Role of Mycorrhizae in Rhizosphere Processes and Phosphorus Dynamics

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The increased soil organic phosphorus (P) mineralization observed under trees compared to pasture has been attributed to the contrasting mycorrhizal associations of the two systems but to date, little work has been conducted comparing P dynamics under different tree species with contrasting mycorrhizal associations. This study investigated rhizosphere P dynamics and P acquisition of three tree species with contrasting mycorrhizal associations (ectomycorrhizal *Pinus radiata*, arbuscular mycorrhizal *Cupressus macrocarpa* and tripartite *Ecualyptus nitens*) using a combination of field and controlled environment studies. Short-term field studies revealed greater acid phosphomonoesterase activity and therefore greater potential organic P mineralization under radiata pine and eucalypt compared with macrocarpa, which correlated with ectomycorrhizal colonization. Related field work revealed that the presence of pasture understory and litter had a significant impact on P dynamics. Pasture acts to increase phosphatase activity and microbial activity within the soil but with a litter layer, the increased activities are mainly above the soil in the overlying litter. A glasshouse study investigated the separate influence of mycorrhizal hyphae and roots and results showed that ectomycorrhizal fungi associated with radiata pine and eucalypt stimulated microbial activity and increased rhizosphere phosphatase activity with a concomitant decline in soil organic P. Macrocarpa and eucalypt roots exuded higher concentrations of low molecular weight organic anions compared with radiata pine which stimulated microbial activity, increased rhizosphere phosphatase activity and decreased soil organic P. A series of experiments were then conducted to further investigate the precise role of mycorrhizae in P dynamics and results showed that ectomycorrhizal colonization increased rhizosphere acid phosphomonoesterase to a greater extent than arbuscular mycorrhizae. Fluorescent staining revealed that the mycorrhizae associated with all three species had similar phosphatase production capacities. The ability of the three species to utilize organic P was investigated in two exhaustive pot experiments. Radiata pine was shown to be the best adapted to utilize organic P, especially in soils with high relative organic P contents. Eucalypt appeared to enhance soil organic P mineralization but did not take up the released inorganic P, while macrocarpa caused limited organic P mineralization compared with radiata pine and eucalypt. This work shows that ectomycorrhizal trees are best adapted to utilize organic P through increased rhizosphere phosphatase activity and stimulated microbial activity. Root exudation of LMWOAs is of higher relative importance to arbuscular mycorrhizal trees to increase microbial activity and consequently phosphatase activities when fewer hyphae are in the rhizosphere are present to produce them.

Keywords: soil, organic phosphorus, phosphatase enzyme activity, mycorrhizae, *Pinus radiata*, *Eucalyptus nitens*, *Cupressus macrocarpa*.

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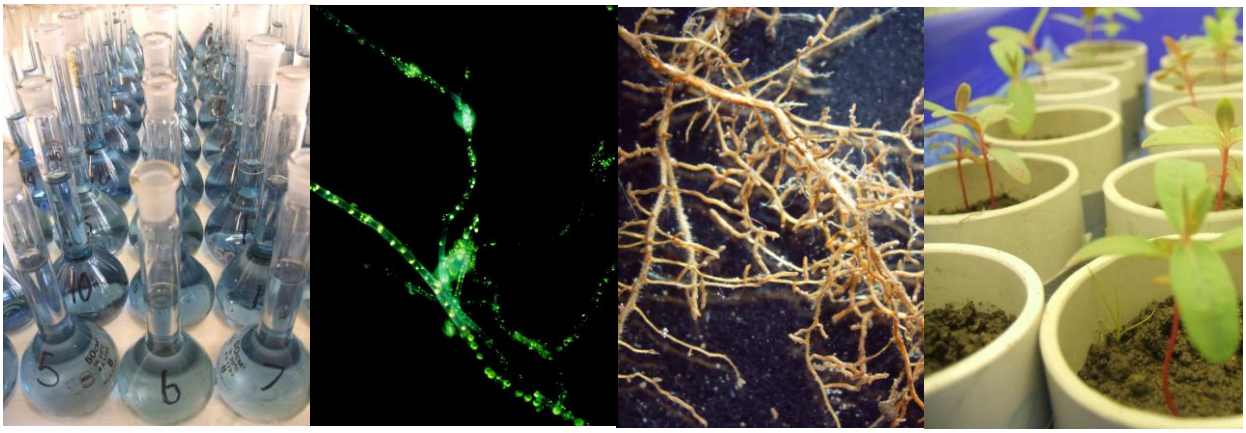

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Chapter 1

Introduction

This chapter firstly details the context of the study with respect to soil phosphorus (P) cycling, the contrast between pasture and forest P cycles and the importance of the rhizosphere and its components on P dynamics. Secondly the aims, objectives and thesis structure are described.

Phosphorus is often the most important element limiting productivity in the world due to its immobility and inaccessibility and so knowledge of its forms and dynamics in the soil is essential to understand the productivity and sustainability of ecosystems. Afforestation with exotic radiata pine in New Zealand has been shown to significantly change P dynamics, decreasing organic P concentrations and increasing inorganic P concentrations. This phenomenon was attributed to the contrasting mycorrhizal associations of pastures and pine forest but the precise mechanisms behind this P change and the effects of other forest species on P dynamics still require investigation. A long term, multispecies afforestation trial in New Zealand provided an opportunity to assess impacts on P dynamics by different tree species with contrasting mycorrhizal associations in the same environment. Field studies from this trial, in conjunction with controlled environment studies, will begin to elucidate and compare the role of different mycorrhizal fungi types in forest P dynamics.

1.1 Background

1.1.1 Soil phosphorus

Phosphorus is one of the 17 essential nutrients required for plant growth and development (Holford, 1997; Vance et al., 2003). Biomolecules such DNA, RNA, ATP and phospholipids all contain P (Bünneman and Condron, 2006) therefore it is involved in a number of essential biological processes such as nucleic acid synthesis, membrane

synthesis, energy generation, photosynthesis, nitrogen fixation and respiration (Vance et al., 2003; Raghothama, 2005). Soil contains most of the P present in terrestrial ecosystems. Total soil P estimates range between 100 and 3000 mg kg^{-1} (Frossard et al., 1995, 2000; Condron and Tiessen, 2005) but despite this, P is often the most important element after nitrogen limiting agricultural and crop production (Holford, 1997; Radersma and Grierson, 2004). Furthermore, primary production is predominantly limited by P deficiency in many forest ecosystems (Lajtha and Harrison, 1995; Chen et al., 2008). This is because P is chemically and biologically reactive resulting in varied inorganic and organic forms bound to soil particles by adsorption or present in mineral forms and precipitated complexes (Hinsinger, 2001) which tend to increase in insolubility over time (Holford, 1997). Generally less than 1% of total soil P is immediately available to plants (Richardson et al., 2005). Plants can only transport P as inorganic P ions from solution and the low solubility of P compounds means the solid phase is favored, making solution P concentration very low (Holford, 1997; Morgan et al., 2005). Plants therefore have to actively transport P across cell membranes from the low solution concentration to the high cell membrane concentration (Schachtman et al., 1998).

1.1.2 Phosphorus cycling

Care must be taken when using the word 'availability' with regards to P as it is a functional concept, not a measureable quantity and is sustained by P cycling. Plant available P is that in solution (orthophosphate) and that which replenishes the solution after uptake and so is time dependant. Because the proportions of organic and mineral P are equal in many soils, unlike nitrogen (N) and sulphur (S) that are predominantly organic forms, P cycling is controlled by a combination of chemical and biological processes (Condron and Newman, 2009). Figure 1.1 is a conceptual diagram of P cycling in soils. Plants take up inorganic P, primarily in the form of orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}) from the soil solution (Magid et al., 1996; Condron and Tiessen, 2005). The concentration of this plant available form in the soil solution at any one time is <2-10µM (Morgan et al., 2005) and must be continually replenished for continued P supply to plants (Condron et al., 2005; Condron and Tiessen, 2005). Replenishment of soil solution phosphate is driven by the decline in solution concentration from root uptake or sorption (Holford, 1997) and involves chemical processes such as desorption

of inorganic P on soil surfaces and dissolution of inorganic P bearing minerals such as calcium, iron or aluminum phosphates from the mineral phase P as well as biochemical processes such as hydrolysis of organic P (Vance et al., 2003; Condron et al., 2005).

