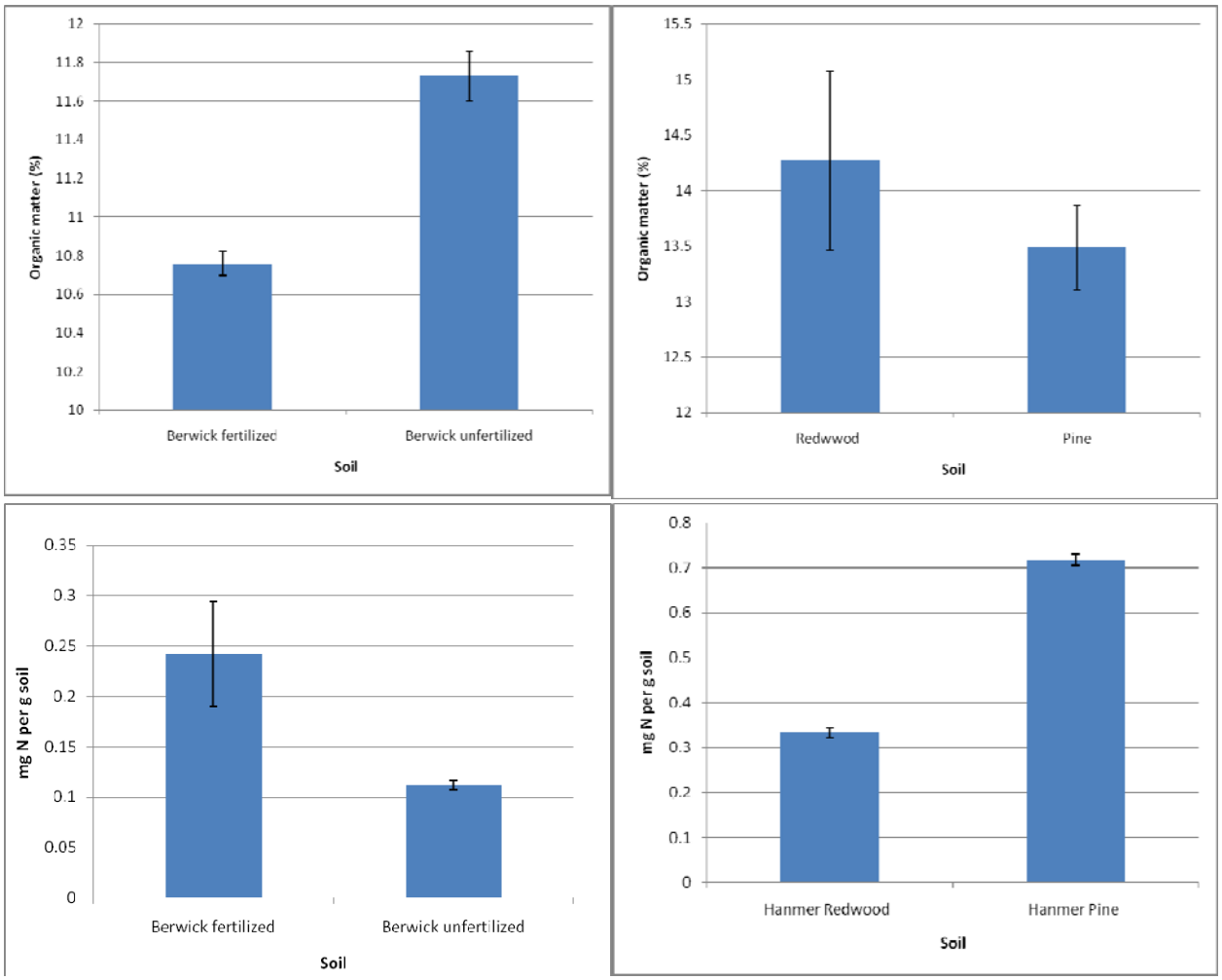

Figure 11: *Top left: Berwick forest loss on ignition soil organic matter content (%). Top right: Hanmer loss on ignition soil organic matter content (%). Bottom left: Berwick forest soil microbial biomass nitrogen. Bottom right: Hanmer soil microbial biomass nitrogen. Values are means and error bars indicate the standard error of the mean.*

3.4. Pot Trial

3.4.1. Direct Plate Count

Plate count results for the first and second pot trials are given in tables 10 and 11 respectively. Bacterial plate counts for trial one did not differ significantly between the species (P=0.9803) nor between fertilized and unfertilized pots (P=0.3112). Bacterial plate counts for the second pot trial also did not differ significantly between the species (P=0.3366) nor between fertilized and unfertilized pots (P=0.9204). Fungal plate counts for the first trial differed significantly only between Sequoia and the control however the effect of fertilizer was not significant (P=0.13380). For the second trial there was no significant effect of species (P=0.3232) or fertilizer (P=0.1145).

Table 10: *Mean plate counts for pot trial one soil samples in CFUs per g soil. S.E.M values are given in parentheses.*

Soil	**Fungi** (CFUs/g)	**Bacteria** (CFUs/g)
Control unfertilized	4.4×10^3 (1.5×10^2)	1.96×10^5 (1.1×10^3)
Control fertilized	3.7×10^3 (1.0×10^2)	1.86×10^5 (1.2×10^3)
Eucalyptus unfertilized	4.8×10^3 (1.7×10^2)	1.83×10^5 (1.1×10^3)
Eucalyptus fertilized	3.9×10^3 (2.3×10^2)	1.88×10^5 (0.7×10^3)
Sequoia unfertilized	3.9×10^3 (2.8×10^2)	1.91×10^5 (0.9×10^3)
Sequoia fertilized	5.6×10^3 (1.2×10^2)	1.91×10^5 (0.6×10^3)
Pine unfertilized	4.0×10^3 (2.1×10^2)	2.06×10^5 (1.1×10^3)
Pine fertilized	4.6×10^3 (1.3×10^2)	1.65×10^5 (0.9×10^3)

Table 11: *Mean plate counts for pot trial two soil samples in CFUs per g soil. S.E.M values are given in parentheses.*

Soil	**Fungi** (CFUs/g)	**Bacteria** (CFUs/g)
Sequoia unfertilized	28×10^3 (2.1×10^2)	2.09×10^5 (0.9×10^3)
Sequoia fertilized	55×10^3 (3.4×10^2)	1.71×10^5 (1.4×10^3)
Pine unfertilized	41×10^3 (2.2×10^2)	1.71×10^5 (1.2×10^3)
Pine fertilized	48×10^3 (1.8×10^2)	1.89×10^5 (0.9×10^3)
Sequoia unfertilized control	50×10^3 (2.1×10^2)	2.13×10^5 (1.0×10^3)
Sequoia fertilized control	38×10^3 (2.5×10^2)	2.10×10^5 (1.0×10^3)
Pine unfertilized control	42×10^3 (2.0×10^2)	1.79×10^5 (1.0×10^3)
Pine fertilized control	36×10^3 (1.8×10^2)	1.97×10^5 (1.4×10^3)

3.4.2. Plant Biomass

Plant biomass results expressed on an oven dry weight basis for the first pot trial are given in figure 12. Ash content in biomass results for pot one and two are given in tables 12 and 13 respectively. The mean total biomass was not significantly different amongst the species (P=0.36615). There was no significant effect of fertilizer on the mean biomass (P= 0.08841). When *P. radiata* is removed from the analysis and the analysis is rerun with only *E. nitens* and *S. sempervirens* the effect of fertilizer on mean biomass is significant (P=0.002417). *S. sempervirens* in the fertilized soil had the largest mean biomass (4.37 g). However, the difference between the species is still not significant (P= 0.536178). Root biomass differed significantly between *E. nitens* and the other species, but not between *P. radiata* and *S. sempervirens*. There was no significant effect of fertilization on root biomass.

Plant biomass results expressed on an oven dry basis for the second pot trial are presented in table 15. Mean total biomass was significantly different between the two species, *P. radiata* and *S. sempervirens*, ($P=6.367\times 10^{-7}$). There was a significant effect of fertilizer

on mean biomass ($P=9.852\times 10^{-12}$). *P. radiata* with N fertilizer treatment had the largest mean biomass (7.22 g) and *S. sempervirens* with N fertilizer treatment had the second largest mean biomass (5.62 g). Overall, for both the fertilized and unfertilized treatments *P. radiata* had the largest mean biomass. Root biomass did not differ significantly between the two speices (P=0.3573). However, there was a significant effect of fertilizer ($P=6.506\times 10^{-7}$) on mean root biomass. Fertilized plants had significantly greater root biomass.

Table 12: *Plant biomass for pot trial one. S.E.M values are given in parentheses.*

Plant	**Root dry weight biomass** (g)	**Shoot dry weight biomass** (g)	**Shoot:Root biomass ratio**	**Total dry weight biomass** (g)	**Ash in biomass content** (%)
Eucalyptus unfertilized	0.72 (0.07)	2.88 (0.21)	4.24 (0.61)	3.61 (0.27)	7.24 (0.57)
Eucalyptus fertilized	0.92 (0.07)	3.16 (0.19)	3.63 (0.28)	4.07 (0.24)	7.00 (0.58)
Sequoia unfertilized	1.55 (0.07)	1.50 (0.11)	1.00 (0.10)	3.06 (0.10)	6.97 (0.92)
Sequoia fertilized	2.08 (0.21)	2.29 (0.14)	1.16 (0.08)	4.37 (0.31)	6.08 (0.64)
Pine unfertilized	1.22 (0.13)	3.07 (0.38)	2.51 (0.21)	4.28 (0.52)	7.05 (1.03)
Pine fertilized	1.69 (0.12)	2.30 (0.15)	1.49 (0.21)	3.98 (0.18)	7.15 (1.12)

Table 13: *Plant biomass for pot trial two. S.E.M values are given in parentheses.*

Plant	**Root dry weight biomass** (g)	**Shoot dry weight biomass** (g)	**Shoot:Root biomass ratio**	**Total dry weight biomasss** (g)	**Ash in biomass content** (%)
Sequoia unfertilized	1.3 (0.06)	1.17 (0.08)	0.91 (0.06)	2.47 (0.12)	6.45 (0.97)
Sequoia fertilized	2.18 (0.25)	3.44 (0.44)	1.60 (0.12)	5.62 (0.67)	5.39 (0.65)
Pine unfertilized	1.39 (0.07)	2.85 (0.20)	2.13 (0.18)	4.23 (0.20)	6.10 (0.56)
Pine fertilized	2.15 (0.17)	5.07 (0.43)	2.62 (0.37)	7.22 (0.39)	6.14 (0.61)

Shoot:Root biomass ratio results for the first pot trial are given table 12. The mean shoot:root biomass ratio of *E. nitens* was the largest followed by *P. radiata* with *S. sempervirens* having the smallest of the three species. The mean shoot:root biomass ratio of the plants differed significantly between species ($P=2\times10^{-16}$). There was a significant effect of fertilizer on the shoot:root biomass ratio of *P. radiata*, but not on *S. sempervirens* or *E. nitens.*

Shoot:Root biomass ratio results for the second pot trial are given in table 13. The mean shoot:root biomass ratio of the plants differed significantly between the two species ($P=4.174\times 10^{-5}$). *P. radiata* had a larger mean shoot:root biomass ratio than *S. sempervirens*. There was no significant effect of fertilization on the shoot:root biomass ratio.

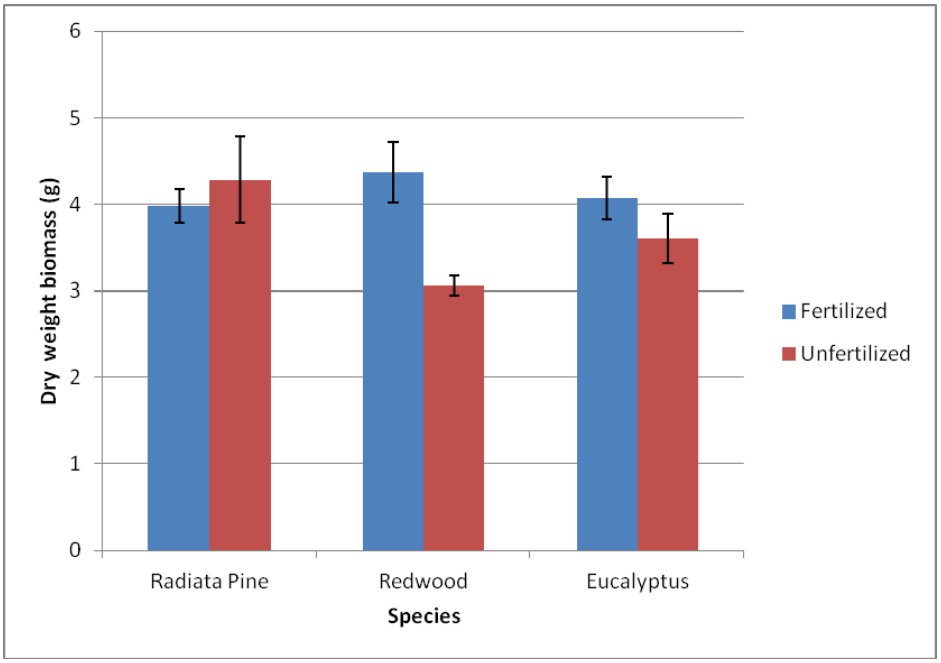

Figure 12: *Plant biomass (g) on an oven dry weight basis for pot trial one. Values are means and error bars indicate the standard error of the mean.*

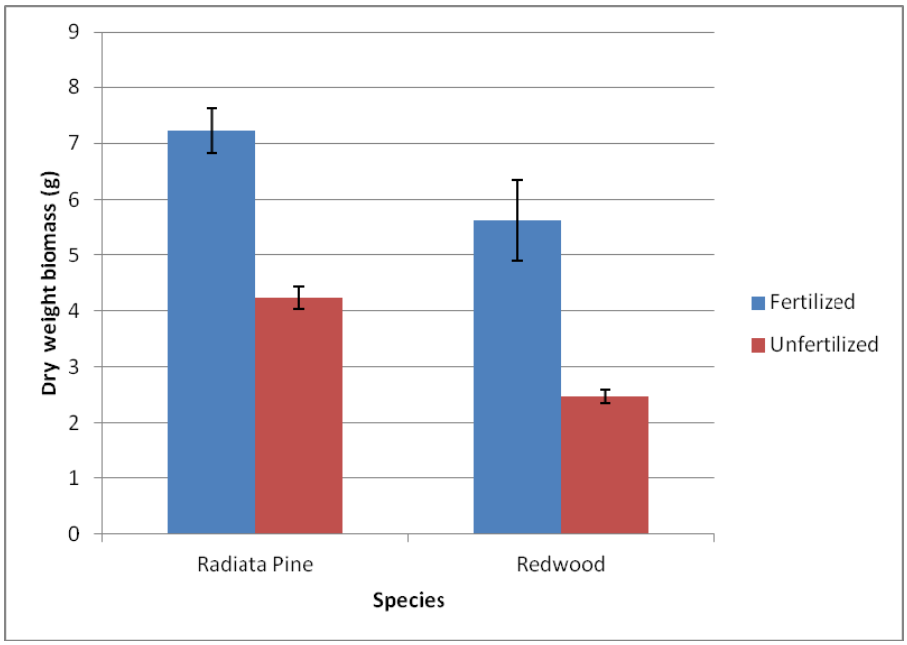

Figure 13: *Plant biomass (g) on an oven dry weight basis for pot trial two. Values are means and error bars indicate the standard error of the mean.*

3.4.3. Carbon and Nitrogen

Total nitrogen results for pot trial one are given in figure 14. Mean total soil N differed significantly between *P. radiata* and both *E. nitens* and *S. sempervirens*. There was a significant effect of fertilizer on total N ($P=3.722\times 10^{-13}$). The plant free controls in fertilized soil had the highest mean total N value at 2.58 mg N per g soil and the *P. radiata* in unfertilized soil had the lowest mean at 1.51 mg N per g soil. Total nitrogen results for pot trial two are given in figure 15. There was a significant effect of fertilizer on soil nitrogen ($P=3.252\times 10^{-7}$). There was a significant interaction effect such that the planted pots only differed significantly from the controls when fertilized.

Table 14: *Total carbon, nitrogen and carbon:nitrogen ratio results for pot trial one. S.E.M values are given in parentheses.*

Soil	**Total C** (mg C per g soil)	**Total N** (mg N per g soil)	**C/N**
Eucalyptus unfertilized	45.82 (0.53)	2.06 (0.08)	22.63 (0.87)
Eucalyptus fertilized	45.85 (0.61)	2.45 (0.05)	18.77 (0.31)
Sequoia unfertilized	42.95 (0.58)	2.05 (0.07)	21.19 (0.80)
Sequoia fertilized	44.84 (0.34)	2.41 (0.06)	18.74 (0.58)
Pine unfertilized	41.55 (0.71)	1.51 (0.07)	28.60 (1.90)
Pine fertilized	45.05 (0.68)	2.43 (0.03)	18.57 (0.26)
Control unfertilized	39.54 (0.54)	2.19 (0.03)	18.08 (0.26)
Control fertilized	42.02 (0.90)	2.58 (0.07)	16.32 (0.19)

Table 15: *Total carbon, nitrogen and carbon:nitrogen ratio results for pot trial two. S.E.M values are given in parentheses.*

Soil	**Total C** (mg C per g soil)	**Total N** (mg N per g soil)	**C/N**
Sequoia unfertilized	52.82 (0.89)	2.65 (0.09)	19.79(0.59)
Sequoia fertilized	53.68 (1.18)	2.82 (0.07)	19.07 (0.17)
Pine unfertilized	54.94 (1.30)	3.47 (0.07)	15.82 (0.22)
Pine fertilized	57.62 (1.13)	3.61 (0.08)	15.99 (0.11)
Sequoia unfertilized control	51.86 (0.74)	2.89 (0.03)	18.29 (0.34)
Sequoia fertilized control	53.51 (1.04)	3.15 (0.08)	17.06 (0.28)
Pine unfertilized control	54.29 (0.87)	3.55 (0.05)	15.30 (0.16)
Pine fertilized control	55.68 (0.73)	3.82 (0.07)	14.60 (0.17)

Total carbon results for pot trial one are given in figure 16. Mean total carbon differed significantly between species ($P=2.636\times 10^{-9}$) with *E. nitens* being significantly different from both *P. radiata* and *S. sempervirens* and all differing significantly from the plant-free controls. *P. radiata* and *S. sempervirens* did not differ significantly from one another. There was a significant effect of fertilizer on *P. radiata* only with fertilized pine having significantly greater mean total C than unfertilized pine. When the relative contributions are examined by subtracting the initial carbon from that at the end of the first pot trial all plants contributed significantly more carbon relative to the controls. Relative to *P. radiata* and *S. sempervirens*, *E. nitens* contributed significantly more carbon (fig. 18). The difference between *P. radiata* and *S. sempervirens* was not significant. The results when looked at relative to both total dry weight biomass and root biomass are similar.