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Dissolution/precipitation and sorption/desorption are the chemical processes of the P cycle. The least soluble P mineral controls dissolution and so solution P concentration. The solution concentration controls sorption/desorption rates and amount of sorbed P determines precipitation so a continuum exists between surface reactions and precipitation (Frossard et al., 1995). Immobilization and mineralization are the biochemical processes of the P cycle. Immobilization (the biological conversion of inorganic P to organic P) occurs when plants uptake inorganic P from solution and return it as organic P in litter and root debris or when inorganic P is taken up during decomposition of organic carbon (C) materials by microorganisms and returned as detritus during microbial turnover, primarily as nucleic acids (Jakobsen et al., 2005). Mineralization is the process whereby inorganic P is released from organic P in the soil.

It has been demonstrated that mineralization of organic P sources is a significant contribution to plant P nutrition (Firsching and Claassen, 1996; Magid et al., 1996; Condron et al., 2005). This is especially important in older and highly weathered tropical Oxisols and Ultisols where inorganic P is strongly fixed as insoluble complexes with iron and aluminum (Vance et al., 2003; Raghothama and Karthikeyan, 2005; Tiessen, 2005; George et al., 2006). Older and more highly weathered soils, such as those in the tropics, contain more secondary P minerals (Fe and Al bound) with a lower solubility than primary (Ca bound) minerals (Walker and Syers, 1976). This creates stronger competition between the biological components of the P cycle (i.e. plants and microbes) and the secondary mineral P sink in the soil. In extreme cases, soil processes maybe excluded almost completely with roots obtaining nutrients directly from an above ground litter layer, thus P is restricted to the biological cycle (Tiessen, 2005).

1.1.3 Forest P dynamics

Much of the research conducted on P cycling and nutrition of forest trees has used commercial forestry species such as *P. radiata* in New Zealand and *P. elliottii* and *P. taeda* in south eastern USA (Comerford and De Barros, 2005). Figure 1.2 represents the nutrient cycling processes influencing P in a forest. Most forest systems are not influenced by external P addition from fertilizers and the soil P cycle is closed, relying on recycling and retranslocation of nutrients within the system (Comerford and De Barros, 2005). Forests are often found on less fertile soils as the most 'desirable' soils have been selected for agricultural use (Pritchett and Fisher, 1987). Forests have an extensive litter and fermentation layer which can represent an extensive P input into the soil. For example, Polglase et al. (1992b) found that P was released from a *P. taeda* forest floor at rates of 0.4-2.3 kg/ha/yr. This was similar to the ranges found for *F. sylvatica*, *P. nigra*, *P. pinaster* and *A. borisii regis* by Kavvadias et al. (2001). Also, Cortina et al. (1995) found that 6% of total P in the forest floor was leached into the underlying soil annually in a mature radiata pine stand demonstrating the importance of the litter layer for retranslocation of nutrients within the forest system.

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The litter layer can be broken down into three layers: the litter layer (L), composed of freshly fallen leaves and containing no roots, the fermentation layer (F), composed of partially decomposed material and the humus layer (H) layer, composed of humified material (Kanerva and Smolander, 2007). The F and H layers will be affected by root turnover and exudation (Comerford and De Barros, 2005). The L layer supports a huge amount of macrofauna which fragment the litter and increase its susceptibility to hydrolysis and inorganic P release by microorganisms (Attiwill and Adams, 1993) in the F and H layer. Chen et al. (2000a) found that 62% of P in the F layer of a radiata pine forest was contained within the microbial biomass demonstrating the importance of microbially mediated processes in the litter layer. This material is gradually incorporated into the underlying mineral soil but is a long term process. Root turnover is a more important organic input into mineral soils of grasslands and this is a faster process than that in forest soils (Chen et al., 2008). Plant uptake of nutrients directly from the litter layer is an important process in tropical forests. Highly weathered soils

are more common in the tropics than temperate areas due to geological stability and high rainfall and temperatures (Lathwell and Grove, 1986). Tropical soils are high in Al and Fe which form stable complexes with P as described above, therefore P is thought to limit ecosystem production in tropical forests. The high P fixing capacity results in a bypass of soil processes in the P cycle as addition of P to soil will render the P inaccessible and so the P cycle is almost entirely biological with nutrients obtained by plants and microorganisms directly from litterfall (Tiessen, 2005). Vitousek (1984) found that litterfall correlated with P availability when reviewing data from 62 tropical forests and concluded that P availability limited litterfall in these systems and further reinforced the importance of litter and the efficient P recycling that occurs in tropical systems. Turnover of litter in tropical systems is faster due to warm, moist climate and a greater diversity of organisms (Pritchett and Fisher, 1987).

Studies have reported greater organic matter mineralization and therefore greater nutrient availability occuring in temperate forest soils compared to grassland soils (Chen et al., 2008). Davis and Condron (2002) in a review of paired site forest and grassland comparison studies demonstrated that there is less C in young forest soils compared to grassland soil due to higher mineralization under forest and less input from litter. The difference in C concentration became less apparent with forest age as C addition from litter begins to counteract this. Many studies have also reported an increase in available P and a decrease in organic P fractions under forest soils compared to grassland soils (Davis and Lang, 1991; Davis, 1994; Chen et al., 2000a). This has been attributed to greater mineralization of the phosphate monoester fraction which includes the inositol phosphates (Condron et al., 1996). This was later confirmed in a 10 month pot trial by Chen et al. (2004b) and by Turner et al. (2005b) when *P. radiata* was found to greatly reduce *scyllo*-inositol hexakisphosphate compared to ryegrass (*Lolium perenne*). Chen et al. (2003b) found in a 40 week glasshouse experiment that *P. radiata* can uptake more P than ryegrass at low P levels and Scott and Condron (2004) found radiata pine had a higher P uptake although inorganic P fractions did not decrease beneath it, verifying conclusions made by Condron et al. (1996) and Chen et al. (2004a) that trees can utilize more recalcitrant forms of organic P, such as *myo*-inositol hexakisphosphate than ryegrass.

The organic inputs added from forest litter are less labile compared to those added in pasture root turnover (Yeates et al., 1997). As a result, there is much less P in the microbial biomass of forest soil (Yeates and Saggar, 1998; Chen et al., 2008) which, in turn, results in a lower phosphatase activity level in forest soils (Chen et al., 2000a). The lower microbial biomass C and P in forest soils is inconsistent with the increased mineralization consistently found under trees. Chen et al., (2000) suggested that the decrease in organic P and other nutrients and increased inorganic P in soils under forest was due to enhanced phosphatase and microbial activity at early growth stages when P uptake is high. A study by Chen et al. (2002) was in agreement with this as microbial biomass P was higher under *P. radiata* seedlings compared with ryegrass in a short term glasshouse experiment where *P. radiata* was in early growth stages.

Plants are considered a major driver in microbial community structure and function, and some studies have demonstrated a difference in microbial function in forests compared to pastures. Stevenson et al. (2004) found forest microbial communities in New Zealand forests to be more responsive to organic acids such as citric acid, possibly reflecting the higher importance of these root exudates for increasing P solubility in forest systems. Indeed it has been shown that organic acids are common in soil solution under tree species (e.g. Fox and Comerford, 1990 and Adams et al., 1999) and that these organic acids increase P solubility (Fox and Comerford, 1992). Trees of boreal and temperate forests, such as those in the above studies, are predominantly ectomycorrhizal where as most pasture and agricultural crop species are arbuscular mycorrhizal (Smith and Read, 1997). This difference in mycorrhizal association will influence the P cycling of these two contrasting ecosystems and will be discussed in detail below.

Forest rooting depth is greater than pasture rooting depth and this will effect a forests available P definition (Comerford and De Barros, 2005). Deep soil layers are of greater importance in determining forest productivity (Pritchett and Fisher, 1987). The deeper forest tree roots may produce a pumping effect, where nutrients are pumped from lower layers by the deeper roots and returned as more labile forms to the surface layers during litterfall (Davis and Lang, 1991). This addition from litter means organic P forms are of greater importance in forest P cycling despite studies showing lower phosphatase activity and that cycling is most active in litter and surface soil. A large proportion of

labile organic P is in diester form in forest soils (Attiwill and Adams, 1993; Grierson and Adams, 2000) likely due to high microbial activity in the litter layer.

1.1.4 Soil organic phosphorus

Soil organic P is defined as P associated with C, either directly bonded (P-C) or more commonly as esters (P-O-C) and is found in the soil from three main sources: microorganisms, soil organic matter and freshly added organic material (Oberson and Joner, 2005). Organic P estimates range from 15 to 80%, although it is commonly between 30 and 65% (Harrison, 1987). The large proportion of organic P is due to the fact that plant, animal and microbial residues containing high proportions of organic P as nucleic acids, membrane phospholipids or monoesterases involved in energy transfer are continually added to the soil (Magid et al., 1996). All organic P is derived from organisms and after release into the soil environment it may be recycled into biomass or stabilized in the soil by association with clays, metal oxides or organic matter (Condron and Tiessen, 2005).

Before the use of ^{31}P nuclear magnetic resonance (NMR) spectroscopy, much less was known about the chemical nature of soil organic P and so it was often considered as a whole or operationally divided into labile, humic, acid soluble and refractory components by fractionation experiments (McKelvie, 2005). Nuclear magnetic resonance spectroscopy uses the magnetic resonance of a nucleus to identify the chemical forms of that nucleus in a sample. Using the only naturally occurring phosphorus isotope (^{31}P) and a suitable extractant, NMR can determine composition of soil organic P and so has advanced knowledge of organic P chemistry considerably over the last two decades (Turner et al., 2003a; Cade-Menun, 2005).

Organic P compounds are classified into orthophosphate esters, phosphonates and anhydrides based on the nature of the P bond (Condron et al., 2005). Despite representing only a small organic P input into soils, approximately 50% of identified organic P compounds in soil are present as phosphomonoesters called inositol phosphates. These compounds have a high sorption capacity to iron oxides and readily form insoluble precipitates in acid or alkaline conditions making them extremely unavailable and stable in soil (Turner et al., 2002). Other phosphomonoesters that are

weakly sorbed, due to having less orthophosphate groups attached to the C moiety, are also abundant in soil and more important in P cycling. These include mononucleotides and sugar phosphates. Less than 10% of organic P is in the form of phosphodiesters such as nucleic acids, phospholipids and phosphoproteins (Richardson et al., 2005). These forms represent much of the organic P input into soils, as they are abundant in all biological tissue, but they are degraded quickly and thus do not accumulate (Bowman and Cole, 1978). Phosphonates are another form of organic P directly bonded to C but only tend to accumulate in soils with wet, cold and acidic conditions as these conditions accommodate few organisms containing phosphonatase enzymes (Condron et al., 2005).

1.1.5 Organic phosphorus cycling

Figure 1.3 is a detailed diagram of the organic P cycle in soils. The main input into the soil system is through plants. Manure can also be a significant source of organic P but it is of limited importance to forest systems and so will not be discussed further here. Plants input organic P through root turnover and litter. Some of this organic P will be incorporated into the microbial biomass through processes described below and become a marginally labile source. Some of the inputted organic P will be in more labile forms, such as simple monoesters like glucose phosphate and unstable diesters such as DNA and RNA which are susceptible to hydrolysis by plant roots, mycorrhizae and microorganisms. Some organic P will become stabilized on soil surfaces such as clays and metal oxides and require solubilization prior to hydrolyzation. All forms of organic P must be mineralized by phosphatase enzymes produced in the rhizosphere to release inorganic orthophosphate for plant and microbial uptake (Condron et al., 2005).

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1.1.6 Microbial P dynamics

Soil hosts a large number and variety of bacteria and fungi which metabolize organic C (Frossard et al., 1995; Manocharachary and Mukerji, 2006). Microbial P can account for 1-10% of total soil P, usually around 5% (Richardson, 2001; Richardson et al., 2009b). Approximately 80% of microbial P is in organic forms including RNA, polyphosphates, phospholipids, DNA and small amounts of inositol phosphates (Oberson and Joner, 2005; Bünemann et al., 2008). The amount of microbial P is linked to concentrations of organic C and so it varies with land use and soil type (Oberson and Joner, 2005). For example, microbial P constituted an average 4.6% of total P in UK permanent grassland soils (Turner et al., 2003b) and 0.5% in planted radiata pine top soils in New Zealand (Chen et al., 2000a) but the high organic C levels of forest floors mean there is a much greater concentration of microbial biomass P in litter compared to the underlying soil, accounting for up to 62% of P in this layer (Chen et al., 2000a).

Microorganisms are primarily responsible for the mineralization of organic C and associated P. The uptake, release and redistribution of P by microorganisms mediate several processes in the P cycle (Stewart and Tiessen, 1987; Jakobsen et al., 2005; Oberson and Joner, 2005; Bünemann et al., 2008). Microorganisms require phosphate and obtain it through mineralization of organic P by release of phosphatase enzymes (Jakobsen et al., 2005). The proportion of phosphate released for plant uptake by

microbial phosphatases compared to plant phosphatases is poorly understood (Richardson et al., 2001). In addition inorganic P can only be taken up over short distances and reacts with the soil matrix to become less soluble over time and so P mobilization must occur in the immediate vicinity of a plant root for it to have a competitive advantage over microbial immobilization and the physiochemical processes such as sorption and precipitation that remove phosphate from soil solution (Magid et al., 1996; Richardson et al., 2005).

Much of the mineralized P is immobilized in the microbial biomass but also subsequently released during turnover as an easily hydrolysable organic form (Macklon et al., 1997). Thein and Myers (1992) demonstrated this by adding C and N to soils and incubating them for 7 days to increase microbial biomass. As a result there was a 280% increase in labile, $NaHCO_3$ extractable P compared to before incubation indicating the microbial biomass has potential to mobilize significant amounts of P to plant available forms. Guggenberger et al. (2000) found, using ^{31}P NMR, that labile P, isolated on a macroporus exchange membrane, was a dynamic pool and a large proportion was phosphodiesters of microbial origin. Seeling and Zasoski (1993) found that dissolved organic P was of microbial origin and had greater mobility in soil solution relative to orthophosphate. The utilization of non plant available forms by microorganisms and subsequent release, in turnover, of more plant available P is a significant means for increasing potential plant P availability in the rhizosphere, consequently, the microbial biomass represents a significant source and sink of potentially plant available P (Jakobsen et al., 2005; Richardson et al., 2005) although the relative contribution of this P to plant nutrition is unknown (Richardson, 2001).

1.1.7 Rhizosphere P dynamics

The rhizosphere is described as roots and their immediate zone of influence and it extends into the soil just a few mm from the root surface (Hinsinger et al., 2009). It is the most chemically, biochemically and biologically active site in the soil and its ecological importance is widely recognized (Lynch, 1982; Walker et al., 2003). Any physical, chemical or biological alterations occurring in the root sphere or directly influenced by root excretions is called a 'rhizosphere effect' (Badalucco and Kuikman, 2001). Between 5 and 21% of all photosynthetically fixed C enters the rhizosphere through root exudation of a vast array of chemicals and compounds and sloughing off of dead root cells (Marschner, 1998; Walker et al., 2003). When taking root influence on water and more mobile nutrients such as nitrate (NO_3^-) into account, the rhizosphere extends more than a few mm into the soil but when a relatively immobile nutrient such as P is considered, the rhizosphere is limited to <1-4 mm (Kovar and Claasen, 2005). There are many rhizosphere effects influencing organic P dynamics and Figure 1.4 illustrates this.

Roots rapidly deplete P in the soil thus establishing a P diffusion gradient from the root surface, out through the rhizosphere soil and into the bulk soil (Gahoonia and Nielsen, 1997). Phosphorus has a low diffusion rate through soil (Kovar and Claasen, 2005) and this limits the supply of P to plant roots unless roots grow to extract P from unexploited soil or chemically modify the soil environment to increase solubility and/or diffusion of P (Richardson et al., 2009a). Roots can change their morphology in P deficient conditions by increasing root growth and branching, by root hair formation and by increasing rooting depth, to increase the volume of soil explored (Gahoonia and Nielsen, 1997; 2004; Richardson et al., 2005) or increasing root density in surface layers where the highest concentrations of P usually are (Manske et al., 2000). Soil microorganisms can further alter root morphology by releasing plant growth regulating hormones (Arshad and Franken-berger, 1998). The rate of orthophosphate diffusion in soil is not sufficient to overcome the concentration gradient resulting from root P uptake and so roots also have the capacity to modify the chemical environment of and P availability in, the rhizosphere through P uptake, exudation of organic anions and active release of phosphatase enzymes (Jones, 1998; Hinsinger, 2001).

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Phosphatase enzymes

Soil organic P mineralization is primarily a 'rhizosphere effect' and provides a major proportion of plant available P in natural ecosystems (Firsching and Claassen, 1996; Magid et al., 1996; Condron et al., 2005). The precise mechanisms involved require investigation but it is thought to be controlled by phosphatase enzymes produced by plant roots, mycorrhizae and micro-organisms (McGill and Cole, 1981; Magid et al., 1996). Synthesis of phosphatases is a ubiquitous response of plants to P deficiency (Vance et al., 2003; Raghothama, 2005) and many studies have demonstrated the excretion of these enzymes, by roots, into the rhizosphere (Goldstein et al., 1988; Hayes et al., 1999; Miller et al., 2001; George et al., 2008). A direct contribution of plants to phosphatase activity is difficult to determine because despite many studies observing a marked increase in phosphatase activity close to roots (e.g. Chen et al., 2002; George et al., 2002b), this was concomitant with increases in microbial activity.