Total carbon results for pot trial two are given in Fig 17. There was a significant species effect ($P=0.00419$) with *P. radiata* and *S. sempervirens* differing significantly from each other. There was a significant effect of fertilizer. When the relative contributions in the second pot trial are examined there was no significant difference between the two species. There was a significant effect of fertilization. However, when the contribution is looked at relative to total dry weight (fig. 19) and root biomass the effect was not apparent.

Carbon:nitrogen ratios for pot trial one are given table 14. Carbon:nitrogen ratios differed significantly between species ($P=2.776\times 10^{-8}$). There was a significant effect of fertilizer on the carbon:nitrogen ratios ($P=2.72\times 10^{-10}$). Carbon:nitrogen ratios for pot trial two are given in table 15. Carbon:nitrogen ratios differed significantly between species and between the species and their respective controls. C:N ratios between fertilized and unfertilized pots did not differ significantly.

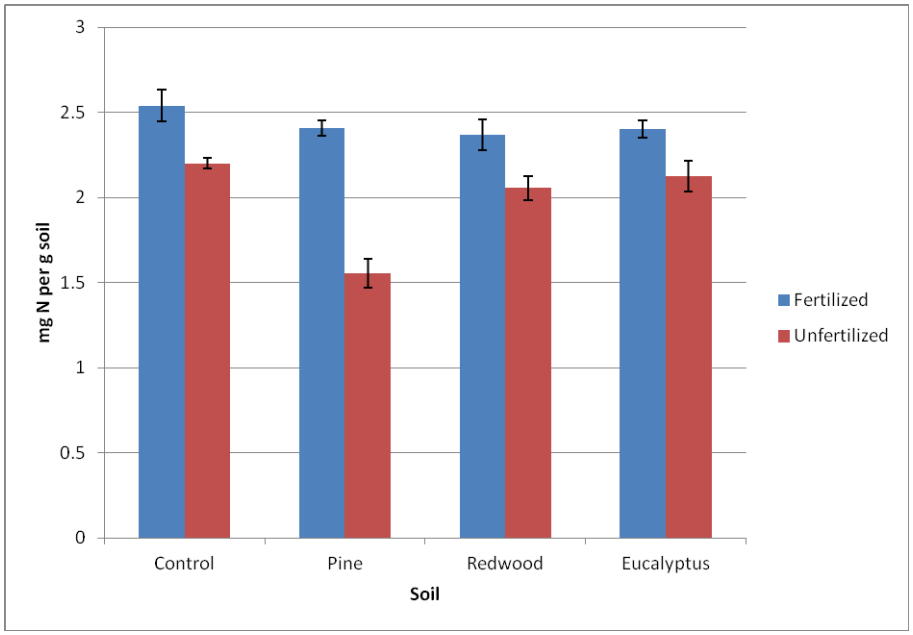

Figure 14: *Total soil nitrogen for pot trial one. Values are means and error bars indicate the standard error of the mean.*

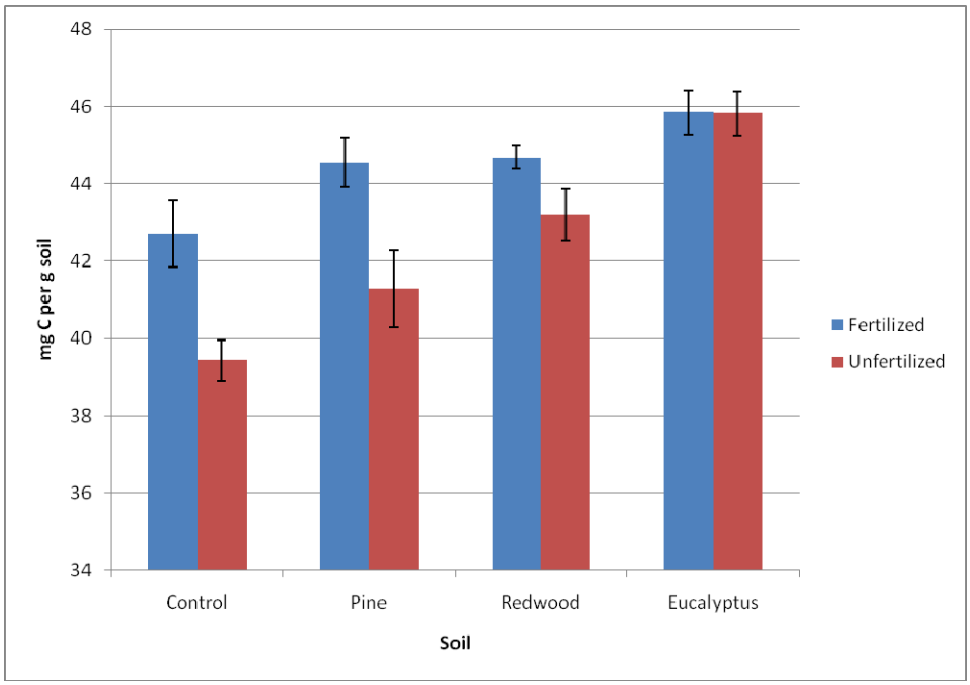

Figure 15: *Total soil carbon for pot trial one. Values are means and error bars indicate the standard error of the mean.*

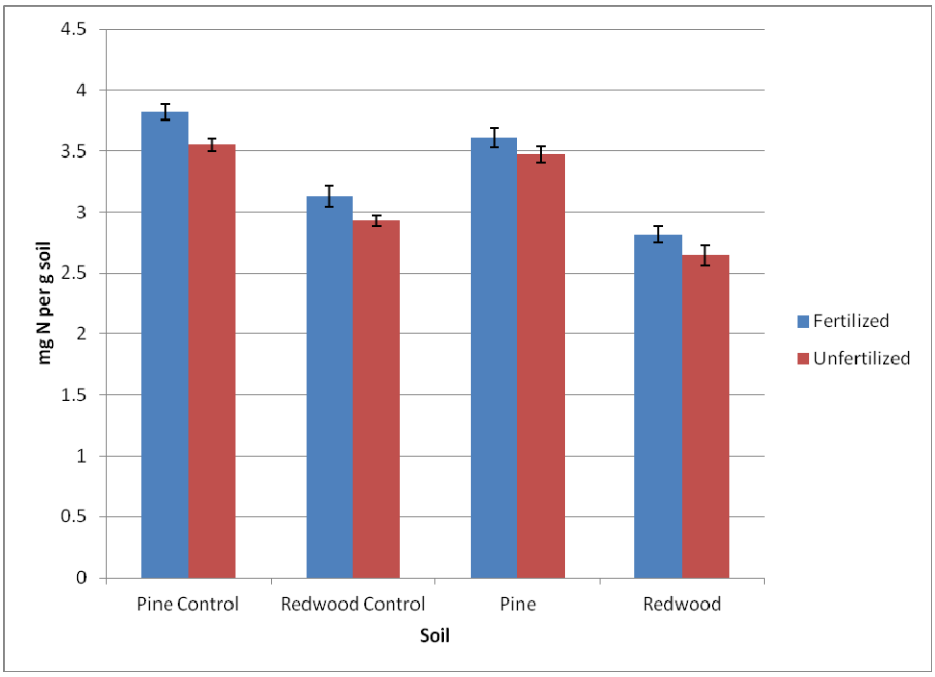

Figure 16: *Total soil nitrogen for pot trial two. Values are means and error bars indicate the standard error of the mean.*

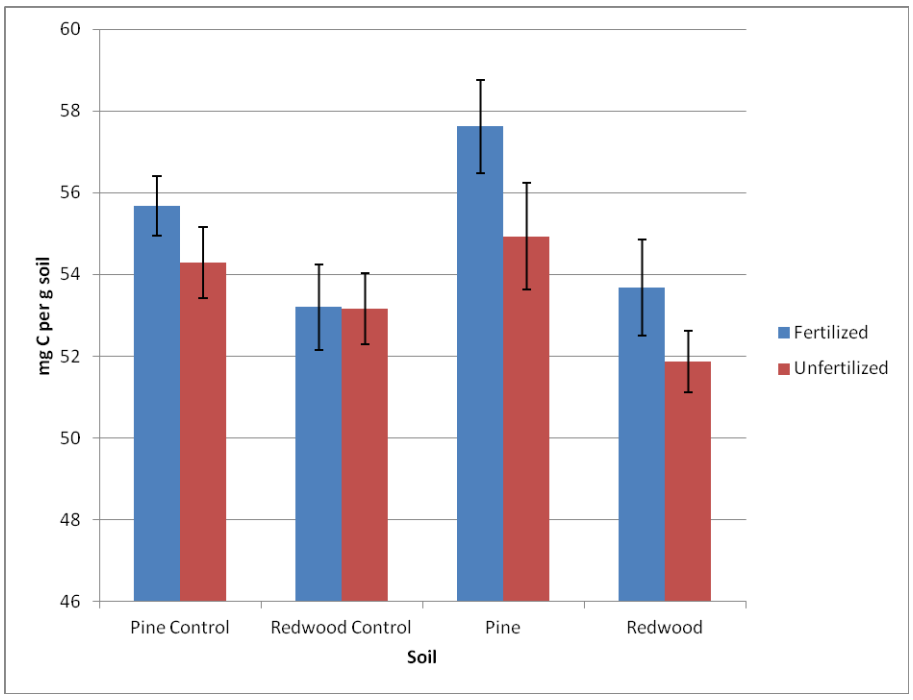

Figure 17: *Total soil carbon for pot trial two. Values are means and error bars indicate the standard error of the mean.*

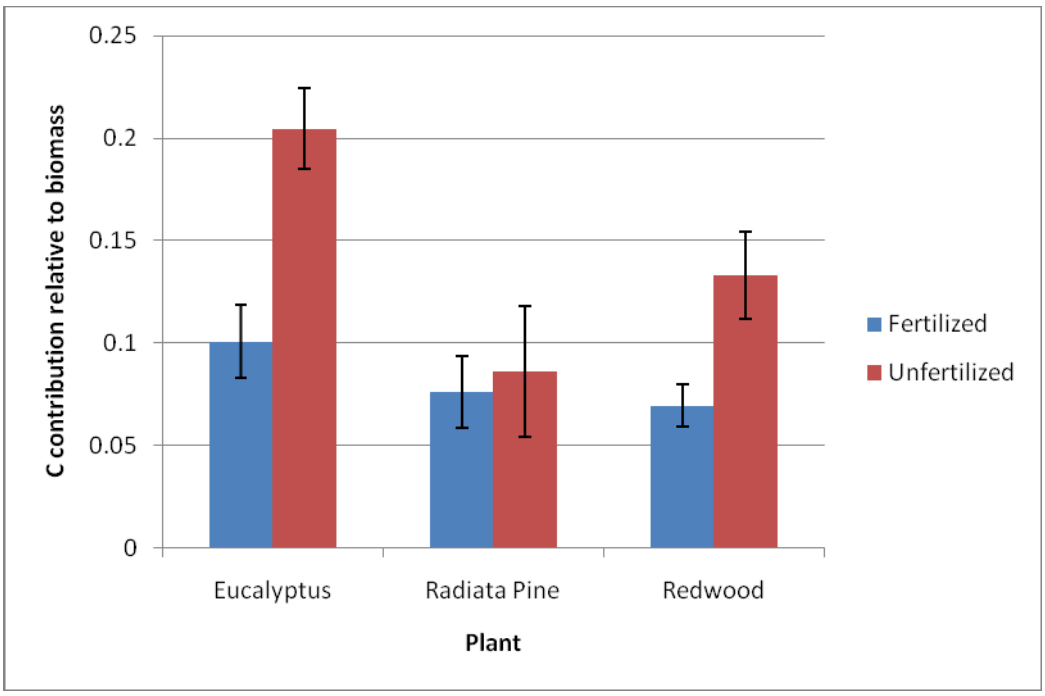

Figure 18: *Carbon contribution relative to plant total dry weight biomass for pot trial one. Values are means and error bars indicate the standard error of the mean.*

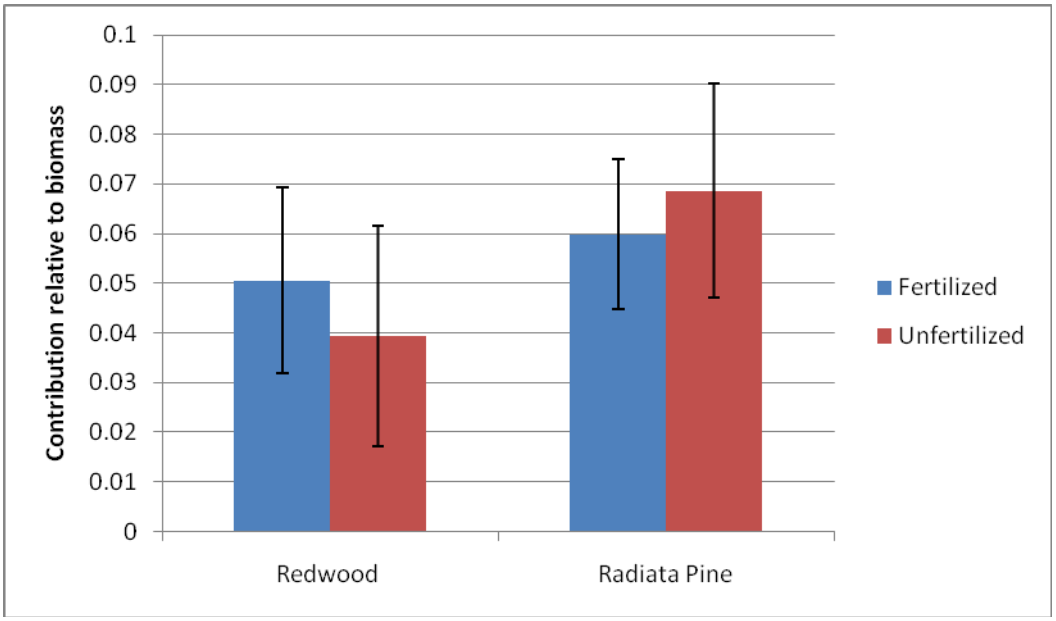

Figure 19: *Carbon contribution relative to plant total dry weight biomass for pot trial two. Values are means and error bars indicate the standard error of the mean.*

3.4.4. Microbial biomass

Microbial biomass carbon results for pot trial one are given in Table 16. Mean microbial biomass carbon ranged from 2.28 mg C per g soil for unfertilized *P.radiata* to 3.08 mg C per g soil for fertilized *E. nitens*. There was a statistically significant effect of fertilization on microbial biomass. The only other statistically significant differences observed were between the planted pots of all three species and the plant-free controls. There were no significant differences between species. Microbial biomass carbon results for pot trial two are given in table 17. There was a significant effect of fertilization and there was a significant difference between *S. sempervirens* and *P. radiata*. The interaction effect between fertilization and species was statistically significant. Microbial quotients for pot trial one are given in table 16. Mean microbial quotients ranged from 5.51% to 6.76%. There were significant differences between plant pots and plant-free controls. The only statistically significant difference between species was that between *P. radiata* and *E. nitens*. Microbial quotient results for pot trial two are given in Table 17. Mean microbial quotients ranged from 5.05% to 7.69%. The only statistically significant difference was that between the planted pots and their respective plant-free controls.