Biochemical P mineralization is thought to be a direct process driven by P demand and independent of the consequential biological mineralization of directly C-bonded N and S which is driven by the demand for C. This is because P is usually ester bound and stabilized by its orthophosphate group whereas other soil nutrients are stabilized in soil by their C moiety (McGill and Cole, 1981). However, phosphonates are directly C bonded and phosphatase enzymes can become stabilized on soil solid surfaces so that the soil phosphatase activity (and organic P hydrolysis) may not reflect the soil P status and P demand (George et al., 2005b).

There are many different types of phosphate esters in soil and so many different types of phosphatase enzymes actively exuded by plant roots and microorganisms to hydrolyze them. Most studies show increased phosphatase activity is a ubiquitous response to P deficiency (Fox and Comerford, 1992; Richardson et al., 2005). Phosphatases are broadly divided into acid and alkaline phosphatases according to the optimal pH for activity (Eivazi and Tabatabai, 1977) and then subclassified according to the substrate on which they work. Phosphomonoesterases, that hydrolyze the simple phosphomonoesters such as sugar phosphates, are the most common phosphatase enzymes exuded by plants, mycorrhizae and other soil microorganisms (Eivazi and Tabatabai, 1977; Raghothama, 2005; Turner, 2008). Phosphodiesterases are required in conjunction with phosphomonoesterases to hydrolyze phosphodiesters such as DNA and RNA (Browman and Tabatabai, 1978; Turner and Haygarth, 2005) and are commonly exuded by plants and also ericoid mycorrhizal fungi that grow on extremely organic soils containing large proportions of phosphodiesters due to slow organic matter turnover, such as heathland and peat soils (Smith and Read, 1997). Phytases are required for the release of phosphate from inositol phosphates (George et al., 2005a,b; Richardson et al., 2006). Phytases have been shown to be absent or only to constitute a small component of exudation and likely work in conjunction with a solubilizing agent to release the inositol phosphate from its binding site (Richardson et al., 2006). The low proportion of extracellular phytase released by plants compared to the high proportion of phytate in soils maybe linked to the high sorption and precipitation capacity of this organic P form rendering the phytase relatively useless (Richardson et al., 2001). For example, Adams and Pate (1992) found that lupins could access phytate when grown in non P fixing sand but not when grown in soil. Direct evidence for the role of

phosphatases in organic P hydrolysis in the rhizosphere is hindered because the increase in rhizosphere activity is a response also induced by low P concentration (Trolove et al., 2003).

There is much contradictory evidence regarding the level of phosphatase activity and P mineralization rates and so the relationship is poorly understood (Condron and Tiessen, 2005). Many studies have shown a correlation between decreased organic P and increased phosphatase activity (Asmar et al., 1995). For example, Liu et al., (2004) and Chen et al., (2002) found increased phosphatase activity did correlate with a decrease in organic P fractions in controlled environment studies. Tarafder and Jungk (1987) correlated depletion of organic P with soil phosphatase activity in rape (*Brassica oleracea*), onion (*Allium cepa*), wheat (*Triticum aestivum*) and clover (*Trofolium alexandrinum*). Häussling and Marschner (1989) found decreased organic P was associated with increased phosphatase activity in rhizosphere soil of Norway Spruce (*Picea abies* (L). Karst.) trees. However, some experiments have found no such correlation (Speir and Ross, 1978). For example, Chen et al. (2000a) in a forest and grassland paired site study found lower phosphatase activity but greater organic P mineralization under forests, and Adams (1992) found that phosphatase activity did not correlate with organic P mineralization in an experiment with *E. diversicolor* forest soils.

It is now established that biological mineralization plays an important role in P mineralization as molecules such as DNA contain abundant C and N as well as P (Condron et al., 2005). Inorganic P can be released as a byproduct from organic matter during biological mineralization of organic matter driven by energy demand (Stewart and Tiessen, 1987). Recent studies have shown the importance of both biochemical and biological processes in organic P mineralization. Organic P use in the soil was shown not to be limited by phosphatase activity but by substrate availability when mineralization of organic P was 20 times higher than plant demand in a study by Tarafdar and Claassen, (1988), despite plant P availability being closely related to phosphatase enzyme activities in many studies (George et al., 2002a). The solubility and hence availability of organic P is linked to the decomposition of organic C constituents in the soil and this in turn affects hydrolysis by phosphatase enzymes therefore a

distinction between biological and biochemical mineralization may not be entirely correct (Magid et al., 1996; Condron and Tiessen, 2005).

Root exudates

In addition, exudation of low molecular weight organic anions (LMWOAs) such as citric acid and oxalic acid has long been known to enhance the solubility of sparingly soluble mineral phosphate (Jones, 1998) by competing with phosphate esters for adsorption sites (Lopez-Hernandez et al., 1986; Hinsinger, 2001) and/or chelation of Al or Fe cations in acidic soil and Ca cations in alkaline soils (Jones, 1998; Jones and Darrah, 1994). Citrate and oxalate are regarded as more effective at nutrient mobilization than other organic acids, this is followed in order by malate, malonate and tartrate and then succinate, fumerate, acetate and lactate (Bar-Josef, 1991, cited in Richardson et al., 2009b). Although precise mechanisms are not well understood, they are speculated to also enhance the solubility and consequently, hydrolysis of organic P. Nardi et al. (2000) demonstrated that LMWOAs can breakdown organic P in humic matter to a lower molecular weight by breaking the hydrophobic bonds holding the molecules together. This is speculated to increase organic P susceptibility to hydrolysis and thus may be a solubilizing agent necessary for the hydrolysis of inositol phosphates. They are thought to dissolve Al and Fe organic P complexes by chelation and so release organic P from them (Chen et al., 2008). Indeed, Tang et al. (2006) found that citrate, oxalate and malate could improve solubility of some phytate salts. It has been suggested that it is the solubility of organic P, not phosphatase activity that controls organic P mineralization (Adams and Pate, 1992).

During carbon metabolism, microorganisms may generate low molecular weight organic acids which may release precipitated P through acidification and/or chelation (Tinker, 1980; Frossard et al., 1995; Jones et al., 2003). These microorganisms are often grouped as phosphate solubilizing microorganisms (Oberson and Joner, 2005). For example, Richardson et al. (2001) found plant utilization of IHP was enhanced by the presence of bacterium isolate CCAR59 when grown on agar and speculated that this bacterium produced more, or a more effective type, of phytase. Hariprasad and Niranjana (2009) found that plant P content and rhizosphere P availability was greater

in tomato plants inoculated with various strains of phosphate solubilizing rhizobacteria than controls.

There is still much debate into the ecological significance of these compounds due to the small amounts exuded in the first place, high adsorption rates limiting zone of influence, as well as the rapid metabolization of these compounds by soil microorganisms resulting in a very short lifespan in the soil (Hinsinger, 2001; Jones, 1998). Organic acids are rapidly metabolized by soil microorganisms and so release of these and other root exuded compounds such as amino acids, sugars and tannins, stimulate and select for microbial activity (Jones, 1998; Walker et al., 2003). This metabolism may also limit organic anion effectiveness at mobilizing P in soil (Gahoonia and Nielsen, 2004). Differences in phosphate solubilizing bacteria were found between plant species growing in the same soil type indicating that populations of these microorganisms are related to root exudation (Reyes et al., 2006). In addition to this, Meharg and Killham (1995) found that rhizosphere microorganisms could enhance root exudation of organic acids further demonstrating the close relationship between rhizosphere components.

Mycorrhizal fungi

Mycorrhizal fungi form a symbiotic relationship with 90% of all terrestrial plants involving a bi-directional nutrient flow of carbon to the fungi (between 10 and 20% of net photosynthates [Jakobsen and Rosendahl, 1990]) and mineral nutrients to the plant (Smith and Read, 1997). The extraradical, biochemically active fungal hyphae explore soil past the root depletion zone. Thus soil and microorganisms influenced by roots and associated mycorrhizal hyphal networks is given the term ‘mycorrhizosphere’ which can extend into the soil more than 10 cm from the root surface (Timonen and Marschner, 2006; Cavagnaro et al., 2001). There are many mechanisms influencing effective P acquisition by mycorrhizal hyphae. Firstly, hyphal diameter is 3-7 μm (Dodd et al., 2000), a diameter smaller than root hairs at 5-20 μm (Wulfsohn et al., 1999) which increases available pore space for exploration, enabling more soil to be utilized for P uptake per unit hyphae surface area than per unit root surface area (Jungk and Claasen, 1989 cited in Marschner and Dell., 1994). This coupled with the longer length of hyphae (Li et al., 1991; Finlay and Read, 1986) increases the volume of soil

explored, increasing the spatial availability of P as well as increasing the surface area for nutrient absorption (Bolan, 1991; Marschner and Dell, 1994; Marschner, 1998; Facelli et al., 2009). Hyphae also form polyphosphates internally to maintain a low phosphate concentration to establish a concentration gradient (Marschner and Dell., 1994). Therefore, in the presence of mycorrhizae, plants can compete efficiently with the microbial biomass for mineralized P and reduce the amount of mineralized P that is adsorbed in soil (Joner and Jakobsen, 1994). Mycorrhizal infection also enhances P flow between roots i.e. from dying roots to live roots thus avoiding loss through adsorption, leaching and immobilization that would occur if this P was released into the soil (Newman, 1988). Much less is understood about the role of different types of mycorrhizae in direct plant utilization of organic P - i.e. do mycorrhizae influence rhizosphere phosphatase enzyme levels and organic P solubility by LMWOA exudation?