Table 16: *Microbial biomass carbon and microbial quotient results for pot trial one. S.E.M values are given in parentheses.*

Soil	**Microbial Biomass Carbon** (mg C per g soil)	**Microbial Quotient** (C_{mic}/C_{org}) (%)
Eucalyptus unfertilized	2.56 (0.10)	5.59 (0.22)
Eucalyptus fertilized	3.08 (0.12)	6.74 (0.28)
Sequoia unfertilized	2.63 (0.09)	6.14 (0.24)
Sequoia fertilized	3.00 (0.21)	6.69 (0.45)
Pine unfertilized	2.28 (0.08)	5.49 (0.17)
Pine fertilized	3.03 (0.07)	6.76 (0.23)
Control unfertilized	2.58 (0.04)	6.52 (0.16)
Control fertilized	2.31 (0.08)	5.51 (0.17)

Table 17: *Microbial biomass carbon and microbial quotient results for pot trial two. S.E.M values are given in parentheses.*

Soil	**Microbial Biomass Carbon** (mg C per g soil)	**Microbial Quotient** (C_{mic}/C_{org}) (%)
Sequoia unfertilized	3.15 (0.13)	6.09 (0.26)
Sequoia fertilized	4.15 (0.30)	7.69 (0.42)
Pine unfertilized	3.53 (0.17)	6.49 (0.37)
Pine fertilized	4.13 (0.20)	7.21 (0.41)
Sequoia unfertilized control	3.14 (0.19)	5.98 (0.37)
Sequoia fertilized control	2.96 (0.15)	5.57 (0.27)
Pine unfertilized control	2.88 (0.04)	5.31 (0.09)
Pine fertilized control	2.80 (0.05)	5.05 (0.12)

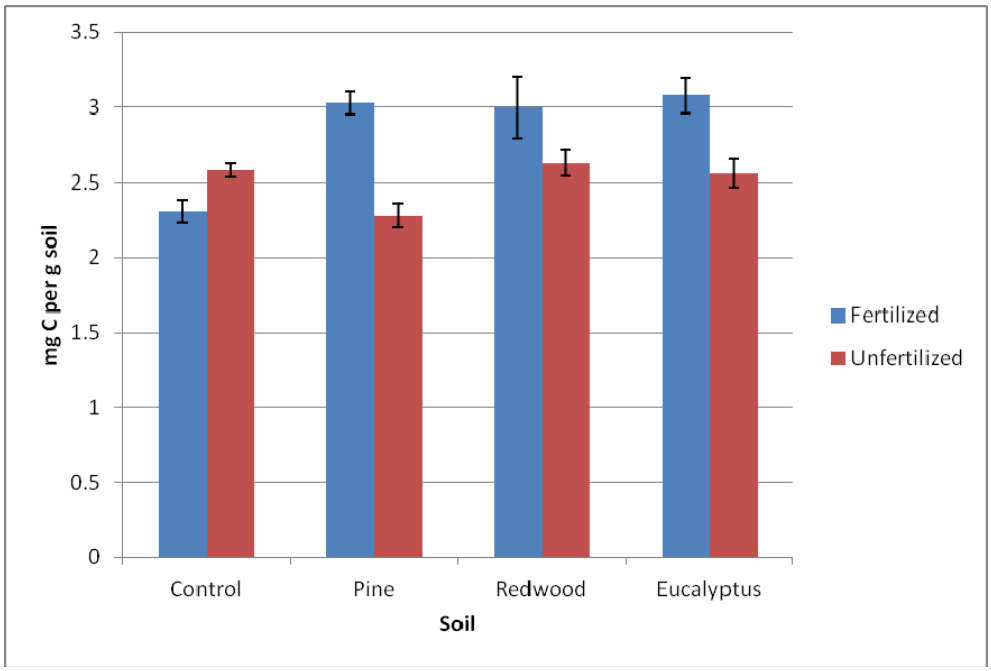

Figure 20: *Microbial biomass carbon for pot trial one. Values are means and error bars indicate the standard error of the mean.*

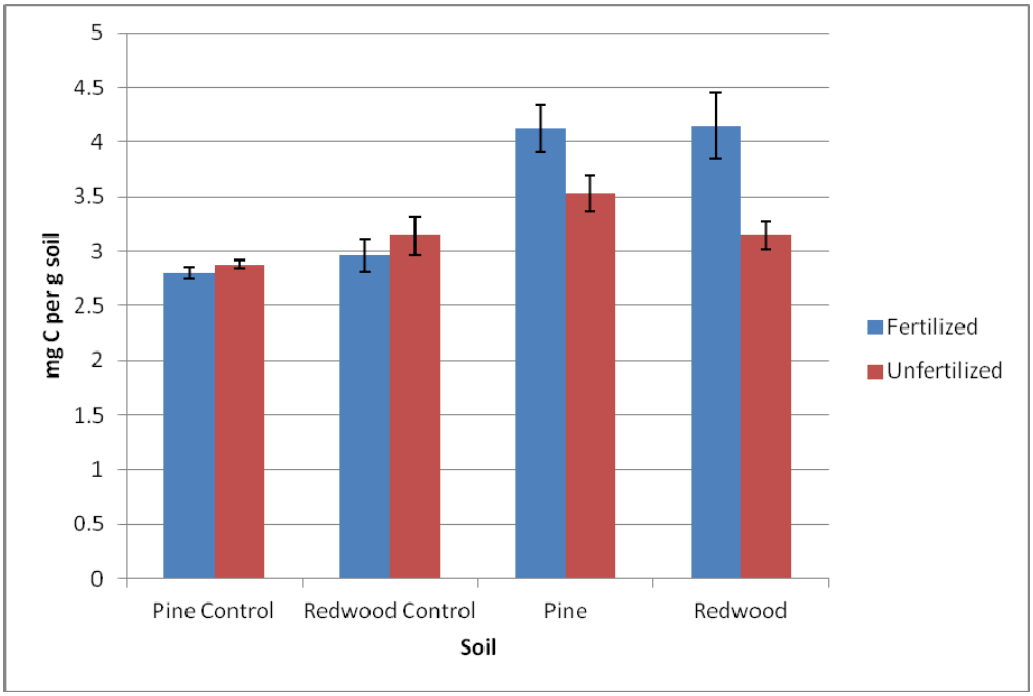

Figure 21: *Microbial biomass carbon for pot trial two. Values are means and error bars indicate the standard error of the mean.*

3.4.5. Community level substrate utilization analysis

The average well colour development (AWCD) of the different carbon substrate groups (carbohydrates, polymers, carboxylic acids, and amino acids) was calculated. There was found to be no significant effect of either tree species or fertilization on average utilization of carboxylic acids, polymers, or amino acids by the microbial community in the first pot trial. The only significant difference in the utilization of carbohydrates was between *P. radiata* and the control, and *E. nitens* and control. For the number of substrates utilized, S, and Shannon's diversity index, H, (figures 21 and 25) the only significant differences that were found were those between *P. radiata* and the control and that between *E. nitens* and the control at $\alpha=0.1$. No significant differences between the species were observed. No significant differences in substrate evenness (figure 23) were observed. PCA was performed to characterize the microbial communities based on the variability in substrate utilization. The substrate utilization patters of the soil microbial communities were not found to be substantially influenced by tree species or fertilization treatments.

In the second pot trial there was no significant effect of tree species or fertilization on the average utilization of carbohydrates, carboxylic acids, polymers, or amino acids by the microbial community in the soil sampled at $\alpha=0.05$. The number of substrates utilized, substrate evenness, and Shannon's diversity index for the second pot trial all showed no significant differences between fertilized and unfertilized pots and no significant difference between the two tree species (figures 22, 24, and 26). Finally, the patterns of substrate utilization by the soil microbial communities were not found to be substantially influenced by tree species or fertilization treatments.

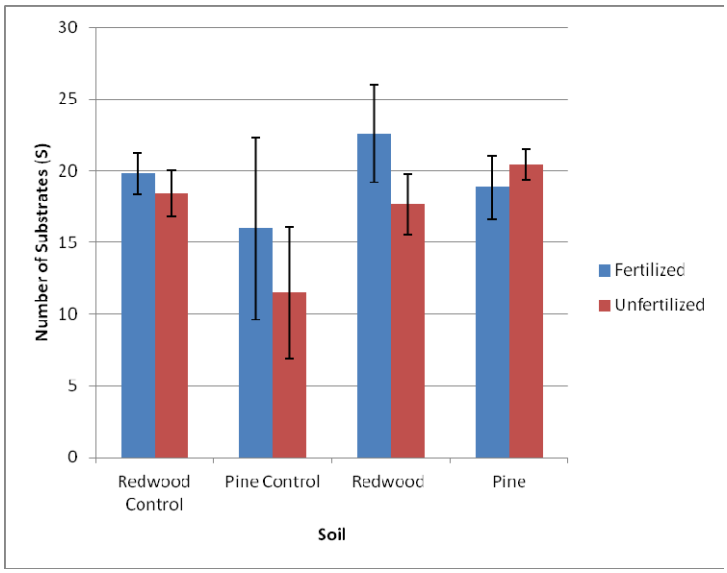

Figure 22: *Mean number of carbon sources utilized by the soil bacterial community in fertilized and unfertilized soil samples under different tree species for pot trial one (mean and standard error).*

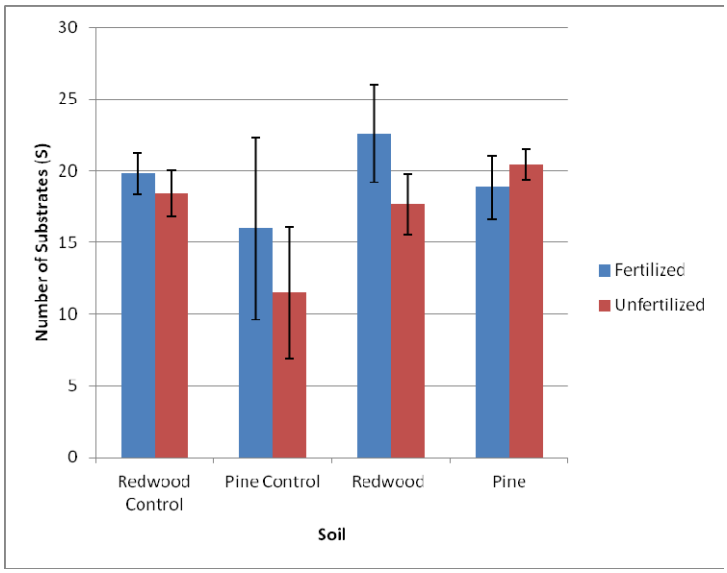

Figure 23: *Mean number of carbon sources utilized by the soil bacterial community in fertilized and unfertilized soil samples under different tree species for pot trial two (mean and standard error).*

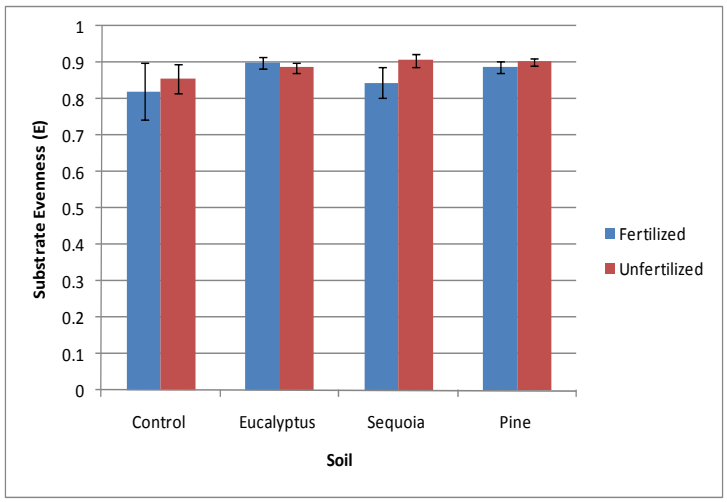

Figure 24: *Evenness values of the carbon sources utilized by the soil bacterial community in fertilized and unfertilized soil samples under different tree species for pot trial one (mean and standard error).*

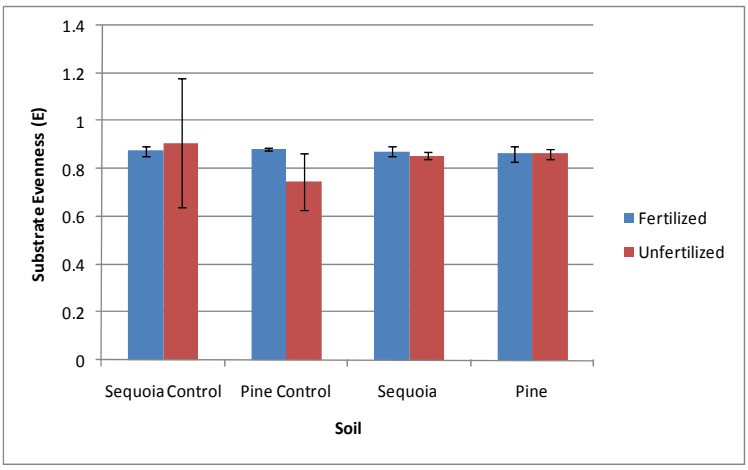

Figure 25: *Evenness values of the carbon sources utilized by the soil bacterial community in fertilized and unfertilized soil samples under different tree species for pot trial two (mean and standard error).*

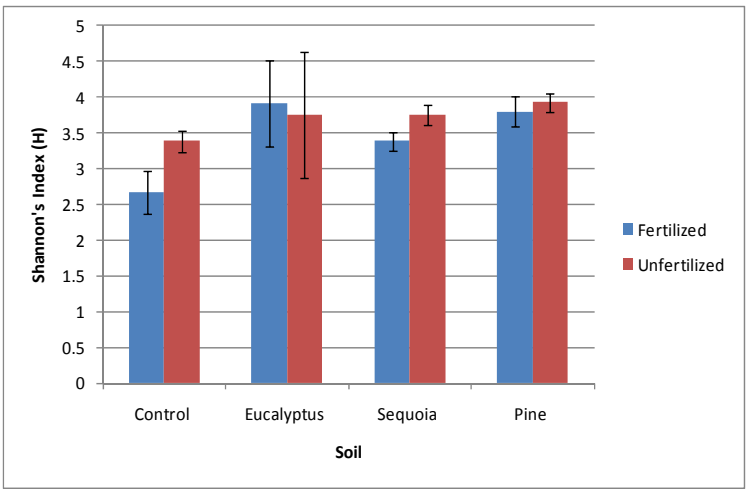

Figure 26: *Shannon's index of the carbon sources utilized by the soil bacterial community in fertilized and unfertilized soil samples under different tree species for pot trial one (mean and standard error).*

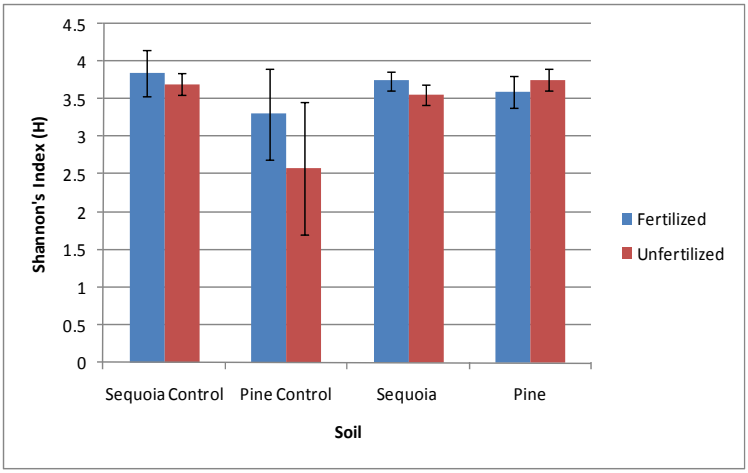

Figure 27: *Shannon's index of the carbon sources utilized by the soil bacterial community in fertilized and unfertilized soil samples under different tree species for pot trial two (mean and standard error).*

Chapter IV

Discussion

4.1. Summary of main findings

Analysis of species effects showed that:

- Soil microbial biomass carbon, nitrogen and the ratio of microbial biomass carbon:nitrogen differed significantly between *P. radiata* and the other tree species sampled at Holt forest. Microbial biomass nitrogen differed significantly between *S. sempervirens* and *P. radiata* at the Hanmer sampling site.
- Soil organic matter was significantly different between *P. radiata* and the other tree species sampled at Holt forest, but not significantly different between *S. sempervirens* and *P. radiata* at the Hanmer sampling site.
- Total soil carbon and nitrogen differed significantly between tree species sampled at Holt forest.
- Total soil carbon differed significantly between *E. nitens* and both *P. radiata* and *S. sempervirens* in the first pot trial and relative to both *E. nitens* contributed significantly more carbon.
- Soil microbial biomass carbon differed significantly between *S. sempervirens* and *P. radiata* in the second glasshouse pot trial.
- No significant species effect on the catabolic diversity of the soil microbial community was found.

Analysis of the effects of fertilizer showed that:

- Soil microbial biomass nitrogen did not differ significantly between fertilized and unfertilized plots at Berwick forest.
- Soil organic matter differed significantly between fertilized and unfertilized plots at Berwick forest being higher under fertilized sites.
- N fertilization had significant effects on both total carbon and nitrogen of the soil in both pot trials.

- N fertilization had a significant effect on soil microbial biomass in both glasshouse pot trials.
- N fertilization was found to have no significant effect on the catabolic diversity of the soil microbial community in both glasshouse pot trials.

4.2. Tree species effect

In the first chapter, four main hypotheses were set out. The first of which stated that:

1. There would be a significant tree species effect on soil carbon.

The results lend some support to this hypothesis. Total C, but not total N differed between the soils of *P. radiata* and *S. sempervirens* at the Hanmer site. Mean total C was significantly higher under *S. sempervirens* at 54.78 mg C per g soil than that under *P. radiata* at 51.53 mg C per g soil. The total C and N for *P. radiata* in the Hanmer and Berwick forest soils were within the range reported in the literature (e.g., Saggar, *et al*., 2001). Total N at Holt forest differed significantly between *P. radiata* and all five of the other species and between *Agathis australis* and both *S. sempervirens* and *Thuja occidentalis*. Very low C and N concentrations in soil and biomass under the *P. radiata* stand at Holt forest were observed. Mean total C was 11.45 mg C per g soil and mean total N was 0.5 mg N per g soil. In contrast mean total C from the fertilized and unfertilized sites at Berwick forest was 42.6 and 39.9 mg C per g soil respectively and total N was 2.64 and 2.33 mg N per g soil respectively. These results were in contrast to the results from the sampling at Hanmer where under the *P. radiata* stand total C, N, and microbial biomass N were significantly higher than that under the *S. sempervirens* stand. Mean total C and N was also substantially higher for *P. radiata* in both pot trials. Loss on ignition (LOI) organic matter for *P. radiata* at 5.31% was particularly low when compared to the other species sampled at Holt forest. This was also approximately half the LOI organic matter observed under *P. radiata* at both the Hanmer and Berwick sampling sites. It is natural to ask what causal factors may explain this. Declining soil C, N and microbial biomass under *P. radiata* has been reported in the literature. For instance,

Saggar, *et al*. (2001), found a 15-25% decline in soil organic C and N under *P. radiata* and a decline in soil microbial biomass relative to grassland.