The two mycorrhizal types associated with most tree species are ectomycorrhizae and arbuscular mycorrhizae (Smith and Read, 1997). Ectomycorrhizae form associations with most trees from the families *Pinaceae* and *Fagaceae* in the northern hemisphere boreal and temperate forests and *Myrtaceae* in the southern hemisphere giving them high economic value in the production of timber (Perry et al., 1986; Smith and Read, 1997; Dahlberg, 2001; Courty et al., 2006). The ectomycorrhizal root has three main structural elements (Figure 1.5). There is a sheath of fungal tissue that encloses the root and can often be seen with the naked eye, a complex of inward hyphal growth between epidermal and cortical cells called the Hartig net and a system of outward growing hyphae which form the connections with the soil environment (Smith and Read, 1997). Ectomycorrhizal hyphae extend for much greater distances in soil than AM hyphae and have been shown to transport P over several metres (Finlay and Read., 1986). It is these hyphae that capture the soil P which is then transferred to the fungal sheath and stored. The P is then released to the plant slowly under high P conditions and faster under P deficient conditions.

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Arbuscular mycorrhizal fungi are the most common underground symbiosis, forming associations with a wide variety of plants (Rillig, 2004; Treseder and Cross, 2006) including most canopy dominants in tropical forests (Perry et al., 1986). Arbuscular mycorrhizae also have three important elements (Figure 1.6). Firstly the root is colonized by fungal structures within the cells (hence the other name endomycorrhizae as opposed to ectomycorrhizae which do not penetrate root cells) then radiates a system of extraradical hyphae into the soil (Smith and Read, 1997). Arbuscular mycorrhizal hyphae have been observed to extend more than 10 cm from the root surface and transport up to 80% of a plants P (Li et al., 1991). The internal structures include hyphae, vesicles and arbuscles which temporarily store nutrients transported to them from the soil through the extraradical hyphae before transfer to the plant cells via the plant-fungi interface (Rillig, 2004).

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It was generally considered that the P capturing extraradical mycorrhizal fungi only access the same P pools that are readily available to plants, however, now it is established that hyphae also have the means to chemically modify the mycorrhizosphere to increase P solubility and/or hydrolysis (Marschner and Dell, 1994; Jakobsen et al.,

2005). Direct evidence for chemical modification of the rhizosphere by mycorrhizal fungi comes mainly from ectomycorrhizal fungi and this symbiosis is considered more efficient than arbuscular mycorrhizae for increasing plant P uptake from sources not immediately available to plants (Lodge, 2000).

There is much evidence that ectomycorrhizal associations increase rhizosphere phosphatase activity (Eleanor and Lewis, 1978; Häussling and Marschner, 1989; Pasqualini et al., 1992; Liu et al., 2004a, b). Antibus et al. (1992) demonstrated that ectomycorrhizae do not increase acid phosphatase activity simply by increasing the surface area on which it can be produced as they found ectomycorrhizae can increase acid phosphatase activity with little increase in surface area. To further verify this, there is much evidence for direct ectomycorrhizal hyphae production of phosphatases from many fungal species which hydrolyze organic P (Ho, 1989; Hilger and Krause, 1989; Lapeyrie et al., 1991; Antibus et al., 1992; Nygren and Rosling, 2009).

Arbuscular mycorrhizal colonization of roots has also been demonstrated to alter rhizosphere P dynamics through increased phosphatase activity. Phosphatase production has been verified in and on the surface of hyphae (Joner and Johansen, 2000; Koide and Kabir, 2000; van Aarle et al., 2001; Feng et al., 2002) but with little evidence for release into the soil and many studies can only relate colonization to generally increasing rhizosphere enzyme activity (Dodd et al., 1987; Tarafdar and Marschner, 1994a, b; Solaiman and Abbott, 2003). Alternatively, Joner et al. (1995) and Joner and Jakobsen (1995) in experiments using cucumber, did not find any increase in extracellular phosphatase in the presence of AM fungi. The confounding results are attributed to the non sterile conditions of the studies resulting in endogenous phosphatase levels masking any mycorrhizae influence on phosphatase levels. This indicates that the influence of arbuscular mycorrhizae on phosphatase enzyme activity is indeed relatively small, possibly due to low biomass in soils (Joner et al., 2000).

Many studies have demonstrated that ectomycorrhizal associations enhance rhizosphere LMWOA production which in turn solubilizes various P types, increasing plant P availability and uptake of P. For example, Cassarin et al. (2003) found that seedlings infected with ectomycorrhizae exuded more oxalate than those without. Liu et al. (2004

a, b) found that seedlings of radiata pine had increased excretion rates of oxalate when infected with ectomycorrhizae and Liu et al. (2005) found that this excretion of oxalate induced dissolution of a phosphate rock as well as mineralization of non-labile organic P fractions. Higher levels of oxalate and dissolved P have also been found in forest soil solution under ectomycorrhizal fungi mats than in soil solution not under a mat (Griffiths et al., 1994). Ectomycorrhizal hyphae have been shown to directly solubilize sparingly soluble forms of inorganic P *in vitro*, by production of oxalic acid when grown without their plant host (Lapeyrie et al., 1987; Lapeyrie, 1988; Lapeyrie et al., 1991). Arbuscular mycorrhizal exudation of LMWOAs has received relatively little attention but Antunes et al. (2007) found that hyphae did not induce localized changes in pH and that they did not release H^+ ions alone or in combination with organic anions. However, potential P solubilization by rhizosphere acidification has been demonstrated in hyphae of both ectomycorrhizae (Liu et al., 2005) and arbuscular mycorrhizae (Li et al., 1991). Therefore rhizosphere acidification by AM fungi needs to be resolved and investigated further.

Hyphae effects can also be indirect through their influence on soil microbial communities (Jeffries et al., 2003; Barea et al., 2005). The production of LMWOAs verified in ectomycorrhizal hyphae will stimulate microbial activity by providing a readily metabolizable C source (Timonen and Marschner, 2006). Exudation by hyphae may also further select for a particular microbial community efficient at P solubilization. For example, Frey-Klett et al. (2005) found that ectomycorrhizae associated with nine month old Douglas fir seedlings selected for bacterial isolates with greater P mobilization abilities when compared to those found in bulk soil. Also, Calvaruso et al. (2007) found that the ectomycorrhizae associated with a mature oak stand selected for a particular microbial community efficient at P solubilization through proton and organic acid release. These two studies indicate the ectomycorrhizosphere influence on microbial functional diversity is quite general, occurring in nursery and field and both old and young trees of different species.

It is well established that microbial community structure associated with arbuscular mycorrhizal roots is different to that associated with non mycorrhizal roots (Fillion et al., 1999; Vasquez et al., 2000) and that those associated with the hyphosphere (soil

zone influenced by hyphae solely, not roots) are different to those associated with the mycorrhizosphere (Andrade et al., 1997). Less attention has been paid to the change in functional diversity that this difference has (Rillig, 2004). Barea et al. (2002) demonstrated that arbuscular mycorrhizal hyphae, through their exploration of a greater soil volume, utilize P solubilized by microbes out of the vicinity of roots and thus termed this a mycorrhizae/microorganism rhizosphere cooperation (Barea, 2005). Jayachandran et al. (1989) found that P released by strong iron chelating agents was available to mycorrhizal but not non mycorrhizal plants. This was attributed to the effective soil exploitation and therefore better competition with resident phosphate solubilizing bacteria. However, Toro et al. (1997) in a soil microcosm experiment found AM formation increased the size of the phosphate solubilizing bacteria population and Ouahmane et al. (2007) found inoculation of *C. atlantica* resulted in a higher excretion of organic acids in the rhizosphere that were effective in mobilizing P from sparingly soluble rock phosphate. They speculated that the effect of the native AM fungi is a result of a multitrophic microbial association and selection of a P solubilizing community. It is likely that mycorrhizal hyphae 'short circuit' the microbial aspect of organic P cycling (Smith and Read, 1997).