There was a significant tree species effect on both total C and total N in the first pot trial, however, there was no significant difference between *P. radiata* and *S. sempervirens*. There was however, a significant difference between *E. nitens* and both *P. radiata* and *S. sempervirens.* Interestingly, *E. nitens* was the only non-coniferous tree species included in this study. All were significantly different from the control. When relative contributions to soil C are examined by subtracting the initial soil C from that at the end of the pot trial as expected all plants contributed relative more than the controls. Relative to *P. radiata* and *S. sempervirens, E. nitens* contributed significantly more C. The difference between *P. radiata* and *S. sempervirens* was not significant. This pattern holds when looked at relative to total and root dry weight biomass with *E. nitens* contributing significantly more C per biomass unit than either *P. radiata* or *S. sempervirens.* These results lent support to my hypothesis. As the plants in the first pot trial were grown in homogenous soil from either fertilized or unfertilized plots at Berwick forest the significant effect of tree species on soil carbon and nitrogen developed over the short time scale of the pot trial. Whereas most studies look at tree species effects over a number of years or examine differences only in mature stands of trees in this study tree species effects were observed over only a matter of months. These initial months after which a tree is planted is a period of rapid growth. The results observed in this study demonstrate how short a period of time over which tree species effects can develop and become apparent. Due to the scale and time-frame of the pot trial in this study no aboveground plant residues were returned to the soil. Any effect would be dominated by root contributions to soil. The small size of the pots used in this study may have accentuated the species effect as a larger proportion of the soil was in contact with the roots. Root inputs to soil represent a substantial proportion of total plant C inputs to soil and in many experiments have been shown to be the dominant contributor (e.g., Campbell *et al*., 1991; Molina *et al*., 2001). For that reason it has been hypothesized that soil C is mostly root derived C (Rasse *et al*., 2005). Root derived carbon is more effectively retained in soil than aboveground litter inputs (Balesdent and Balabane, 1996)

and ^{13}C labeling experiments have demonstrated the dominance of root derived C in soil (Mendez-Millan *et al*., 2010). The significant differences in soil carbon and nitrogen between *P. radiata* and *S. sempervirens* seedlings seen in the second pot trial are the expected result as the tree seedlings were grown in the same soil from which mature specimens of the same species had been growing and between which significant differences in soil carbon and nitrogen had previously been determined (see table 8). The seedlings contributed relatively more C to the soil relative to the plant-free controls. However, there was no significant difference in relative contributions between the two species.

Soil microbes influence soil carbon not only through decomposition, but because microbes and microbial products are substantial components of soil organic C (Kögel-Knabner, 2002). In particular, microbial biomass C comprises a significant proportion of the labile fraction of soil organic C (Sparling, 1992). As such it is expected to vary on short time scales and has been suggested as an early indicator of the effects of soil management on soil organic matter quality (Haynes, 2000). When the results for the first pot trial are examined the only significant differences at $\alpha=0.05$ in microbial biomass C were between the planted pots and the plant-free controls. These differences were expected, but the lack of any significant difference between different species does not lend any support to my hypothesis. There was a significant tree species effect on microbial biomass C in the second pot trial which does lend some support to my hypotheses. This fits with evidence from past studies which have found tree species effects on soil microbial biomass (e.g., Bauhus *et al*., 1998).

The results indicated differences in soil microbial biomass C under the six different tree species sampled at Holt forest. Analysis showed that the statistically significant differences were between *P. radiata* and all five of the other species sampled; *Agathis australis, P. strobus, S. sempervirens, Thuja occidentalis,* and *Dacrydium cupressinum.* Differences between those species were not statistically significant. These results were mirrored by the microbial biomass N results. Mean microbial biomass C was 0.99 mg C per g soil. Although low the microbial biomass results here were higher than those

reported by Ros *et al*. (1995), on coastal soil in New Zealand under *P. radiata* where microbial C was up to 0.39 mg per g soil and by O'Brien *et al*., (2003) in south eastern Australia where microbial C ranged from 0.3-0.6 mg per g soil. Because of differences in methods used to determine microbial biomass and the correction factor used, microbial biomass results reported in the literature will vary making comparisons within this study, rather than within this and other studies, most relevant (O'Brien *et al*., 2003). The high microbial biomass C:N ratios of Holt forest soils seen in this study are characteristic of fungal dominated forest ecosystems (Anderson and Domsch, 1980). There were significant differences in soil microbial biomass under *S. sempervirens* and *P. radiata* at the Hanmer sampling site. This is consistent with the findings from other studies e.g., Grayston and Prescott, (2005), Priha *et al*. (2001), and other studies mentioned in the literature review which found differences in soil microbial biomass between different tree species. These differences may be attributable to differing characteristics of the tree species studied.

Due to the more responsive nature of microbial biomass to changes in the soil environment than organic C, the microbial quotient, the proportion of organic C present as microbial C, can reveal changes occurring in soil organic matter and can be a more sensitive measure than organic C alone (Sparling, 1992). Microbial biomass C quotients generally range from 1-5% averaging 2-3% (Jenkinson and Ladd, 1981). The range reported by Bauhus and Khanna (1999) was 0.3-9.9 %. In comparison the microbial biomass C quotients determined from the total C and microbial biomass C results in this study from Holt forest, with the exception of *P. radiata,* ranged from 2-3% (see table 4). Microbial biomass N quotients at the Holt forest site determined from the total N and microbial biomass N results ranged from 2-7% within the range of N quotients seen in the literature. The microbial biomass quotients for the first pot trial ranged from 5-6% and those for the second pot trial ranged from 5-7% (see tables 16 and 17 respectively) well within the range reported by Bauhus and Khanna (1999). As for microbial biomass results some of the variation in microbial quotients reported in the literature will be due to differences in the method used to determine microbial biomass and the correction factor used (O'Brien *et al*., 2003). Due to this, comparisons of microbial quotients are

most relevant within this study. When dealing with the effect of different tree species on soil comparisons can be very difficult as many factors including soil type, stand age, management, undergrowth, and land-use history can confound results. As adjacent stands of the relatively small (15 hectares) Holt forest the stands sampled were growing on the same soil type, have been managed in the same way, and have the same land-use history. The sites were specifically chosen for their lack of undergrowth. This increases confidence in the results. However, the stands were not replicated and although the trees were at the same stage of maturity the stands had different ages. Given the identical growing conditions and the identical initial state of the soil derived from either fertilized or unfertilized plots at Berwick forest and the total absence of any other plants the effects seen in the first glasshouse pot trial gives greater confidence that results were a consequence of the tree species acting upon the soil.

The relation between carbon and nitrogen is often expressed using the carbon to nitrogen ratio. The C:N ratio is the ratio of the percentage of C and the percentage of N. It gives an indication of the quality of soil organic matter N and the degree of organic matter decomposition. A low ratio indicates high quality N and a high ratio indicates low quality N (Killham, 1994). A high ratio therefore reflects low quality N availability for plants and poorly decomposed organic matter and a low ratio reflects high quality N availability for plants and highly decomposed organic matter.

The varying C:N ratios and for that reason N availability of the soil under the six different tree species sampled at Holt forest (see table 2) may be a result of varying leaf litter C:N ratios and/or faster decomposition of leaf litter and forest floor organic matter (Killham, 1994). Decomposition rates are directly related to leaf and root litter quality. Increased decomposition directly contributes to increased soil carbon content. However, I cannot distinguish between these potential causal mechanisms without further investigation. There was a significant effect of both species and fertilizer on the C:N ratios in the both the first and second pot trials. In the case of the first pot trial this cannot be a result of differing leaf litter C:N ratios as there was none evident.

4.3. Effect of fertilizer

The second hypotheses stated that:

2. The application of N fertilization would have a significant effect upon soil carbon.

The results presented in the preceding chapter lend some support to this hypothesis. Conventionally one would expect that N fertilizer application would result in increased N concentration in soils. The higher total nitrogen under the fertilized plots at Berwick forest is expected and reflects higher nitrogen accumulation as a result of the fertilizer application. This increase in soil N in response to N fertilization agrees with the literature (Jandl *et al*., 2003; Smaill *et al*., 2008). Increased soil N may either be caused directly via the retention of the additional N in the soil or indirectly through increased litter inputs to soil. LOI organic matter determined also differed significantly between fertilized and unfertilized plots. LOI organic matter was greater in samples from the fertilized site than those from the unfertilized site. As the sites sampled at Berwick forest were of the same species (*P. radiata*) the same management (weed control and log only harvest) and the trees were all mature and of the same age, the only difference of significance was the fertilizer treatment. This gave me confidence that the results observed were due to the effect of fertilizer and not the result of some confounding factor.

The relationship between soil C and N availability in soil is complex and the results of different studies on soil C and N availability have often been conflicting (Hyvonen et al, 2006). In the first trial there was a significant effect of fertilizer on *P. radiata* only with fertilized pine having significantly greater mean total C than unfertilized pine. When the contributions to soil C are examined relative to plant dry weight biomass the fertilized plants contribute relatively less C than the unfertilized plants. This indicates that the increased biomass of the plants as a result of fertilization is not matched by an increased contribution of carbon to soil. However, in the second pot trial there was no significant effect of fertilization. Although when the relative contributions to soil C are examined

there was a significant effect of fertilizer, but when the relative contributions are looked relative to dry weight plant and root biomass this effect disappears. This does not lend any support to the hypothesis that the application of N fertilizer would have a significant effect upon soil C.

The significantly narrower C:N ratio seen in the Berwick forest soil is the expected result reflecting the N inputs from N fertilization. The mean C:N ratio of the fertilized plots was 16.1 whereas that for the unfertilized plots was 17.1. In the first pot trial there was a significant effect of fertilizer on C:N ratios. This is the expected result and is consistent with what was observed at Berwick forest. C:N ratios were generally lower in fertilized than unfertilized pots in the second trial, for instance, unfertilized *S. sempervirens* seedlings in the second trial had a mean C:N ratio of 19.79 whereas the fertilized *S. sempervirens* seedlings had a mean C:N ratio of 19.07, however this effect was not statistically significant. The lower C:N ratios in fertilized soils agrees with other published results (Gurlevik, *et al*., 2004; Smaill *et al*., 2008).

The shoot:root biomass ratio of *P. radiata* was significantly smaller in the fertilized soil from Berwick forest in the first pot trial. The effect was not significant on either *S. sempervirens* or *E. nitens*. This response of the shoot:root biomass ratio of the seedlings (a relative decline in root growth and relative increase in shoot growth) to additional N in the pot trials is the expected result (Ericsson, 1995). That the effect of tree species was more significant than the effect of fertilizer on the shoot:root biomass ratio was also the expected result as one would anticipate species to be the greatest determinant of the shoot:root biomass ratio. In the first pot trial *E. nitens* had on average the highest shoot:root biomass ratio followed by *P. radiata* with *S. sempervirens* having the lowest. Similarly in the second pot trial *P. radiata* had on average a larger shoot:root biomass ratio than *S. sempervirens*. However, there was no significant effect of fertilization on the shoot:root biomass ratio in the second pot trial.

Microbial biomass at the fertilized Berwick forest plots was on average higher (0.242 mg N per g soil) than in the unfertilized plots (0.112 mg N per g soil). This increase may

have been a response to fertilization; however, the effect was not statistically significant. N fertilization is often found to lead to a decrease in soil microbial biomass in the long-term. For instance, Wallenstein, *et al*. (2006), found that in three long-term experiments on forest soils N fertilization experiments soil microbial biomass declined. Because microbial biomass under the different tree species at Holt forest and Hanmer did differ significantly, but did not differ significantly between the fertilized and unfertilized plots at Berwick forest may suggest that tree species differences have a greater effect on the soil microbial community than the effect of N fertilization. Fertilization had a significant effect on microbial biomass in the second pot trial. Mean microbial biomass was greater in fertilized *S. sempervirens* and *P. radiata* pots. However, microbial biomass in the plant-free controls was not greater. In the first pot trial mean microbial biomass was greater in the fertilized pots for all three species, however this was not the case for the plant-free controls. The increased microbial biomass seen in this study may reflect the short time scale of the trial. Long-term studies in the literature have shown decreases in microbial biomass with N fertilization e.g., Smolander, *et al*. (1994), and Wallenstein, *et al*., (2006). Consistent with the findings of this study Zhang and Zak (1998) found a short-term positive effect on microbial biomass of N addition but, no long-term effect. A meta-analysis of published data by Treseder, K.K. (2008), concluded that N fertilization has a negative effect on soil microbial biomass. That the results of this study showed no significant difference in microbial biomass between fertilized and unfertilized plant-free control, but microbial biomass did differ between fertilized and unfertilized pots which did contain seedlings suggests that the positive effect on biomass may be an indirect result of N fertilization acting upon the plant rather than direct effect of N fertilization on the soil microorganisms and may suggest that the microbial community was not N limited. This is supported by the observation that both total dry weight plant and root biomass was significantly and positively affected by N fertilization. Leckie *et al*. (2004), suggested similar results in a study of regenerating conifer forests which found that it was not the direct effects of fertilization which were the influencing factor on the microbial communities, but rather differences in plant growth rates. Miegroet and Jandl (2007) hypothesized that N additions potentially lead to C loss due to increased rates of C mineralization by soil microorganisms. The reasoning behind this is that immobilization

of excess N by soil microorganisms necessitates microbial biomass formation which will lead to increased respiratory C losses and there is some empirical evidence to support this view (e.g., Jandl *et al*., 2003; Khan *et al*., 2007). Microbial biomass results from the Berwick forest sites in this study do not support this hypothesis as microbial biomass did not differ significantly between fertilized and unfertilized plots. In contrast to the hypothesis of Miegroet and Jandl, a meta-analysis by Janssens., *et al*. (2010), suggested that in soil where nitrogen is not a limiting factor for microbial growth that N additions stimulate carbon sequestration by impeding organic matter decomposition.

The question becomes whether the microbial biomass results seen here are due to the direct effect of N fertilization or are due to differing plant growth rates. In the first pot trial there was no significant effect of N fertilizer on plant biomass for *P. radiata*, but the effect was significant for both *S. sempervirens* and *E. nitens*. In the second pot trial where N fertilizer was added directly during the course of the trial plant biomass was significantly greater with additional N for both *P. radiata* and *S. sempervirens*. The dry weight biomass for both was on average 3 g greater for fertilized over unfertilized plants. Root biomass on a dry weight basis in particular was significantly greater in fertilized over unfertilized plants in the second trial, but not in the first trial. For instance, mean dry weight root biomass for *P. radiata* in the second trial was 1.39 g for unfertilized plants as opposed to 2.15 g for fertilized plants (see table 13).

4.4. Effects of fertilization and tree species on microbial diversity

Two hypotheses regarding the soil microbial community were put forth in the first chapter. Firstly it was hypothesized that:

- There would be a significant tree species effect on the soil microbial community.

and secondly that:

- The application of N fertilization would have a significant effect on the soil microbial community.

In order to test these hypotheses and to determine the degree to which microbial communities in the soil under the different tree species and fertilizer treatments varied community level physiological profiles (CLPP) were constructed using Biolog ecoplates which contain 31 environmentally relevant carbon substrates (see table 1). This technique has been used to characterize soil microbial communities including that under different tree species (e.g., Priha and Smolander, 1999; Priha, *et al*., 1999; Priha, *et al*., 2001; Grayston and Prescott, 2005). In comparing microbial communities in humus and soil under birch, pine and spruce in Finland Priha *et al*. (2001), were unable to distinguish communities using carbon utilization profiles, but were able to by profiling phospholipid fatty acids (PLFA) extracted from the soil. In contrast Grayston and Prescott (2005) were able to distinguish differences using both PLFA and carbon utilization profiles under four different tree species in coastal British Columbia, western red cedar (*Thuja plicata*) , hemlock (*Tsuga heterophylla*) , Douglas fir (*Pseudotsuga menziesii*), and Sitka spruce (*Picea sitchensis*).

Culture dependent methods such as plate counting suffer from the problem of selection bias. That is, they will only select for those microorganisms which are culturable. In as complex a microbial community as those in soil habitats this presents a significant problem. Based on a comparison of direct microscope counts of microbes in soil samples and colony forming units in cultures it has been estimated that less than 0.1% of soil microorganisms are culturable using current culture media (Hill, *et al*., 2000). The results of the direct plate counts carried out in this study revealed no significant differences in either fungal or bacterial counts in both pot trials. This could be due to these problems associated with culture based methods. As Biolog is a culture dependent method it, like plate culture techniques, still encounters the same bias problems associated with those techniques. Even so it is still a widely used technique which provides valuable information on the potential catabolic diversity of microorganisms in soil habitats. Biolog plates measure the catabolic diversity of the soil microbial community. They do not provide a direct measurement of the species present in the soil microbial community. Rather, the measurement of catabolic diversity provided by the Biolog plates is used as a relative measure of species diversity present in the soil microbial community (Zak, *et al*.,

1994). The measurement of catabolic or functional diversity of the soil microbial communities studied should not be considered to be categorical because it is still a culture dependent method and reflects the environment of the culture plates used. Instead it is a measure of the potential catabolic diversity of the soil microbial community in question. As the aims of this study were to examine the species and fertilization effects on the soil microbial community the use of Biolog was considered appropriate to provide information on the relative differences between tree species and fertilization treatments. It is known that the bulk of substrate utilization that occurs in Biolog plates is due to the bacterial component of the soil microbial community (Preston-Mafham, *et al*., 2002). For the purposes of this study in comparing the relative differences between tree species and fertilization this was considered sufficient.

The incubation temperature used in this study was 24ºC. This temperature was chosen to reflect the temperature of the glasshouse used in the pot trials. A lower temperature may have selected for species that were not representative of those most prominent in the growing conditions. Most Biolog studies use incubation temperatures between 15 and 28ºC (Preston-Mafham *et al*., 2002). To determine a suitable incubation time a small trial was carried out from which an incubation time of 72 hours between the initial and final readings was decided upon to give interpretable results, as utilization patterns were apparent in this time and carbon source utilization had not reached an end point.

The average well colour development (AWCD) of the different carbon substrate groups (carbohydrates, polymers, carboxylic acids, and amino acids) was calculated. Carbohydrates, carboxylic acids, and amino acids were of particular interest as they made up the majority of substrates in Biolog ecoplates and are readily available in soil as plant root exudates. At least nine of the 31 carbon substrates present in Biolog ecoplates are reported to be plant root exudates (Campbell, *et al*., 1997; see table 18). Amines and phenolic acids were not examined due to the low number of substrates of each group present in Biolog ecoplates. There was a significant effect of tree species on average carbohydrate utilization by the microbial community in samples from the first pot trial. However, when examined it was found this difference was only between *P. radiata* and

the control, and *E. nitens* and control. The difference between the species was not significant. There was found to be no significant effect of either tree species or fertilization on average utilization of carboxylic acids, polymers, or amino acids by the microbial community in the first pot trial. For the number of substrates utilized, which is equivalent to species richness, and Shannon's diversity index the only significant difference that was found was that between *P. radiata* and the control at $\alpha=0.05$. The difference between *E. nitens* and the control was not significant at $\alpha=0.05$, but was at $\alpha=0.1$. No significant differences between the species were observed. No significant differences in substrate evenness were observed. Although some differences were found to be significant as these difference were between a particular species and the plant-free controls this does not lend any support to my hypothesis. The substrate utilization patterns of the soil microbial communities were not found to be substantially influenced by tree species or fertilization treatments.

In the second pot trial there was no significant effect of tree species or fertilization on the average utilization of carbohydrates, carboxylic acids, polymers, or amino acids by the microbial community in the soil sampled at $\alpha=0.05$. The number of substrates utilized, Shannon's diversity index, substrate evenness, and patterns of substrate utilization for the second pot trial also showed no significant differences between fertilized and unfertilized pots and no significant difference between the two tree species.

Together, the results from the carbon utilization could not provide any evidence to substantiate either of the two main hypotheses. Although no significant results from the carbon utilization patterns were apparent this does not preclude the possibility that the communities in question are in actuality differing in some way. It merely means that no difference in the potential catabolic diversity of relatively fast-growing culturable component of the soil community was found. Past studies combining both substrate utilization profiles and other techniques have found that although there may be no apparent difference in substrate utilization patterns there are significant differences in the soil microbial community when measured by other methods. For example, Leckie, *et al*., (2004) did not find differences in carbon utilization profiles where differences in PLFA

profiles in response to tree species and fertilization were found in a study of microbial community responses in a regenerating coniferous forest.

Table 18: *List of carbon sources in Biolog ecoplate reported in Campbell, et al., (1997) as constituents of plant root exudates.*

Carbohydrates	Carboxylic acids	Phenolic acids	Amino acids
D-xylose	D-Malic Acid	2-Hydroxy Benzoic acid	L-Arginine
		4-Hydroxy Benzoic acid	L-Asparagine
			L-Phenylalanine
			L-Serine
			L-Threonine

4.5. Conclusions

My original hypotheses stated that:

1. There would be a significant tree species effect on soil carbon.
2. The application of N fertilization would have a significant effect upon soil carbon.
3. There would be a significant tree species effect on the soil microbial community.
4. The application of N fertilization would have a significant effect on the soil microbial community.

Those significant effects of both tree species and fertilizer on soil carbon and soil microbial biomasses that were observed in this study lends partial support to these hypotheses. However, the results of the carbon substrate utilization results do not provide any support to the hypotheses. It is difficult to make any generalizations based on the results here. Making any generalizations in soil studies is problematic and difficult. Site specific factors in particular make any generalizations difficult. The short time scale of

the pot trials in this study limits its relevance. However, a number of inferences can be made from the results obtained here and a careful review of the literature. Over the short-term the effects of N fertilization on soil microbial biomass is likely to be positive. This is supported by the pot trial results and previously reported results from short-term experiments. However, it seems based on published results over the long-term the effect of N fertilization on soil microbial biomass is more likely to be negative or neutral. Although no significant differences in soil microbial community function were observed in this study, from the available evidence it is probable that the composition of the microbial community is likely to be tilted in favor of the fungal component with higher quantities of N added. Of particular note was the short-time period in which tree species effects became apparent. This occurred without any aboveground inputs to the soil. These results emphasise the influence that plant roots and in particular root carbon exert on soil. As a strategy for C sequestration N fertilization may not always be successful. The application of fertilizers to forests in New Zealand is a common practice and is usually carried out with the intention of increasing productivity or preventing nutrient deficiencies not for the purposes of carbon sequestration. The results obtained in this study and from the literature suggest that tree species is likely to be a greater determinant of soil carbon than N fertilization.

4.6. Recommendations for further work

Since significant variation was shown with some of the variables measured in this study and not others further work should be carried out to confirm these findings and explore further the effects investigated in this study.

While Biolog can provide important information on the potential function of soil microbial communities it suffers from the problem of culturable bias. Culture independent methods such as DNA fingerprinting or non-genetic molecular techniques such as PLFA that, although still face problems of bias, do not suffer from the same selection bias associated with culture dependent methods, but also do not provide functional information on the soil microbial community, could be used in conjunction

with culture based methods such as Biolog, which would build a more complete picture of the soil microbial community. To further explore the functional diversity of the soil microbial community enzyme analysis could be considered. Enzyme analysis has been used in past studies investigating tree species effects on the soil microbial community e.g., Kourtev, *et al*. (2002), and Waldrop *et al*. (2000). In particular, enzymes associated with carbon substrates for instance, β –glucosidase, phenol oxidase, and cellobiohydrolase would be of interest particularly in connection to carbon utilization data gathered through use of Biolog. Enzymes associated with N metabolism, for example, urease would also have been relevant to this study and provide finer grained detail as to the changes taking place within the soil. Such analysis would also have the added benefit of describing the functional diversity of both the fungal and bacterial component of the soil microbial community.

Other methods of measuring soil microbial biomass and activity should be investigated to complement the results found here to provide more precision in the quantification of microbial biomass. For instance, substrate induced respiration (SIR) could be looked at as an alternative or complement to the fumigation-extraction technique used to determine microbial biomass carbon in this study.

A more critical consideration for future research into the tree species effects, fertilization and their relationships to each other is not connected with any particular microbiological technique that could be explored, but rather the experimental design. For instance, in the field component of this study only a single one off sampling time was used. Sampling at different times throughout the year should be considered as it may provide different results than that from a single sample time. This is particularly important for the soil microbial biomass results as microbial biomass has been shown to vary at different times in the year (Wardle, 1998). A single measurement of soil microbiological properties merely provides a snapshot of single point in time and does not necessarily provide an accurate representation of those properties at other times nor does it show how these properties develop over time. The need for more long-term soil experiments in answering questions about C cycling and the effects of management practices has been recognized

(Richter *et al*., 2007). In the pot trial components of this study the differences that did exist were shown to have developed from an initial identical state in a relatively short period of time. Although not practical, given time constraints, a more comprehensive study could track these changes with multiple sampling times over a longer period of the tree's life than just the early stages of seedling growth.

This study was confined to surface soil, but there is evidence that tree species can affect deep soil (e.g., Dijkstra and Smits, 2002) and soil carbon and nitrogen is known to differ significantly with depth (Jobbágy and Jackson, 2000). A more comprehensive future study would look at soil carbon and nitrogen throughout the soil profile not simply the shallow depth used in field samples for this study.

The tree species used in the pot trials in this study were chosen for their commonality and commercial availability. A widening of the study to include more species would further our knowledge of the tree species effects investigated in this study. Native New Zealand species such as those sampled from Holt forest in this study, for example *Agathis australis* and *Dacrydium cupressinum* or other natives such as tōtara (*Podocarpus totara*) and kahikatea (*Dacrycarpus dacrydioides*) if used in a pot trial would provide valuable information. Other non-native, but commercially important plantation species in New Zealand could also have been considered for example, Douglas fir (*Pseudotsuga menziesii*), other Eucalyptus plantation species such as *Eucalyptus regnans* and *Eucalyptus saligna*, or cypresses such as Macrocarpa (*Cupressus macrocarpa*) and Lusitanica (*Cupressus lusitanica*). Furthermore, the experimental design in this study included only the presence or absence of N fertilization. A more comprehensive investigation could study varying levels of N fertilization.

Literature Cited

Allen, M.F. (1992). *Mycorrhizal functioning: an integrative plant-fungal process*. Chapman and Hall, London. 534p.

Allison, S.D., Hanson, C.A., and Treseder, K.K. (2007). Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. *Soil Biology and Biochemistry* **39:** 1878-1887.

Anderson, J.P.E., and Domsch, K.H. (1980). Quantities of plant nutrients in the microbial biomass of selected soils. *Soil Science* **130:** 211-216.

Aneja, K.A. (2003). *Experiments in microbiology, plant pathology and biotechnology*. New Age International, New Delhi. 607p.

Augusto, L., Ranger, J., Binkley, D., and Rothe, A. (2002). Impact of several common tree species of European temperate forests on soil fertility. *Annals of Forest Science* **59:** 233-253.

Ayres, E., Steltzer, H., Berg, S., Wallenstein, MD., Simmons, BL., and Wall DH. (2009). Tree species traits influence soil physical, chemical and biological properties in high elevation forests. *PLoS ONE* **4:** e5964.

Bauhus, J., Khanna, P.K. (1999). The significance of microbial biomass in forest soils. In: Rastin, N., Bauhus, J. Going underground – Ecological studies in forest soils. Research Signpost, Trivandrum, India, pp. 77–110.

Bauhus, J., Pare, D., Cote L. (1998). Effects of tree species, stand age and soil type on microbial biomass and its activity in a southern boreal forest. *Soil Biology and Biochemistry* **30:** 1077-1089.

Balesdent, J., and Balabane, M. (1996). Major contribution of roots to soil carbon storage inferred from maize cultivated soils. *Soil Biology and Biochemistry* **28:** 1261-1263.

Bardgett, R.D., Mawdsley, J.L., Edwards, S., Hobbs, P.J., Rodwell, J.S., and Davies, W.J. (1999). Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. *Functional Ecology* **13:** 650-660.

Bardgett, R.D., Usher, M.B., and Hopkins, D.W. (2005). *Biological Function and Diversity in Soils*. Cambridge University Press, Cambridge. 411p.

Bardgett, R.D., and Wardle, D.A. (2010). *Aboveground-Belowground Linkages: Biotic Interactions, Ecosystem Processes, and Global Change*. Oxford University Press, Oxford. 301p.

Binkley, D., and Giardina, C. (1998). Why do tree species affect soil? The Warp and Woof of tree soil interactions. *Biogeochemistry* **42:** 89-106.

Blanco-Canqui, H., and Lal, R. (2004). Mechanisms of carbon sequestration in soil aggregates. *Critical Reviews in Plant Sciences* **23:** 481-504.

Brechet, L., Ponton, S., Roy, J., Freycon, V., Couteaux, M-M., Bonal, D., and Epron, D. (2009). Do tree species characteristics influence soil respiration in tropical forests? A test based on 16 tree species planted in monospecific plots. *Plant and Soil* **319:** 235-246.

Brookes, P.C., Landman, A., Pruden, G., and Jenkinson, D.S. (1985). Chloroform fumigation and the release of soil nitrogen – a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry* **17:** 837-842.

Campbell, C.D., Grayston, S.J., and Hirst, D.J. (1997). Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. *Journal of Microbiological Methods* **30:** 33-41.

Campbell, C.A., Lafond, G.P., Zentner, R.P., and Biederbeck, V.O. (1991). Influence of fertilizer and straw bailing on soil organic matter in a thick black chernozem in western Canada. *Soil Biology and Biochemistry* **23:** 443-446.

Carney, K.M., and Matson, P.A. (2006). The influence of tropical plant diversity and composition on soil microbial communities. *Microbial Ecololgy* **52:** 226–238.

Carter, M.R., and Gregorich, E.G. (2008). *Soil Sampling and Methods of Analysis*. 2nd Edition. CRC Press, Boca Raton. 1224p.

De Dyne, G.B., Cornelissen, J.H.C., and Bardgette, R.D. (2008). Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology Letters* **11:** 516-531.

Demoling, F., Nilsson, LO., and Baath, E. (2008). Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. *Soil Biology and Biochemistry* **40:** 370-379.

Dijkstra, F.A., and Smits, M.M. (2002). Tree species effects on calcium cycling: The role of calcium uptake in deep soils. *Ecosystems* **5:** 385-398.

Epelde, L., Becerril, J.M., Hernandez-Allica, J., Barrutia, O., and Garbisu, C. (2008). Functional diversity as indicator of the recovery of soil health derived from *Thlaspi caerulescens* growth and metal phytoextraction. *Applied Soil Ecology* **39:** 299-310.

Ericsson, T. (1995). Growth and shoot:root ratio of seedlings in relation to nutrient availability. *Plant and Soil* **168-169:** 205-214.

Frey, S.D., Knorr, M., Parrent, J.L., and Simpson, R.T. (2004). Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management* **196:** 159-171.

Galloway, J.N., Schlesinger, W.H., Hiran Levy II, Michaels, A., and Schnoor, J.L. (1995). Nitrogen fixation: anthropogenic enhancement – environmental response. *Global Biogeochemical Cycles* **9:** 235-252.

Garland, J.L., and Mills, A.L. (1991). Classification and characterization of heterotrophic microbial communities on the basis of community level sole source carbon utilization. *Applied and Environmental Microbiology* **57:** 2351-2359.

Goulding, K.W.T., Bailey, N.J., Bradbury, N.J., Hargreaves, P., Howe, M., Murphy, D.V., Poulton, P.R., and Willison, T.W. (1998). Nitrogen deposition and its contribution to nitrogen cycling and associated soil processes. *New Phytologist* **139:** 49-58.

Grayston, S.J. (2000). Rhizodeposition and its impact on microbial community structure and function in trees. *Phyton* **40:** 27-36.

Grayston, S.J., and Campbell, C.D. (1996). Fucntional biodiversity of microbial communities in the rhizosphere of hybrid larch (*Larix eurolepsis*) and Sitka spruce (*Picea sitchensis*). *Tree Physiology* **16:** 1031-1038.

Grayston, S.J., Campbell, C.D., Bardgett, R.D., Mawdsley, J.L., Clegg, C.D., Ritz, K., Griffiths, B.S., Rodwell, J.S., Edwards, S.J., Davies, W.J., Elston, D.J., and Millard, P. (2004). Assessing shifts in microbial community structure across a range of grasslands differing in management intensity using CLPP, PLFA, and community DNA techniques. *Applied Soil Ecology* **25:** 63-84.

Grayston, S.J., Griffith, G.S., Mawdsley, J.L., Campbell, C.D., and Bardgett, R.D. (2001). Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Biology and Biochemistry* **33:** 533-551.

Grayston, S.J., and Prescott, C.E. (2005). Mircobial communities in forest floors under four tree species in coastal British Columbia. *Soil Biology and Biochemistry* **37:** 1157-1167.

Gurlevik, N., Kelting, D.L., and Allen, H.L. (2004). Nitrogen mineralization following vegetation control and fertilization in a 14-year old loblolly pine plantation. *Soil Science Society of America Journal* **68:** 272-281.

Hackl, E., Zechmeister-Boltenstern, S., Bodrossy, L., and Sessitsch, A. (2004). Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils *Applied and Environmental Microbiology* **70:** 5057-5065.

Hagen-Thorn, A., Callesen, I., Armolaitis, K., and Nihlgard, B. (2004). The impact of six European tree species on the chemistry of mineral topsoil in forest plantations on former agricultural land. *Forest Ecology and Management* **195:** 373-384.

Haynes, R.J. (2000). Labile organic matter as an indicator of organic matter quality in arable and pastoral soils in New Zealand. *Soil Biology and Biochemistry* **32:** 211-219.

Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., Maldonado-Ramirez, S., Lynch, S.T., and Nelson, E.B. (2000). Methods for assessing the composition and diversity of soil microbial communities. *Applied Soil Ecology* **15:** 25-36.

Hobbie, SE. (1992). Effects of plant species on nutrient cycling. *Trends in Ecology and Evolution* **7:** 336-339.

Hogberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Hogberg, M.N., Nyberg, G., Ottosson-Lofvenius, M., and Read, D.J. (2001). Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* **411:** 789-792.

Hogberg, P., Hogberg, M.N., Gottlicher, S.G., Betson, N.R., Keel, S.G., Metcalfe, D.B., Campbell, C., Schindlbacher, A., Hurry, V., Lundmark, T., Linder, S., and Nasholm, T. (2008). High temporal tracing of photosynthate carbon from the tree canopy to forest soil microorganisms. *New Phytologist* **177:** 220-228.

Hooper, D.U. and Vitousek P.M. (1997). The effects of plant composition and diversity on ecosystem processes. *Science* **277:** 1302-1305.

Hyvonen, R., Agren, G.I., Linder, S., Persson, T., Cotrufo, M.F., Ekblad A., Freeman, M., Grelle, A., Janssens, I.A., Jarvis, P.G., Kellomaki, S., Lindroth, A., Loustau, D., Lundmark, T., Norby, R.J., Oren, R., Pilegaard, K., Ryan, M.G., Sigurdsson, B.D., Stromgren, M., Van Oijen, M., Wallin, G. (2007). The likely impact of elevated CO_2 nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. *New Phytologist* **173:** 463-480.

Iovieno, P., Alfani, A., and Baath, E. (2010). Soil microbial community structure and biomass as affected by *Pinus pinea* plantation in two Mediterranean areas. *Applied Soil Ecology* **45:** 56-63.

Jandl, R., Lindner, M., Vesterdal, L., Bauwens, B., Baritz, B., Hagedorn, F., Johnson, D.W., Minkkenin, K., and Byrne, K.A. (2007). How Strongly Can Forest Management Influence Soil Carbon Sequestration? *Geoderma* **137:** 253-268.

Jandl, R., Kopeszki, H., Bruckner, A., and Hager, H. (2003). Forest soil chemistry and mesofauna 20 years after an amelioration fertilization. *Restoration Ecology* **11:** 239-246.

Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J-A., Reichstein, M., Ceulemans, R., Ciais P., Dolman, A. J., Grace, J., Matteucci, G., Papale, D., Piao, S. L., Schulze, E-D., Tang J., and Law B.E. (2010). Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience* **3:** 315-322.

Jha, P., and Mohapatra, KP. (2010). Leaf litter, fine root production and turnover in four major tree species of the semi-arid region of India. *Plant and Soil* **326:** 481-491.

Jiang, P-K., and Xu, Q-F. (2006). Abundance and dynamics of soil labile carbon pools under different types of forest vegetation. *Pedosphere* **16:** 505-511.