1.1.8 Rhizosphere methodology

The study of rhizosphere properties and processes is limited by being able to distinguish between rhizosphere and non rhizosphere soil. One approach, used mainly for rhizosphere definition in the field, was originally described by Hendriks and Jungk (1981) and described soil adhering to roots after hand shaking as rhizosphere soil, which is then removed by careful scraping, brushing or washing, and the remaining soil as bulk or non rhizosphere. This simple method has been used in many studies (e.g. Häussling and Marschner, 1989; Scott and Condron, 2003; Yanai et al., 2003; Liu et al., 2004; Philips and Fahey, 2006). However, using this approach it is not possible to obtain samples at known distances from the root, and the mycorrhizosphere is not considered as hyphae are severed and removed at all stages of separation.

The necessarily crude field methods for measuring rhizosphere soil meant that the development of specific methodology has been necessary to advance understanding of rhizosphere processes. These studies, based on the original design by Farr et al. (1969)

include a root and mycorrhizal hyphae exclusion zone and plane. Here, plants are grown in a container, split into two parts by mesh of appropriate pore size up to which roots and/or hyphae could grow but not penetrate. A mesh size of 20-30 μm is sufficient to exclude roots but allow penetration of root hairs and fungal hyphae. This mesh essentially forms a rhizoplane. Membranes of 0.45-1 μm effectively exclude roots hairs and fungal hyphae but allow for exchange of soil solution and gases. This mesh effectively creates a hyphoplane or mycorrhizoplane (Luster et al., 2009). Soil or artificial substrate below the membrane is determined as rhizosphere soil. Kuchenbuch and Jungk (1982) further modified this technique and introduced microtome slicing of the rhizosphere soil for sampling at various distances from the root plane. If the root mat is dense enough, a gradient of rhizosphere effects can be observed from the root plane and smaller, usually undetected effects are consequently enhanced and so become detectable (Luster et al., 2009). However, effects such as phosphatase levels in rhizosphere soil maybe enhanced by the severing of mycorrhizal hyphae by the microtome. This releases normally internal phosphatases and so makes soil activity higher than would normally be in soils influenced by hyphae (Joner et al., 2000). Many other studies have used a similar two part design (e.g. Gahoonia and Neilsen, 1991; Zoysa et al., 1997, 1998; Chen at al., 2002). Li et al. (1991) modified this technique again by creating a root exclusion zone (30 μm) with a hyphae exclusion zone below it (0.45 μm), thus creating a rhizosphere and rhizoplane as well as a hyphosphere and hyphoplane. This means that there is a zone without roots - driven primarily by mycorrhizal hyphae and a zone below this without roots or hyphae - non rhizosphere soil. The difference between this and zones created with a 0.45-1 μm mesh in other studies is that this design created a hyphoplane, not a mycorrhizoplane as roots have been excluded already at the previous barrier. These systems confine and enhance rhizosphere effects that are too small to be measured by conventional methods. For example, P depletion around single mycorrhizal hyphae is not measurable but when a dense number of hyphae are collected in a distinct compartment, effects are measurable in the compartment soil. This enhancement means that the potential contribution of each component to rhizosphere effects is measured and not the actual contribution under field conditions (Li et al., 1991; Luster et al., 2009).

1.2 Hypotheses, aims and objectives of this research

This study aims to further investigate the hypothesis that increased P mineralization under radiata pine forest compared to pasture is due to the pines ectomycorrhizal association and increased organic acid excretion compared to the arbuscular mycorrhizal association of pasture. Phosphorus dynamics and rhizosphere processes under tree species with contrasting mycorrhizal associations will be investigated to eliminate the confounding effects of contrasting tree and pasture physiologies. This will consequently provide further evidence on the roles of ectomycorrhizal and arbuscular mycorrhizal fungi in forest tree P nutrition.

The following hypotheses are to be tested by this study:

1. Utilization of organic P will vary significantly with tree species.
2. Differences in organic P utilization and dynamics in the soil are directly related to the exudation of different phosphatase enzymes and LMWOAs by various rhizosphere components and the interactions between these components.
3. Differences in LMWOAs and phosphatase enzymes are directly influenced by the mycorrhizal association of the host tree.

This project will compare rhizosphere processes and organic P utilization of different tree species. It aims to discover if varying rates of organic P turnover and utilization are due to differences in microbial activity, phosphatase enzymes and/or LMWOAs and whether these differences can be attributed to the influence of mycorrhizae.

The following objectives were established to accomplish these aims:

1. To assess and compare the microbial activity, phosphatase enzyme activities, LMWOA exudation and soil P concentrations in the rhizosphere of radiata pine (*Pinus radiata*), eucalypt (*Eucalyptus nitens*) and macrocarpa (*Cupressus*

macrocarpa) growing in the same environment in both a long term and short term situation.

2. To relate the differences in these rhizosphere properties to the influence of different rhizosphere components (roots, mycorrhizal hyphae and microorganisms).
3. To examine, in further detail, the influence of mycorrhizal associations on the phosphatase enzyme activities and LMWOA exudation of different tree species.
4. To assess the uptake and utilization of organic P by different tree species on soils with different proportions and qualities of organic P.

A long term replicated field trial established to investigate long term effects of plantation forest species on soil properties and processes as used. In this trial ectomycorrhizal *Pinus radiata*, arbuscular mycorrhizal *Cupressus macrocarpa* and tripartite (associated with both types) *Eucalyptus nitens* are growing, thus it provided an excellent opportunity to assess the impact of tree species with contrasting mycorrhizal associations on P dynamics in the same field environment and in conjunction glasshouse and controlled environment studies it will begin to elucidate the mechanisms behind the changes in soil P to gain knowledge and understanding of the long term impacts of this land use change on the soil system and on the P nutrition of forest trees.

Firstly, short term rhizosphere processes of radiata pine, eucalypt and macrocarpa were measured in the field in an artificial rhizosphere. Following this short term investigation, long term rhizosphere effects were measured by sampling rhizosphere and non rhizosphere soil from under nine year old trees. Results from initial field experiments are presented in Chapter 2. To gain further insight into rhizosphere effects by enhancing and isolating the rhizosphere components, a glasshouse study was completed. Here, rhizosphere effects on P dynamics were linked to tree roots, mycorrhizal associations and microbial activity and the interactions between them. Results are presented in Chapter 3. To further the results obtained from the hyphal compartments of this study, and gain more direct evidence for the role of mycorrhizae in P dynamics under these species, a series of controlled environment studies were undertaken. Here fungal hyphae were extracted from the rhizosphere of each tree species and stained to show phosphatase activity and sterile tree seedlings were

inoculated with specific mycorrhizal species and the soil properties were compared to those of the controls. Results from these studies are presented in Chapter 4. Lastly, to assess the organic P utilization capacity of these species under P stress, glasshouse studies were undertaken to place the species in P deficient environments. This included planting the trees in a series of soils with varying proportions and qualities of organic P and also an exhaustive pot trial where the trees were grown in a confined space for an extended period of time to create near P exhaustion. Results are presented in Chapter 5. Figure 1.7 shows how the various experiments are related to the hypotheses and objectives of the study. Overall conclusions and future research priorities will be outlined in Chapter 6.