Jobbágy, E.G., and Jackson, R.B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications* **10:** 423-436.

Joergensen, R.G. (1996). The fumigation-extraction method to estimate soil microbial biomass: calibration of the k_{EC} value. *Soil Biology and Biochemistry* **28:** 25-31.

Joergensen, R.G., and Mueller, T. (1996). The fumigation-extraction method to estimate soil microbial biomass: calibration of the k_{EN} value. *Soil Biology and Biochemistry* **28:** 33-37.

Johnson, M.J., Lee, K.Y., and Scow, K.M. (2003). DNA fingerprinting reveals links among agricultural crops, soil properties, and the composition of soil microbial communities. *Geoderma*, **114:** 279-303.

Killham, K. (1994). *Soil Ecology*. Cambridge University Press, Cambridge. 242p.

Khan, S.A., Mulvaney, R.L., Ellsworth, T.R., and Boast, C.W., (2007). The myth of nitrogen fertilization for soil carbon sequestration. *Journal of Environmental Quality* **36:** 1821-1832.

Kögel-Knabner, I. (2002). The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biology and Biochemistry*. **34:** 139-162.

Kourtev, P.S., Ehrenfeld, G.J., and Haggblom, M. (2002). Exotic plant species alter the microbial community structure and function in the soil. *Ecology* **83:** 3152-3166.

Kumar, H.D. (2006). *Agricultural Ecology*. APH Publishing, New Delhi. 320 pp.

Kutsch, W.L., Bahn, M., and Heinemeyer, A. (2009). *Soil carbon dynamics an integrated methodology.* Cambridge University Press, Cambridge. 286p.

Lal, R. (2004). Soil carbon sequestration to mitigate climate change. *Geoderma* **123:** 1-22.

Leckie, S.E. (2005). Methods of microbial community profiling and their application to forest soils. *Forest Ecology and Management* **220:** 88-106.

Leckie, SE., Prescott, CE., and Grayston, SJ. (2004). Forest floor microbial community response to tree species and fertilization of regenerating coniferous forests. *Canadian Journal of Forest Research* **34:** 1426-1435.

Lejon, D., Chaussod, R., Ranger, J., and Ranjard, L. (2005). Microbial Community Structure and density under Different Tree Species in an Acid Forest Soil (Morvan, France). *Microbial Ecology* **50:** 614-625.

Lemenih, M., Olsson, M., and Karltun, E. (2004). Comparison of soil attributes under *Cupressus lusitanica* and *Eucaplytus saligna* established on abandoned farmlands with continuously cropped farmlands and natural forest in Ethiopia. *Forest Ecology and Management*, **195:** 57-67.

Mendez-Milan, M., Dignac, M.F., Rumpel, C., Rasse, D.P., and Derenne, S. (2010). Molecular dynamics of shoot vs root biomarkers in an agricultural soil estimated by natural abundance of ^{13}C labeling. *Soil Biology and Biochemistry*. **42:** 169-177.

Mertens, J., Van Nevel, L., De Schrijver, A., Piesschaert, F., Oosterbaan, A., Tack, F.M.G., and Verheyen, K. (2007). Tree species effect on the redistribution of soil metals. *Environmental Pollution* **149:** 173-181.

Miegroet, H.V., and Jandl, R. (2007). Are nitrogen-fertilized forest soils sinks or sources of carbon? *Environmental Monitoring and Assessment* **128:** 121-131.

Molina, J.A.E., Clapp, C.E., Linden, D.R., Allmaras, R.R., Layese, M.F., Dowdy, R.H., and Cheng, H.H., (2001). Modeling the incorporation of corn (*Zea mays* L.) carbon from roots and rhizodeposition into soil organic matter. *Soil Biology and Biochemistry* **33:** 83-92.

Nef, J.C., Townsend, A.R., Gleixner, G., Lehman, S.J., Turnbull, J., and Bowman, W.D. (2002). Variable effects of nitrogen on the stability and turnover of soil carbon. *Nature* **419:** 915-917.

O'Brien, N.D., Attiwill, P.M., and Weston, C.J. (2003). Stability of soil organic matter in *Eucalyptus regnans* forests and *Pinus radiata* plantations in south eastern Australia. *Forest Ecology and Management* **185:** 249-261.

Opik, H., Rolfe, S.A., Willis, A.J., and Street, H.E. (2005). *The physiology of flowering plants.* 3rd Edition. Cambridge University Press, Cambridge. 392p.

Pellissier, F., and Souto X.C. (1999). Allelopathy in northern temperate and boreal semi-natural woodland. *Critical Reviews in Plant Sciences* **18:** 637-652.

Philips, R.P., and Fahey, T.J. (2006). Tree species and mychorrizal associations influence the magnitude of rhizosphere effects. *Ecology* **87:** 1302-1313.

Pollierer, M.M., Langel, R., Korner, C., Maraun, M., and Scheu, S. (2007). The underestimated importance of belowground carbon input for forest soil animal food webs. *Ecology Letters* **10:** 729-736.

Powlson, D.S., Brookes, P.C., and Christensen, B.T. (1987). Measurement of soil microbial biomass provides an early indication in changes to total organic matter due to straw incorporation. *Soil Biology and Biochemistry* **19:** 159-164.

Prescott, CE. (2002). The Influence of the Forest Canopy on Nutrient Cycling. *Tree Physiology* **22:** 1193-1200.

Prescott, C.E., Zabak, L.M., Staley, C.L., and Kabzems, R. (2000). Decomposition of broadleaf and needle litter in forests of British Columbia: Influence of litter type, forest type, and litter mixtures. *Canadian Journal of Forest Research* **30:** 1742-1751.

Preston-Mafham, J., Boddy, L., and Randerson, P.F. (2002). Analysis of microbial community functional diversity using sole-carbon-source utilization profiles – a critique. *FEMS Microbiology Ecology* **42:** 1-14.

Priha, O., Grayston, SJ., Hiukka, R., Pennanen, T., and Smolander, A. (2001). Microbial community structure and characteristics of the organic matter in soils under *Pinus sylvestris*, *Picea abies*, and *Betula pendula* at two forest sites. *Biology and Fertility of Soils* **33:** 17-24.

Priha, O., Grayston, SJ., Pennanen, T., and Smolander, A. (1999). Microbial activities related to C and N cycling and microbial community structure in the rhizospheres of Pinus sylvestris, Picea abies and Betula pendula seedlings at two forest sites. *Biology and Fertility of Soils*, **33:** 17-24.

Priha, O., and Smolander, A. (1998). Nitrogen transformations in soil under Pinus sylvestris, Picea abies and Betual pendula at two forest sites. *Soil Biology and Biochemistry* **31:** 965-977.

Raich, J.W. and Potter C.S. (1995). Global patterns of carbon dioxide emissions from soils. *Global Biogeochemical Cycles* **9:** 23-36.

Raich, J.W., Russel, A.E., and Bedoya-Arrieta, R. (2006). Lignin and enhanced litter turnover in tree plantations of lowland Costa Rica. *Forest Ecology and Management* **239:** 128-135.

Raich, J.W., Russel, A.E., and Valverde-Barrantes, O. (2009). Fine root decay rates vary widely among lowland tropical tree species. *Oecologia* **161:** 325-330.

Rasse, D.P., Rumpel, C., and Dignac, M.F. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilization. *Plant and Soil* **269:** 341-356.

Resh, S.C., Binkley, D., and Parrotta, J.A. (2002). Greater soil carbon sequestration under nitrogen-fixing trees compared with *Eucalyptus* species. *Ecosystems* **5:** 217-231.

Richter, D.D., Hofmockel, M.F., Callaham, M.A., Powlson, D.S., and Smith, P. (2007). Long-term soil experiments: keys to managing Earth's rapidly changing ecosystems. *Soil Science Society of America Journal* **71:** 266-279.

Ros, M., Goberna, M., Pascaul, J.A., Klammer, S., and Insam, H. (2008). 16s rDNA analysis reveals low microbial diversity in community level physiological profile assays. *Journal of Microbiological Methods* **72:** 221-226.

Ross, D.J., Sparling, G.P., Burke, C.P., and Smith, C.T. (1995). Microbial biomass C and N and mineralizable-N, in litter and mineral soil under *Pinus radiata* on coastal sand: Influence of stand age and harvest management. *Plant and Soil* **175:** 167-177.

Russel, A.E., Cambardella, C.A., Ewell, J.J., and Parkin, T.B. (2004). Species, rotation, and life-form diversity effects on soil carbon in experimental tropical ecosystems. *Ecological Applications* **14:** 47-60.

Russel, AE., Raich, JW., Valverde-Barrantes, OJ., and Fisher, RF. (2007). Tree species effects on soil properties in experimental plantations in tropical moist forest. *Soil Science Society of America* **71:** 1389-1397.

Saggar, S., Hedley, C.B., and Salt, G.J. (2001). Soil microbial biomass, metabolic quotient, and carbon and nitrogen mineralization in 25 year-old *Pinus radiata* agroforestry regimes. *Australian Journal of Soil Research* **39:** 491-504.

Schulp, C.J.E., Nabuurs, G.J., Verburg, P.H., and de Waal, R.W. (2008). Effect of tree species on carbon stocks in forest floor and mineral soil and implications for soil carbon inventories. *Forest Ecology and Management* **256:** 482-490.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, T., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C,. Nannipieri, P., Rasse, D.P., Weiner, S., and Trumbore, S.E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature* **478:** 49-56.

Schweitzer, J.A., Bailey, J.K., Fischer, D.G., LeRoy, C.J., Lonsdorf, E.V., Whitham, T.G., and Hart, S.C. (2008). Plant–soil–microorganism interactions: heritable relationship between plant genotype and associated soil microorganisms. *Ecology* **89:** 773-781.

Selamnts, P.C., Hart, S.C., Boyle, S.I., and Stark, J.M. (2005). Red alder (*Alnus rubra*) alters community-level soil microbial function in conifer forests of the of the Pacific Northwest, USA. *Soil Biology and Biochemistry* **37:** 1860-1868.

Smaill S.J., Clinton, P.W., and Greenfield, L.G. (2008). Nitrogen fertilization effects on litter fall, FH layer and mineral soil characteristics in New Zealand *Pinus radiata* plantations. *Forest Ecology and Management* **256:** 564-569.

Smolander, A., Kurka, A., Kitunen, V., and Malkonen, E. (1994). Microbial biomass C and N, and respiratory activity in soil of repeatedly limed and N-and P-fertilized Norway Spruce stands. *Soil Biology and Biochemistry* **26:** 957-962.

Sparling, G.P. (1992). Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. *Australian Journal of Soil Research* **30:** 195-207.

Stapleton, LM., Crout, NMJ., Sawstrom, C., Marshall, WA., Poulton, PR., Tye, AM., and Laybourn-Parry, J. (2005). Microbial carbon dynamics in nitrogen amended Arctic tundra soil: Measurement and modeling testing. *Soil Biology and Biochemistry* **37:** 2088-2098.

Strobel, B.W., Hansen, H.C., Borggaard, O.K., Andersen, M.K., and Raulund-Rasmussen, K. (2001) Composition and reactivity of DOC in forest floor soil solutions in relation to tree species and soil type. *Biogeochemistry* **56:** 1-26.

Swift, M.J., Heal, O.W., and Anderson, J.M. (1979). *Decomposition in terrestrial ecosystems* Vol 5. University of California Press, Berkeley. 372p.

Swift, R.S. (2001). Sequestration of carbon by soil. *Soil Science* **166:** 858-871.

Tate K.R,. and Parshotam, A. (1995) Soil carbon storage and turnover in temperate forests and grasslands A New Zealand perspective. *Journal of Biogeography* **22:** 695-700.

Thomas, K.D., and Prescott, C.E. (2000). Nitrogen availability in forest floors of three tree species on the same site: the role of litter quality. *Canadian Journal of Forestry Research* **30:** 1698-1706.

Torsvik, V.L., Sorheim, R, and Goksoyr, J. (1996). Total bacterial diversity in soil and sediment communities – a review. *Journal of Industrial Microbiology* **17:** 170-178.

Townsend, A.R., Braswell, B.H., Holland, E.A., and Penner, J.E. (1996). Spatial and temporal patterns in terrestrial carbon storage due to deposition of fossil fuel nitrogen. *Ecological Applications* **6:** 806-814.

Treseder, KK. (2008). Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters* **11:** 1111-1120.

Tyler, G. (1992). Tree species affinity of decomposer and ectomychorrizhal macrofungi in beech (*Fagus sylvatica* L.), oak (*Quercus robur* L.) and hornbeam (*Carpinus betulus* L.) forests. *Forest Ecology and Management* **47:** 269-284.

Ushio, M., Waiga, R., Balser, TC., and Kitayama, K. (2008). Variations in the soil microbial community composition of tropical montane forest: Does tree species matter? *Soil Biology and Biochemistry* **40:** 2699-2702.

Vesterdal, L., and Raulund-Rasmussen, K. (1998). Forest floor chemistry under seven tree species along a soil fertility gradient. *Canadian Journal of Forest Research* **28:** 1636-1647.

Vesterdal, L., Schmidt, I.K., Callesen, I., Nilsson, L.O., and Gundersen, P. (2008). Carbon and nitrogen in forest floor and mineral soil under six common European tree species. *Forest Ecology and Management* **255:** 35-48.

Waldrop, M.P., Balser, T.C., and Firestone, M.K. (2000). Linking microbial community composition to function in a tropical soil. *Soil Biology and Biochemistry* **32:** 1837-1846.

Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., Gallo, M., and Lauber, C. (2004). Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecological Applications* **14:** 1172-1177.

Wardle, D.A. (1998). Controls on temporal variability of the soil microbial biomass: A global scale synthesis. *Soil Biology and Biochemistry* **30:** 1627-1637.

Wardle, D.A. (2002*). Communities and ecosystems: linking the aboveground and belowground components*. Princeton University Press. 392p.

Wardle, D.A., Barker, G.M., and Bonner K.I. (1998). Can comparative approaches based on ecophysiological traits predict the nature of biotic interactions and individual plant species effects in ecosystems? *Journal of Ecology* **86:** 405-420.

Wallenstein, M.D., McNutty, S., Fernandez, I.J., Boggs, J., and Schlesinger, W.H. (2006). Nitrgoen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. *Forest Ecology and Management* **222:** 459-468.

White, R.E. (1997). *Principles and practice of soil science: the soil as a natural resource*. Blackwell Publishing, Oxford. 348p.

Wieland, G., Neumann, R., and Backhaus, H. (2001). Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. *Applied and Environmental Microbiology* **67:** 5849-5854.

Zak, J.C., Willig, M.R., Moorhead, D.L., and Wildman, H.G. (1994). Functional diversity of microbial communities: a quantitative approach. *Soil Biology and Biochemistry* **26:** 1101-1108.

Zhang, P., Tian, X., He, X., Song, F., Ren, L., and Jiang, P. (2009). Effect of quality on its decomposition in broadleaf and coniferous forest. *European Journal of Soil Biology* **44:** 392-399.

Zhang, Q., and Zak, J.C. (1998). Effects of water and nitrogen amendment on soil microbial biomass and fine root production in a semi-arid environment in West Texas. *Soil Biology and Biochemistry* **30:** 39-45.

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Appendix

Appendix A: Hanmer Soil Moisture Content

Soil	Mass of crucible (g)	Mass moist soil + crucible (g)	Mass of oven dry soil + crucible (g)	Moisture content (%)
Redwood	26.21	37.40	36.18	10.9
Redwood	19.41	28.27	27.31	10.8
Redwood	11.09	20.56	19.48	11.4
Redwood	16.16	26.02	24.89	11.5
Pine	15.92	26.80	25.96	7.7
Pine	16.15	26.06	25.29	7.8
Pine	22.36	31.48	30.79	7.6
Pine	15.70	25.74	24.95	7.9

Moisture content calculated as per equation 1.

Appendix B: Hanmer Soil Loss on Ignition Organic Matter Content

Soil	Mass of crucible (g)	Original mass	Mass remaining after furnace	Organic matter (%)
Redwood	26.21	36.18	34.59	15.9
Redwood	19.41	27.31	26.29	12.9
Redwood	11.09	19.48	18.40	12.9
Redwood	16.16	24.89	23.55	15.3
Pine	15.92	25.96	24.68	12.7
Pine	16.15	25.29	24.11	12.9
Pine	22.36	30.79	29.60	14.1
Pine	15.70	24.95	23.64	14.2

Loss on ignition organic matter (%) calculated as per equation 2.

Appendix C: Berwick forest Soil Moisture Content

Soil	Mass of crucible (g)	Mass of moist soil + crucible (g)	Mass of oven dry soil + crucible (g)	Moisture content (%)
Berwick unfertilized	22.37	32.73	31.94	7.6
Berwick unfertilized	15.70	25.15	24.46	7.3
Berwick unfertilized	15.92	26.81	25.99	7.5
Berwick unfertilized	11.09	22.03	21.18	7.8
Berwick fertilized	19.41	29.64	28.89	7.3
Berwick fertilized	26.21	36.02	35.32	7.1
Berwick fertilized	16.16	26.22	25.49	7.3
Berwick fertilized	22.36	31.14	30.46	7.7

Moisture content calculated as per equation 1.

Appendix D: Berwick forest Soil Loss on Ignition Organic Matter Content

Soil	Mass of crucible (g)	Original mass	Mass remaining after furnace	Organic matter (%)
Berwick unfertilized	22.37	31.94	30.90	10.9
Berwick unfertilized	15.70	24.46	23.51	10.8
Berwick unfertilized	15.92	25.99	24.91	10.7
Berwick unfertilized	11.09	21.18	20.11	10.6
Berwick fertilized	19.41	28.89	27.76	10.9
Berwick fertilized	26.21	35.32	34.24	11.9
Berwick fertilized	16.16	25.49	24.39	11.8
Berwick fertilized	22.36	30.46	29.54	11.4

Loss on ignition organic matter (%) calculated as per equation 2.

Appendix E: Hanmer soil total carbon and nitrogen

Soil	Total Carbon (mg C per g soil)	Total Nitrogen (mg N per g soil)	C/N
Redwood	49.7	2.7	18.4
Redwood	54.5	3.1	17.6
Redwood	56.5	3.2	17.7
Redwood	58.4	3.1	18.8
Pine	49.9	4.1	12.2
Pine	46.1	2.4	19.2
Pine	54.3	4.3	12.6
Pine	55.8	4.38	12.7

Appendix F: Berwick soil total carbon and nitrogen

Soil	Total Carbon (mg per g soil)	Total Nitrogen (mg per g soil)	C/N
Berwick unfertilized	40.7	2.5	16.3
Berwick unfertilized	35.3	2.1	16.8
Berwick unfertilized	39.4	2.3	17.1
Berwick unfertilized	44.0	2.4	18.3
Berwick fertilized	40.8	2.4	17.0
Berwick fertilized	41.4	2.7	15.3
Berwick fertilized	41.3	2.6	15.9
Berwick fertilized	46.9	2.87	16.3

Appendix G: Holt forest Soil Moisture Content

Soil	Mass of crucible (g)	Mass of moist soil + crucible (g)	Mass of oven dry soil + crucible (g)	Moisture content (%)
Agathis australis	22.373	32.165	29.005	32.27
Agathis australis	19.413	28.502	25.135	37.04
Agathis australis	14.632	23.704	20.404	36.38
Agathis australis	10.174	17.003	14.561	35.76
Pinus strobus	11.964	20.016	17.664	29.21
Pinus strobus	15.929	25.936	22.405	35.29
Pinus strobus	9.604	15.608	13.654	32.54
Pinus strobus	11.211	16.150	14.554	32.31
Sequoia sempervirens	11.101	18.576	14.515	54.33
Sequoia sempervirens	19.119	28.121	23.212	54.53
Sequoia sempervirens	10.198	15.082	12.790	46.93
Sequoia sempervirens	10.742	16.964	14.518	39.31
Pinus radiata	11.693	19.847	18.267	19.38
Pinus radiata	16.164	28.132	26.346	14.92
Pinus radiata	11.155	20.472	17.640	63.66
Pinus radiata	11.984	20.336	18.823	18.12
Thuja occidentalis	26.217	34.958	30.869	46.78
Thuja occidentalis	11.518	19.808	16.952	34.45
Thuja occidentalis	9.560	16.067	13.663	36.94
Thuja occidentalis	17.359	26.782	23.334	36.60
Dacrydium cupressinum	9.386	14.926	12.753	39.22
Dacrydium cupressinum	10.352	14.981	12.929	44.33
Dacrydium cupressinum	9.247	14.830	12.750	37.26
Dacrydium cupressinum	8.837	14.414	12.545	33.51

Moisture content calculated as per equation 1.

Appendix H: Holt forest Soil Loss on Ignition Organic Matter Content

Soil	**Mass of crucible (g)**	**Mass of oven dry soil + crucible (g)**	**Mass remaining after furnace (g)**	**Organic matter (%)**
Agathis australis	22.373	29.005	27.991	15.29
Agathis australis	19.413	25.135	24.139	17.41
Agathis australis	14.632	20.404	18.965	24.93
Agathis australis	10.174	14.561	13.733	18.87
Pinus strobus	11.964	17.664	16.905	13.32
Pinus strobus	15.929	22.405	21.222	18.27
Pinus strobus	9.604	13.654	12.467	29.31
Pinus strobus	11.211	14.554	13.875	20.31
Sequoia sempervirens	11.101	14.515	13.461	30.87
Sequoia sempervirens	19.119	23.212	21.992	29.81
Sequoia sempervirens	10.198	12.790	12.256	20.60
Sequoia sempervirens	10.742	14.518	13.901	16.34
Pinus radiata	11.693	18.267	17.959	4.69
Pinus radiata	16.164	26.346	26.003	3.37
Pinus radiata	11.155	17.640	17.127	7.91
Pinus radiata	11.984	18.823	18.462	5.28
Thuja occidentalis	26.217	30.869	29.461	30.27
Thuja occidentalis	11.518	16.952	16.058	16.45
Thuja occidentalis	9.560	13.663	12.970	16.89
Thuja occidentalis	17.359	23.334	22.426	15.20
Dacrydium cupressinum	9.386	12.753	12.095	19.54
Dacrydium cupressinum	10.352	12.929	12.365	21.89
Dacrydium cupressinum	9.247	12.750	12.174	16.44
Dacrydium cupressinum	8.837	12.545	12.043	13.54

Loss on ignition organic matter (%) calculated as per equation 2.

Appendix I: Holt forest Soil Total Carbon and Nitrogen

Species	**Total C** (mg C per g soil)	**Total N** (mg N per g soil)	**C/N**
Agathis australis	225.8	7.8	28.95
Agathis australis	204.8	6.7	30.57
Agathis australis	69.6	3.8	18.32
Agathis australis	134.1	4.8	27.94
Dacrydium cupressinum	70.6	4.0	17.65
Dacrydium cupressinum	84.2	4.4	19.14
Dacrydium cupressinum	76.4	4.1	18.63
Dacrydium cupressinum	60.5	3.4	17.79
Pinus radiata	9.3	0.4	23.25
Pinus radiata	9.3	0.4	23.25
Pinus radiata	16.1	0.7	23.00
Pinus radiata	11.1	0.5	22.20
Pinus strobus	46.9	2.3	20.39
Pinus strobus	83.1	4.5	18.47
Pinus strobus	67.3	3.4	19.79
Pinus strobus	60.3	3.7	16.30
Thuja occidentalis	97.6	4.9	19.92
Thuja occidentalis	58.4	3.5	16.69
Thuja occidentalis	58.9	3.4	17.32
Thuja occidentalis	48.6	2.8	17.36
Sequoia sempervirens	88.2	4.2	21.00
Sequoia sempervirens	107.3	4.3	24.95
Sequoia sempervirens	65.6	3.0	21.89
Sequoia sempervirens	62.5	2.9	21.55

Appendix J: Holt forest Microbial Biomass Carbon and Nitrogen

Species	**Microbial Biomass Carbon** (μg C per g soil)	**Microbial Biomass Nitrogen** (μg N per g soil)	**MBC:MBN**
Agathis australis	1803.63	133.47	13.51
Agathis australis	2915.67	205.49	14.19
Agathis australis	2905.72	149.27	19.47
Agathis australis	2270.09	116.80	19.44
Dacrydium cupressinum	1863.33	169.54	10.99
Dacrydium cupressinum	2472.33	177.44	13.93
Dacrydium cupressinum	2452.29	134.21	18.27
Dacrydium cupressinum	1968.60	101.63	19.37
Pinus radiata	959.20	41.11	23.33
Pinus radiata	401.47	22.00	18.25
Pinus radiata	1357.06	28.97	46.85
Pinus radiata	1221.23	54.69	22.33
Pinus strobus	1498.25	152.79	9.81
Pinus strobus	1996.84	191.77	10.41
Pinus strobus	2162.63	126.69	17.07
Pinus strobus	2556.06	197.46	12.94
Thuja occidentalis	2078.14	218.72	9.50
Thuja occidentalis	1777.32	183.75	9.67
Thuja occidentalis	2622.11	197.79	13.26
Thuja occidentalis	2172.14	132.48	16.40
Sequoia sempervirens	1760.50	190.41	9.25
Sequoia sempervirens	2224.67	261.06	8.52
Sequoia sempervirens	2056.69	105.78	19.44
Sequoia sempervirens	1845.13	105.66	17.46

Microbial biomass carbon and nitrogen calculated as per equation 3 and 6 respectively.

Appendix K: Pot Trial One Plant Biomass

Plant	Total weight (g)	Root weight (g)	Shoot weight (g)	Root:Shoot ratio	Ash Content (%)
E-1	3.63	0.68	2.95	4.34	6.81
E-2	3.36	0.44	2.92	6.64	11.16
E-3	3.08	0.39	2.69	6.90	5.15
E-4	4.19	0.73	3.46	4.74	6.16
E-5	1.36	0.30	1.06	3.53	6.03
E-6	3.20	0.80	2.40	3.00	9.35
E-7	3.79	0.84	2.95	3.51	3.54
E-8	4.87	1.10	3.77	3.43	6.22
E-9	2.75	0.63	2.12	3.36	8.75
E-10	3.22	0.57	2.65	4.65	10.34
E-11	4.35	1.07	3.28	3.07	6.49
E-12	5.54	1.19	4.35	3.66	7.40
E-13	3.53	0.66	2.87	4.35	6.72
E+1	3.85	0.97	2.88	2.97	7.12
E+2	5.45	1.31	4.23	3.23	6.05
E+3	4.25	1.02	3.23	3.17	7.74
E+4	4.41	0.66	3.75	5.68	8.69
E+5	2.96	0.42	2.54	6.05	5.76
E+6	3.61	0.91	2.70	2.97	3.72
E+7	5.33	1.12	4.21	3.76	8.36
E+8	4.33	0.76	3.57	4.70	3.33
E+9	4.06	0.89	3.17	3.56	9.55
E+10	3.47	0.89	2.58	2.90	7.62
E+11	2.31	0.55	1.76	3.20	8.42
E+12	3.54	0.86	2.68	3.12	10.50
E+13	5.63	1.48	4.15	2.80	7.76
E+14	3.84	1.04	2.80	2.69	3.41
S-1	2.61	1.57	1.04	0.66	8.89
S-2	2.67	1.77	0.90	0.51	4.31
S-3	2.70	1.19	1.51	1.27	10.98
S-4	3.63	1.65	1.98	1.20	4.80
S-5	3.29	2.03	1.26	0.62	8.38
S-6	2.85	1.45	1.40	0.97	3.15
S-7	3.23	1.25	1.98	1.58	5.73
S-8	2.78	1.70	1.08	0.64	5.25
S-9	3.04	1.44	1.60	1.11	3.01
S-10	3.35	1.50	1.85	1.23	11.56
S-11	3.49	1.54	1.95	1.27	10.59
S+1	3.81	2.08	1.73	0.83	8.10
S+2	3.99	1.83	2.16	1.18	6.50
S+3	5.45	2.75	2.70	0.98	7.21
S+4	3.76	1.59	2.17	1.36	3.97
S+5	3.71	1.52	2.19	1.44	4.92
S+6	2.77	1.07	1.70	1.59	8.24
S+7	3.57	1.77	1.80	1.02	9.54
S+8	4.74	2.06	2.68	1.30	3.83
S+9	5.82	2.90	2.92	1.01	3.60
S+10	6.10	3.25	2.85	0.88	4.87
P-1	2.89	0.95	1.94	2.04	12.83
P-2	1.80	0.81	0.99	1.22	10.39

P-3	1.68	0.51	1.17	2.29	3.33
P-4	3.86	1.15	2.71	2.36	3.52
P-5	5.40	1.33	4.07	3.06	4.84
P-6	2.85	1.24	1.61	1.30	6.72
P-7	7.22	1.57	5.65	3.60	1.26
P-8	3.96	1.20	2.76	2.30	11.10
P-9	4.09	0.97	3.12	3.22	9.80
P-10	3.84	0.91	2.93	3.22	11.34
P-11	7.44	2.52	4.92	1.95	4.57
P-12	5.33	1.44	3.89	2.70	7.69
P-13	5.31	1.21	4.10	3.39	4.25
P+1	3.24	1.36	1.88	1.38	8.79
P+2	4.70	1.52	3.18	2.09	4.25
P+3	3.37	1.53	1.84	1.20	15.45
P+4	3.49	1.72	1.77	1.03	2.17
P+5	3.71	1.88	1.83	0.97	8.98
P+6	4.11	1.79	2.32	1.30	2.12
P+7	5.36	2.58	2.78	1.08	11.42
P+8	3.86	0.84	3.02	3.60	5.22
P+9	4.13	1.81	2.32	1.28	5.57
P+11	4.36	1.77	2.69	1.52	3.43
P+12	3.43	1.79	1.64	0.92	10.09

Ash content in biomass (%) calculated as per equation 10. Shoot:root ratio calculated as per equation 11

Key: + = fertilized, - = unfertilized, P = *Pinus radiata,* E = *Eucalyptus nitens*, S = *Sequoia sempervirens*

Appendix L: Pot Trial Two Plant Biomass

Plant	Total weight (g)	Root weight (g)	Shoot weight (g)	Root:Shoot ratio	Ash Content (%)
S-1	1.86	0.9	0.96	1.07	3.28
S-2	2.25	1.26	0.99	0.79	13.50
S-3	2.42	1.51	0.91	0.60	10.31
S-4	2.63	1.18	1.45	1.23	2.90
S-5	2.96	1.54	1.42	0.92	3.15
S-6	2.46	1.27	1.19	0.94	6.99
S-7	2.23	1.32	0.91	0.69	9.22
S-8	1.93	1.07	0.86	0.80	4.96
S-9	2.72	1.20	1.52	1.27	6.39
S-10	3.21	1.62	1.59	0.98	3.87
S-11	2.49	1.43	1.06	0.74	6.41
S+1	2.94	1.07	1.87	1.75	3.97
S+2	6.81	2.65	4.16	1.57	5.12
S+3	6.77	2.80	3.97	1.42	5.19
S+4	5.29	1.80	3.49	1.94	3.87
S+5	7.65	2.67	4.98	1.87	5.18
S+6	4.28	2.09	2.19	1.05	8.65
P-1	4.09	1.19	2.90	2.44	7.30
P-2	4.56	1.08	3.48	3.22	8.50
P-3	3.77	1.97	1.80	0.91	5.05
P-4	3.09	1.12	1.97	1.76	7.93
P-5	3.78	1.05	2.73	2.60	3.61
P-6	3.70	1.28	2.42	1.89	4.03
P-7	3.29	1.43	1.86	1.30	8.43
P-8	4.81	1.62	3.19	1.97	8.46
P-9	4.30	1.20	3.10	2.58	6.59
P-10	5.87	1.43	4.44	3.10	3.02
P-11	3.90	1.48	2.42	1.64	4.44
P-12	4.00	1.58	2.42	1.53	2.87
P-13	5.29	1.29	4.00	3.10	7.20
P-14	4.83	1.73	3.10	1.79	7.95
P+1	9.91	1.45	8.46	5.83	8.94
P+2	6.24	2.08	4.16	2.00	7.31
P+3	7.84	2.12	5.72	2.70	8.92
P+4	7.32	2.11	5.21	2.47	8.81
P+5	8.45	1.75	6.70	3.83	3.41
P+6	6.05	2.64	3.41	1.29	7.57
P+7	6.10	3.18	2.92	0.92	3.33
P+8	7.35	2.48	4.87	1.96	4.82
P+9	7.65	1.75	5.90	3.37	3.43
P+10	8.78	3.23	5.55	1.72	6.47
P+11	5.96	1.36	4.60	3.38	4.36
P+12	5.04	1.67	3.37	2.02	6.23

Ash content in biomass (%) calculated as per equation 10. Shoot:root ratio calculated as per equation 11.

Key: + = fertilized, - = unfertilized, P = *Pinus radiata,* S = *Sequoia sempervirens*

Appendix M: Pot Trial One Soil Moisture Content

Soil	Mass of crucible (g)	Mass of moist soil + crucible (g)	Mass of oven dry soil + crucible (g)	Moisture content (%)
C-1	13.63	18.68	16.78	37.62
C-2	13.53	18.67	17.66	19.65
C-3	13.43	18.63	16.77	35.77
C-4	13.46	18.42	17.01	28.43
C-5	13.67	18.57	16.76	36.94
C-6	13.50	18.69	16.63	39.69
C-7	13.63	18.66	16.48	43.34
C-8	13.59	18.59	16.63	39.20
C-9	13.48	18.65	16.87	34.43
C-10	13.56	18.58	17.19	27.69
C+1	13.67	18.54	16.69	37.99
C+2	13.58	18.49	16.59	38.70
C+3	13.56	18.48	16.61	38.01
C+4	13.48	18.34	16.73	33.13
C+5	13.56	18.49	16.94	31.44
C+6	13.69	18.68	17.12	31.26
C+7	13.66	18.61	16.64	39.78
C+8	13.70	18.66	16.91	35.28
C+9	13.42	18.46	16.76	33.73
C+10	13.66	18.56	17.07	30.41
E-1	13.50	18.53	16.97	31.01
E-2	13.49	18.42	17.07	27.38
E-3	13.49	18.48	16.75	34.67
E-4	13.58	18.51	16.75	35.70
E-5	13.65	18.58	16.88	34.48
E-6	13.55	18.5	16.67	36.97
E-7	13.65	18.52	16.76	36.14
E-8	13.48	18.63	16.5	41.36
E-9	13.51	18.46	16.74	34.75
E-10	13.49	18.4	16.91	30.35
E-11	13.42	18.65	17.05	30.59
E-12	13.48	18.69	17.22	28.21
E-13	13.44	18.5	16.7	35.57
E+1	13.58	18.51	16.88	33.06
E+2	13.63	18.59	16.88	34.48
E+3	13.54	18.57	17.24	26.44
E+4	13.48	18.34	16.93	29.012
E+5	13.47	18.55	16.94	31.69
E+6	13.56	18.32	16.52	37.82
E+7	13.46	18.41	16.74	33.74
E+8	13.54	18.52	16.94	31.73
E+9	13.48	18.5	16.97	30.48
E+10	13.61	18.68	16.82	36.69
E+11	13.49	18.58	16.79	35.17
E+12	13.64	18.61	16.91	34.21
E+13	13.50	18.55	17.1	28.71
E+14	13.46	18.49	17.04	28.83
S-1	13.63	18.62	16.93	33.87
S-2	13.54	18.53	16.67	37.27

S-3	13.47	18.48	16.78	33.93
S-4	13.43	18.49	16.52	38.93
S-5	13.41	18.59	16.77	35.14
S-6	13.63	18.69	16.9	35.38
S-7	13.67	18.48	16.32	44.91
S-8	13.59	18.67	16.5	42.72
S-9	13.58	18.63	16.89	34.46
S-10	13.58	18.4	16.44	40.66
S-11	13.49	18.44	16.59	37.37
S+1	13.62	18.7	16.89	35.63
S+2	13.64	18.66	16.85	36.06
S+3	13.64	18.63	16.97	33.27
S+4	13.47	18.54	16.8	34.32
S+5	13.62	18.64	17.13	30.08
S+6	13.49	18.5	16.74	35.13
S+7	13.61	18.67	17.08	31.42
S+8	13.54	18.52	16.7	36.55
S+9	13.51	18.48	16.55	38.83
S+10	13.55	18.59	16.66	38.29
P-1	13.75	18.69	17.1	32.18
P-2	13.63	18.38	16.69	35.58
P-3	13.41	18.62	17.04	30.33
P-4	13.60	18.58	16.71	37.55
P-5	13.47	18.5	16.47	40.36
P-6	13.55	18.52	16.57	39.24
P-7	13.48	18.63	16.7	37.48
P-8	13.58	18.46	16.82	33.61
P-9	13.56	18.4	16.57	37.81
P-10	13.53	18.59	17.2	27.47
P-11	13.44	18.59	16.84	33.98
P-12	13.45	18.48	16.62	36.98
P-13	13.42	18.5	16.75	34.45
P+1	13.47	18.59	16.82	34.57
P+2	13.61	18.65	16.97	33.33
P+3	13.55	18.51	16.59	38.71
P+4	13.58	18.6	16.86	34.66
P+5	13.47	18.59	16.67	37.50
P+6	13.47	18.57	16.76	35.49
P+7	13.46	18.44	16.58	37.35
P+8	13.54	18.56	16.67	37.65
P+9	13.7	18.57	16.68	38.81
P+11	13.43	18.54	16.73	35.42
P+12	13.48	18.46	16.6	37.35

Moisture content calculated as per equation 1.