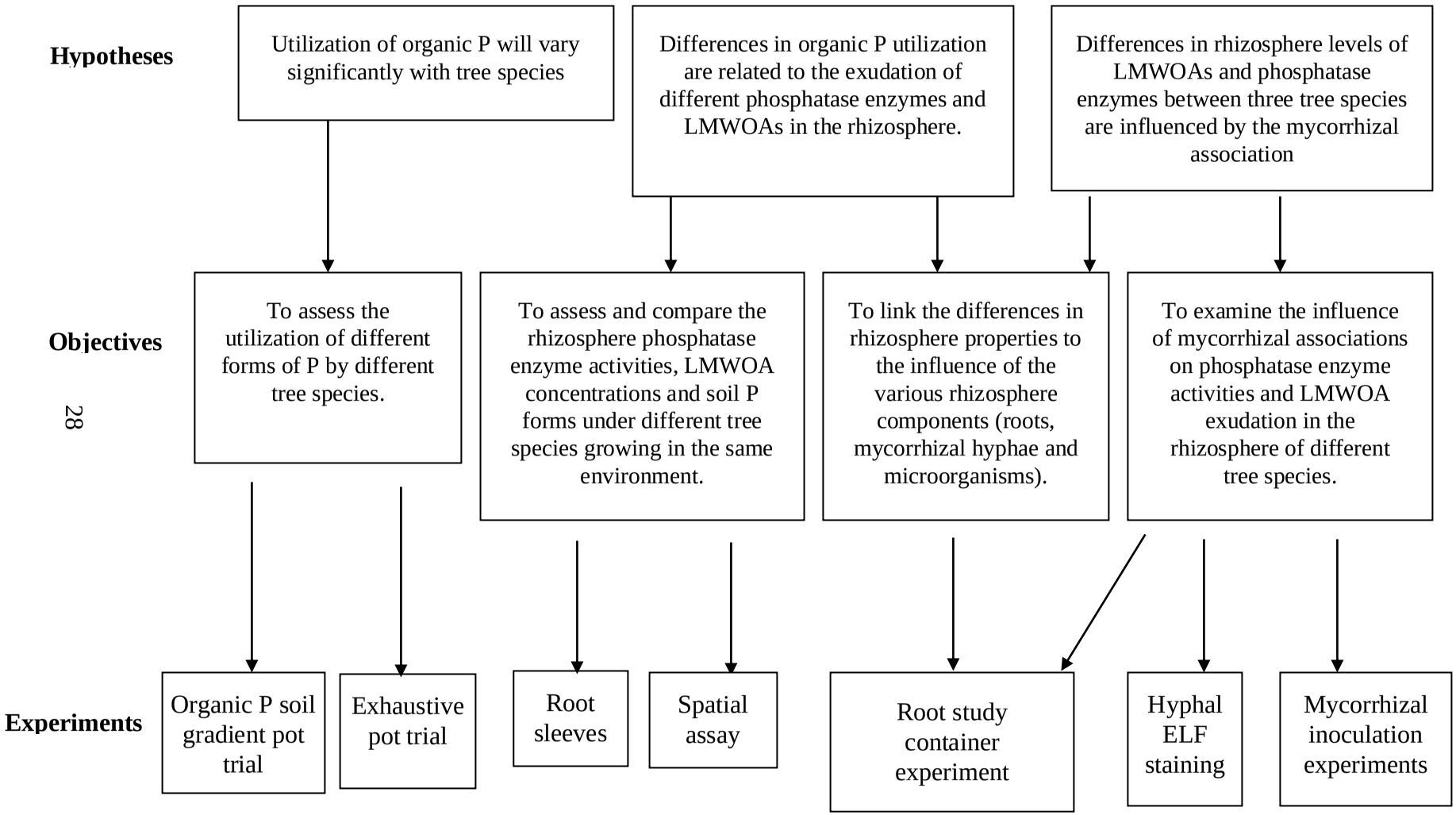

Figure 1.7 Organization of hypotheses, objectives and experiments in the thesis.

Chapter 2

Soil and Rhizosphere Phosphorus Dynamics in a Plantation Forest System

2.1 Introduction

The rhizosphere is the most chemically, biochemically and biologically active site in the soil and its ecological importance is widely recognized (Lynch, 1982). Microbial activity and nutrient transformations are stimulated in the rhizosphere (Marschner, 1998; Walker et al., 2003). Defining rhizosphere and therefore quantifying rhizosphere effects in the field is challenging because it is "discontinuous in time and space and variable in extension and character" (Smith, 1990) and hence there is no definite boundary between it and bulk soil. Furthermore, 90% of all terrestrial plant species are symbiotically associated with mycorrhizal fungi and the extraradical, biochemically active fungal hyphae explore soil past the root depletion zone and this mycorrhizosphere can extend more than 10 cm from the root surface (Timonen and Marschner, 2006; Cavagnaro et al., 2001; Martin et al., 2001).

In this chapter, two field based studies were used to advance understanding of rhizosphere processes and P dynamics in radiata pine, eucalypt and macrocarpa trees. Firstly, a novel root sleeve method for sampling rhizosphere soil in the field was used as a short term, *in situ* assessment of rhizosphere P dynamics. Here, intact roots of seven year old macrocarpa, radiata pine and eucalypt trees were enclosed in fresh pasture soil for seven months creating a manipulated, temporary rhizosphere to investigate short term rhizosphere dynamics. Based on findings from the root sleeve experiment, a destructive root sampling experiment was conducted comparing bulk with rhizosphere soil at different depths and different distances from radiata pine, eucalypt and macrocarpa trees at the same site. This experiment was designed to investigate the

cumulative effect of nine years tree growth on rhizosphere soil with no manipulation and reveal the long term impacts of these species on soil P dynamics.

2.2 Materials and Methods

2.2.1 Study Site

The chosen study site is an afforestation trial located at Orton Bradley Park on Banks Peninsula, South Island (Figure. 2.1). It is a replicated field trial established to investigate the long term effects of plantation forest species on the properties and processes that influence soil quality and fertility in hill country. Large scale change in land use will affect soil physical, chemical and biological properties and these will change with time and differ with species planted so it is important to quantify long term impacts of afforestation with different exotic species on soil properties and fertility. To do this, a long term field trial was established in 1999 at Orton Bradley Park (S 43^{o} 39' 48.3''; E 172^{o} 42'13.2'') in a 1.92 ha area of Takahe silt loam (Mottled Fragic Pallic soil, NZ classification; Typic Fragiustept, USDA classification) derived from greywacke loess. The soil had been developed under grazed pasture with limited fertilizer inputs (see Table 2.1 for initial soil properties before planting). Altitude at the site is 100-150 m, annual rainfall is 990 mm and mean annual temperature is 12.1^{o}C. The trial consists of four replicates of three commercial short rotation plantation forest tree species (radiata pine, eucalypt and macrocarpa) in randomized 30 x 30 m plots (see Figures 2.2 and 2.3). The trees were seven and nine years old when the root sleeve and root sampling experiments, respectively were carried out. The plots have not been grazed since trial establishment and the trees have been pruned and thinned in accordance with commercial silviculture practice. The trees have varying growth rates and Table 2.2 shows the average number of stems and the live and total standing volume (m_3) per hectare for each species in 2009.

Table 2.1 Selected chemical properties of soil to 30 cm depth determined at the Orton Bradley Park field site at establishment in 1999.

Depth (cm)	pH	Total C (%)	Total N (%)	Total P (mg/kg)	Organic P (mg/kg)
0-5	5.1	5.01	0.43	630	495
5-10	5.2	3.47	0.32	561	455

10-20	5.4	2.30	0.23	471	378
20-30	5.7	1.35	0.15	356	278

Figure 2.1 The location of Orton Bradley Park.

Table 2.2 Mean stem count and live and total standing volume across plots of radiata pine, eucalyptus and macrocarpa at the Orton Bradley Park field site in 2009.

Species	Live stems (count/ha)	Live standing volume (M_3/ha)	Total standing volume (M_3/ha)
Radiata pine	533	212	212
Eucalypt	800	165	165
Macrocarpa	779	81*	109

Data from L M Condron, personal communication.
***value is less than total due to the presence of disease in macrocarpa plots**