Key: + = fertilized, - = unfertilized, P = *Pinus radiata,* E = *Eucalyptus nitens*, S = *Sequoia sempervirens*

Appendix N: Pot Trial Two Soil Moisture Content

Soil	Mass of crucible (g)	Mass of moist soil + crucible (g)	Mass of oven dry soil + crucible (g)	Moisture content (%)
PC-1	13.52	18.47	16.89	31.92
PC-2	13.54	18.46	17.07	28.25
PC-3	13.50	18.35	16.81	31.75
PC-4	13.48	18.56	16.74	35.83
PC-5	13.49	18.66	17.33	25.73
PC-6	13.48	18.45	16.9	31.19
PC-7	13.69	18.64	16.82	36.77
PC-8	13.43	18.59	16.74	35.85
PC-9	13.68	18.7	17.21	29.68
PC-10	13.52	18.61	16.96	32.42
PC+1	13.57	18.66	16.82	36.15
PC+2	13.46	18.52	16.77	34.58
PC+3	13.63	18.34	16.78	33.12
PC+4	13.45	18.53	16.9	32.09
PC+5	13.49	18.33	16.58	36.16
PC+6	13.43	18.45	17.02	28.49
PC+7	13.64	18.7	16.66	40.32
PC+8	13.41	18.48	16.54	38.26
PC+9	13.56	18.52	16.68	37.10
PC+10	13.57	18.69	16.5	42.77
SC-1	13.6	18.44	16.39	42.36
SC-2	13.45	18.52	16.44	41.03
SC-3	13.6	18.68	16.68	39.37
SC-4	13.53	18.34	16.21	44.28
SC-5	13.6	18.53	16.67	37.73
SC-5	13.52	18.49	17.13	27.36
SC-6	13.64	18.6	16.82	35.89
SC-7	13.48	18.5	16.92	31.47
SC-8	13.42	18.42	16.93	29.80
SC-9	13.48	18.52	16.78	34.52
SC-10	13.6	18.44	16.39	42.36
SC+1	13.66	18.28	16.55	37.45
SC+2	13.57	18.42	16.5	39.59
SC+3	13.43	18.33	16.36	40.20
SC+4	13.54	18.6	16.65	38.54
SC+5	13.77	18.72	16.97	35.35
SC+6	13.46	18.42	16.73	34.07
SC+7	13.63	18.69	16.95	34.39
SC+8	13.56	18.59	16.84	34.79
SC+9	13.45	18.44	17.04	28.06
SC+10	13.62	18.7	16.89	35.63
S-1	13.53	18.58	16.58	39.60
S-2	13.65	18.57	16.75	36.99
S-3	13.55	18.61	17.17	28.46
S-4	13.40	18.4	16.72	33.60
S-5	13.42	18.43	17.31	22.36
S-6	13.48	18.55	16.78	34.91
S-7	13.55	18.56	16.83	34.53
S-8	13.46	18.5	16.88	32.14

S-9	13.48	18.54	16.79	34.58
S-10	13.52	18.53	16.84	33.73
S-11	13.70	18.64	16.99	33.40
S+1	13.54	18.67	16.96	33.33
S+2	13.57	18.56	16.93	32.67
S+3	13.44	18.69	16.76	36.76
S+4	13.53	18.42	16.82	32.72
S+5	13.6	18.4	16.74	34.58
S+6	13.49	18.56	16.77	35.31
P-1	13.57	18.39	16.95	29.88
P-2	13.5	18.35	17.04	27.01
P-3	13.76	18.71	17.14	31.72
P-4	13.59	18.66	16.83	36.09
P-5	13.51	18.53	16.5	40.44
P-6	13.56	18.54	16.66	37.75
P-7	13.65	18.7	16.72	39.21
P-8	13.5	18.55	16.78	35.05
P-9	13.45	18.49	17.05	28.57
P-10	13.62	18.66	17.23	28.37
P-11	13.53	18.61	16.8	35.63
P-12	13.5	18.6	16.99	31.57
P-13	13.52	18.59	16.91	33.14
P-14	13.57	18.62	16.74	37.23
P+1	13.6	18.67	16.93	34.32
P+2	13.42	18.44	16.87	31.27
P+3	13.64	18.68	17.22	28.97
P+4	13.7	18.7	16.99	34.20
P+5	13.59	18.61	16.87	34.66
P+6	13.63	18.6	16.69	38.43
P+7	13.51	18.54	16.72	36.18
P+8	13.47	18.5	16.76	34.59
P+9	13.41	18.51	16.8	33.53
P+10	13.53	18.59	16.79	35.57
P+11	13.43	18.44	16.74	33.93
P+12	13.65	18.68	16.98	33.80

Moisture content calculated as per equation 1.

Key: + = fertilized, - = unfertilized, P = *Pinus radiata,* S = *Sequoia sempervirens*

Appendix O: Pot Trial One Total Carbon and Nitrogen

Soil	Total Nitrogen (mg N per g soil)	Total Carbon (mg C per g soil)	C/N
C-1	2.0	36.5	18.25
C-2	2.3	39.3	17.09
C-3	2.1	41.6	19.81
C-4	2.3	40.1	17.43
C-5	2.3	39.8	17.30
C-6	2.2	41.4	18.82
C-7	2.1	36.7	17.48
C-8	2.2	40.2	18.27
C-9	2.2	40.3	18.32
C-10	2.3	38.4	16.70
C+1	2.4	41.9	17.46
C+2	2.6	43.8	16.85
C+3	2.7	44	16.30
C+4	2.3	38.2	16.61
C+5	2.5	39.8	15.92
C+6	2.7	44.1	16.33
C+7	2.5	39.7	15.88
C+8	2.8	42.7	15.25
C+9	3.0	47.6	15.87
C+10	1.9	45.4	23.89
E-1	2.5	48.9	19.56
E-2	1.9	47.3	24.89
E-3	2.2	42.3	19.23
E-4	1.6	43.6	27.25
E-5	2.2	45.1	20.50
E-6	2.5	49	19.60
E-7	2.0	47.3	23.65
E-8	2.2	45.1	20.50
E-9	1.8	45.3	25.17
E-10	1.6	46.2	28.88
E-11	2.3	43.9	19.09
E-12	2.1	46.3	22.05
E-13	2.7	48.7	18.04
E+1	2.5	42.7	17.08
E+2	2.6	46.4	17.85
E+3	2.3	42	18.26
E+4	2.3	43.7	19.00
E+5	2.2	42.9	19.50
E+6	2.6	47.7	18.35
E+7	2.7	48.3	17.89
E+8	2.1	46.6	22.19
E+9	2.4	44.1	18.38
E+10	2.5	47.4	18.96
E+11	2.5	49.0	19.60
E+12	2.5	46.8	18.72
E+13	2.4	45.6	19.00
E+14	2.0	44.1	22.05
S-1	2.3	45.6	19.83
S-2	2.0	38.9	19.45

S-3	1.9	39.7	20.89
S-4	2.3	41.8	18.17
S-5	1.8	43.9	24.39
S-6	2.0	44	22.00
S-7	2.1	43.3	20.62
S-8	2.1	44.2	21.05
S-9	1.6	43.5	27.19
S-10	2.5	43.5	17.40
S-11	2.0	46.8	23.40
S+1	2.2	43.8	19.91
S+2	2.6	44.8	17.23
S+3	2.4	45.4	18.92
S+4	2.4	46.7	19.46
S+5	2.5	44	17.60
S+6	2.5	44.1	17.64
S+7	2.6	43.9	16.88
S+8	2.3	43.8	19.04
S+9	2.6	45.1	17.35
S+10	1.6	45.3	28.31
P-1	1.3	46.6	35.85
P-2	1.4	38.7	27.64
P-3	1.5	40	26.67
P-4	1.4	38.6	27.57
P-5	1.8	39.9	22.17
P-6	1.6	39.2	24.50
P-7	0.9	44	48.89
P-8	1.8	43.3	24.06
P-9	1.4	42.9	30.64
P-10	1.4	39.1	27.92
P-11	1.6	41.5	25.94
P-12	1.9	41	21.58
P-13	2.2	41.8	19.00
P+1	2.4	46.3	19.29
P+2	2.3	41.7	18.13
P+3	2.5	47.2	18.88
P+4	2.5	42.2	16.88
P+5	2.6	45.1	17.35
P+6	2.5	46.2	18.48
P+7	2.4	47.2	19.67
P+8	2.4	42.7	17.79
P+9	2.5	48	19.20
P+11	2.4	47.1	19.63
P+12	2.0	36.5	18.25

Key: + = fertilized, - = unfertilized, P = *Pinus radiata,* E = *Eucalyptus nitens*, S = *Sequoia sempervirens*

Appendix P: Pot Trial Two Total Carbon and Nitrogen

Soil	**Total Nitrogen** (mg N per g soil)	**Total Carbon** (mg C per g soil)	**C/N**
PC-1	3.5	52.7	15.06
PC-2	3.3	51.8	15.70
PC-3	3.6	52.3	14.53
PC-4	3.5	50.4	14.40
PC-5	3.8	57.9	15.24
PC-6	3.6	56.6	15.72
PC-7	3.6	56.3	15.64
PC-8	3.5	56.2	16.06
PC-9	3.8	57.6	15.16
PC-10	3.3	51.1	15.49
PC+1	3.9	55.2	14.15
PC+2	3.8	55.9	14.71
PC+3	4.2	59.7	14.21
PC+4	3.5	53.4	15.26
PC+5	4.0	54.4	13.60
PC+6	3.8	54.0	14.21
PC+7	3.7	53.5	14.46
PC+8	4.0	58.9	14.73
PC+9	3.8	58.4	15.37
PC+10	3.5	53.4	15.26
SC-1	2.9	51.0	17.59
SC-2	3.0	52.9	17.63
SC-3	2.8	53.5	19.11
SC-4	2.9	58.7	20.24
SC-5	3.0	54.8	18.27
SC-6	2.9	50.3	17.34
SC-7	2.8	50.9	18.18
SC-8	2.8	56.0	20.00
SC-9	3.0	50.5	16.83
SC-10	3.3	56.6	17.15
SC+1	2.8	44.9	16.04
SC+2	3.0	54.4	18.13
SC+3	3.3	56.3	17.06
SC+4	3.5	55.8	15.94
SC+5	3.1	53.1	17.13
SC+6	3.2	56.2	17.56
SC+7	2.9	53.0	18.28
SC+8	2.8	50.3	17.96
SC+9	3.1	52.8	17.03
SC+10	3.6	55.2	15.33
S-1	2.8	50.5	18.04
S-2	3.2	56.8	17.75
S-3	3.0	51.1	17.03
S-4	2.3	52.2	22.70
S-5	2.8	56.3	20.11
S-6	2.7	48.1	17.81
S-7	2.3	50.6	22.00
S-8	2.4	52.3	21.79

S-9	2.7	51.7	19.15
S-10	2.6	49.9	19.19
S-11	2.3	51.0	22.17
S+1	2.9	53.3	18.38
S+2	2.9	56.7	19.55
S+3	2.8	53.1	18.96
S+4	2.8	52.6	18.79
S+5	2.5	48.8	19.52
S+6	3.0	57.6	19.20
P-1	3.4	53.6	15.76
P-2	3.5	57.4	16.40
P-3	3.7	61.2	16.54
P-4	3.7	61.8	16.70
P-5	3.7	56.1	15.16
P-6	3.6	60.6	16.83
P-7	3.9	54.0	13.85
P-8	3.5	56.3	16.09
P-9	3.1	47.3	15.26
P-10	3.1	45.7	14.74
P-11	3.6	56.3	15.64
P-12	3.5	58.1	16.60
P-13	3.1	49.0	15.81
P-14	3.2	51.7	16.16
P+1	3.8	60.7	15.97
P+2	3.9	59.4	15.23
P+3	3.9	61.3	15.72
P+4	3.2	52.1	16.28
P+5	3.7	58.4	15.78
P+6	3.4	53.2	15.65
P+7	3.8	61.6	16.21
P+8	3.5	57.6	16.46
P+9	3.2	52.8	16.50
P+10	3.2	52.4	16.38
P+11	4.0	64.0	16.00
P+12	3.7	57.9	15.65

Key: + = fertilized, - = unfertilized, P = *Pinus radiata,* S = *Sequoia sempervirens*

Appendix Q: Pot Trial One Microbial Biomass Carbon

Soil	**Microbial Biomass Carbon** (mg C per g soil)
C-1	2.63
C-2	2.48
C-3	2.43
C-4	2.55
C-5	2.42
C-6	2.87
C-7	2.74
C-8	2.51
C-9	2.50
C-10	2.65
C+1	1.75
C+2	2.44
C+3	2.71
C+4	2.46
C+5	2.23
C+6	2.21
C+7	2.38
C+8	2.46
C+9	2.29
C+10	2.22
E-1	2.66
E-2	2.86
E-3	1.89
E-4	3.15
E-5	2.26
E-6	2.49
E-7	3.01
E-8	2.65
E-9	2.38
E-10	2.56
E-11	2.44
E-12	2.05
E-13	2.89
E+1	3.03
E+2	3.64
E+3	3.24
E+4	1.98
E+5	3.12
E+6	3.46
E+7	3.31
E+8	2.46
E+9	3.25
E+10	3.28
E+11	3.66
E+12	3.02
E+13	3.04
E+14	2.67
S-1	2.65
S-2	2.75

S-3	3.20
S-4	2.23
S-5	2.34
S-6	2.22
S-7	3.08
S-8	2.74
S-9	2.63
S-10	2.60
S-11	2.49
S+1	2.26
S+2	3.14
S+3	4.18
S+4	2.44
S+5	4.01
S+6	2.55
S+7	2.27
S+8	3.11
S+9	2.74
S+10	3.30
P-1	2.58
P-2	1.92
P-3	2.32
P-4	1.96
P-5	1.99
P-6	2.03
P-7	2.39
P-8	2.74
P-9	2.55
P-10	2.34
P-11	1.92
P-12	2.59
P-13	2.27
P+1	3.17
P+2	3.04
P+3	2.76
P+4	3.11
P+5	3.67
P+6	2.74
P+7	2.77
P+8	3.12
P+9	3.15
P+11	2.77
P+12	3.06

Key: + = fertilized, - = unfertilized, P = *Pinus radiata,* E = *Eucalyptus nitens*, S = *Sequoia sempervirens*

Appendix R: Pot Trial Two Microbial Biomass Carbon

Soil	**Microbial Biomass Carbon** (mg C per g soil)
PC-1	3.01
PC-2	2.69
PC-3	2.95
PC-4	2.69
PC-5	2.97
PC-6	2.71
PC-7	3.00
PC-8	2.88
PC-9	2.99
PC-10	2.91
PC+1	2.54
PC+2	3.09
PC+3	2.69
PC+4	3.01
PC+5	2.94
PC+6	2.80
PC+7	2.78
PC+8	2.75
PC+9	2.73
PC+10	2.71
SC-1	3.74
SC-2	3.25
SC-3	4.61
SC-4	2.58
SC-5	3.21
SC-6	2.65
SC-7	2.78
SC-8	2.76
SC-9	2.96
SC-10	2.91
SC+1	2.39
SC+2	2.42
SC+3	3.41
SC+4	3.67
SC+5	2.44
SC+6	3.37
SC+7	2.72
SC+8	2.70
SC+9	3.57
SC+10	2.93
S-1	2.55
S-2	3.17
S-3	3.75
S-4	3.49
S-5	2.90
S-6	2.59
S-7	3.74
S-8	3.66
S-9	2.72

S-10	2.97
S-11	3.14
S+1	3.67
S+2	5.22
S+3	3.41
S+4	3.71
S+5	3.74
S+6	5.12
P-1	3.06
P-2	3.45
P-3	4.97
P-4	3.42
P-5	3.80
P-6	3.26
P-7	2.78
P-8	2.72
P-9	4.74
P-10	3.85
P-11	3.37
P-12	3.63
P-13	3.13
P-14	3.21
P+1	3.40
P+2	5.65
P+3	3.50
P+4	4.32
P+5	3.77
P+6	4.39
P+7	3.69
P+8	5.52
P+9	4.29
P+10	3.52
P+11	4.13
P+12	3.35

Key: + = fertilized, - = unfertilized, P = *Pinus radiata,* S = *Sequoia sempervirens*