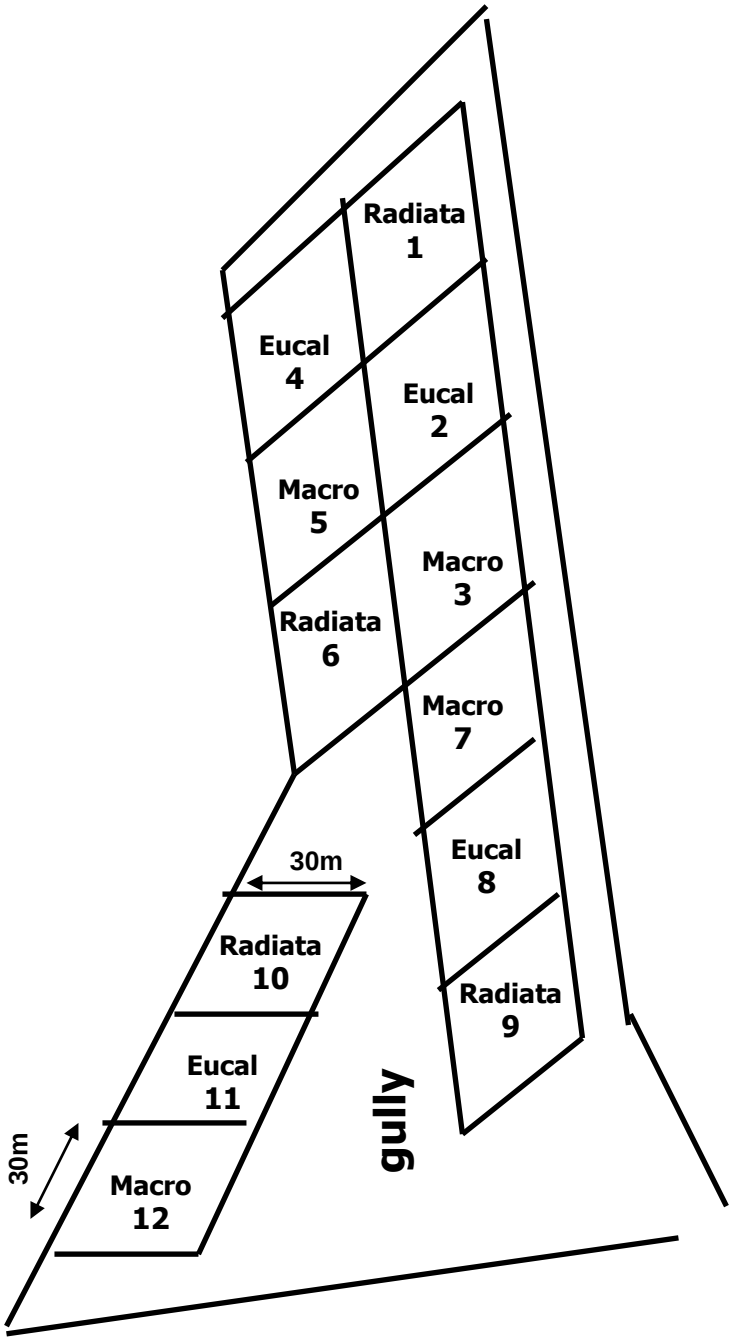
Radiata
1
Eucal
4
Eucal
2
Macro
5
Macro
3
Radiata
6
Macro
7
Eucal
8
Radiata
9
30m
Radiata
10
Eucal
11
Macro
12
30m
gully

Figure 2.2 The randomized plot design of the afforestation trial site at Orton Bradley Park.

Figure 2.3 The afforestation trial site at Orton Bradley Park in October 2006; a) macrocarpa plot, b) eucalypt plot, c) radiata pine plot, d) the whole trial with species marked with red letters. m = macrocarpa, e = eucalypt, r = radiata pine.

2.2.2 Root Sleeve Experiment

In October 2006, three 7 year old trees of each species were chosen in the middle of selected plots at the Orton Bradley Park field site (plot 9 for radiata pine, plot 8 for eucalypt and plot 7 for macrocarpa [Figure 2.4]).

Figure 2.4 Plots 9 (radiata pine), 8 (eucalypt) and 7 (macrocarpa) and the adjacent pasture used for the root sleeve experiment in October 2006 at the Orton Bradley Park field site.

Roots from distances of 50 and 100 cm from the tree bole were carefully excavated and freed of as much soil as possible (Figure 2.5a). Care was taken to keep the roots moist whilst exposed to the atmosphere by spraying them with a fine mist every five minutes to avoid desiccation. Around the exposed section of root a 15 x 15 cm piece of 25 µm nylon mesh was placed and sealed at each end with plastic cable ties to create a sleeve (Figure 2.5b). The sleeve was then filled with 4 mm sieved soil taken from adjacent pasture, sewed together with nylon thread and reburied (Figure 2.5c). The roots then continued to grow but could not grow outside the 25 µm mesh thus creating an intact rhizosphere. Eight replicates of these root sleeves were placed in each selected plot around three trees of each species giving 24 root sleeves in total. Eight 10 x 10 cm control sleeves of 1 µm mesh were also buried randomly in the plots for the same period of time (Figure 2.4d). These bags were filled with the same pasture soil, but not placed around root sections. In May 2007, after seven months, the sleeves were harvested by exposing the sleeve, cutting the root either side of the mesh and removing it from the ground. All soil inside the sleeves was determined to be rhizosphere soil and was stored at 4^{o}C until processing with a subsample dried at 20^{o}C. Control sleeve soil was treated in the same way. Roots were washed and stored at 4^{o}C until processing.

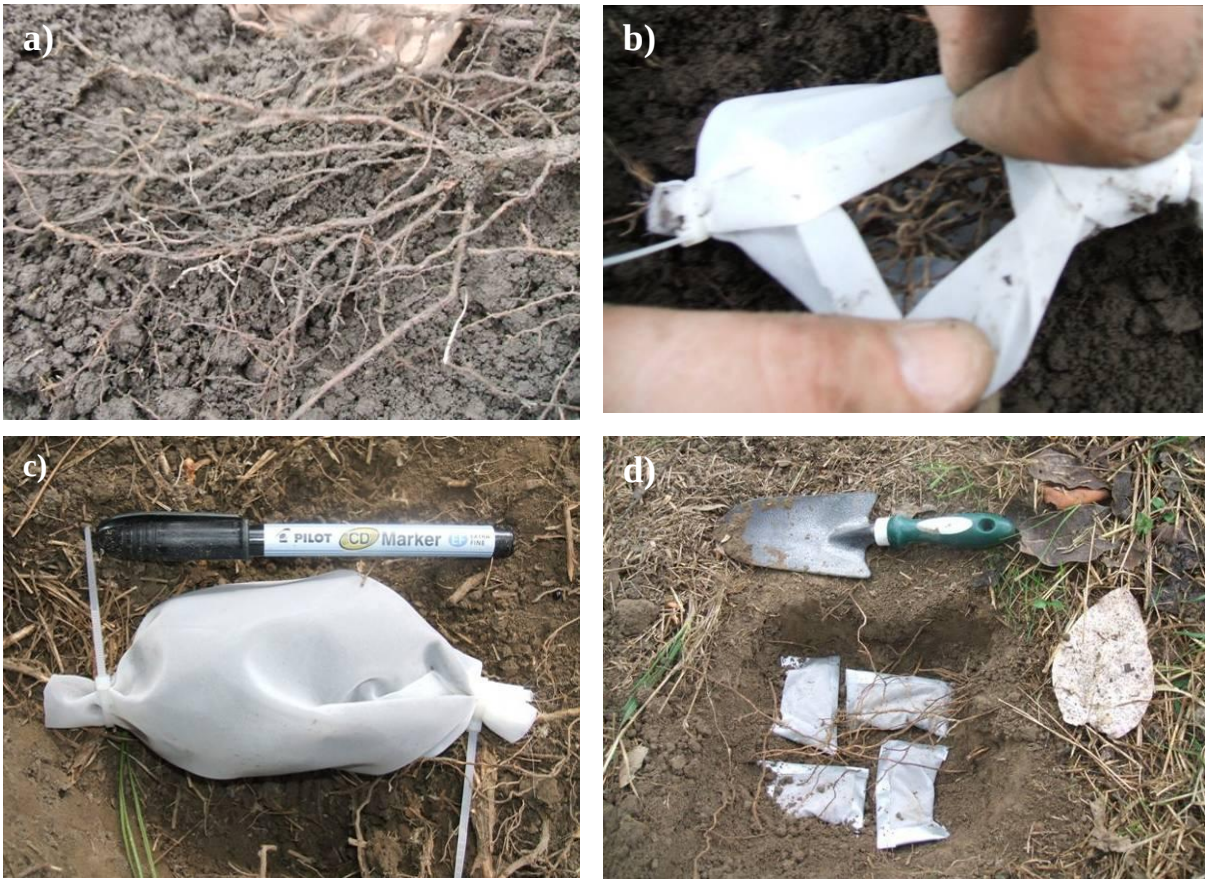

Figure 2.5 Different stages of the root sleeve preparation: a) a root section is excavated and exposed, b) exposed roots are wrapped in 25 µm nylon mesh and sealed at each end to create a sleeve, c) the sleeve is filled with 4 mm sieved soil from adjacent pasture and sewn together to create and intact section of soil, d) control sleeves containing no roots were made and sealed with duct tape and buried.

2.2.3 Root Sampling Experiment

This study was conducted at the Orton Bradley Park field site in December 2008 when the trees were nine years of age. Three replicate trees were randomly chosen from each species in selected plots (plot 12 for macrocarpa, plot 11 for eucalypt and plot 10 for radiata pine) and samples were taken with a 7 cm diameter corer at two distances from the tree (50 cm and 100 cm) and at two depths in the soil (0-10 cm and 10-15 cm). These distances were chosen because fine root biomass has been shown to decrease with increasing distance from tree base in similar species (e.g. Bouillet et al., 2002;

Gautam et al., 2002; O'Grady et al., 2005) and 100 cm was the furthest distance possible before encroaching on the adjacent tree. Little work has been conducted on horizontal distribution of rhizosphere effects. Samples at each distance and depth were taken at four places around the tree and bulked to provide sufficient soil for analysis (Figure 2.6).

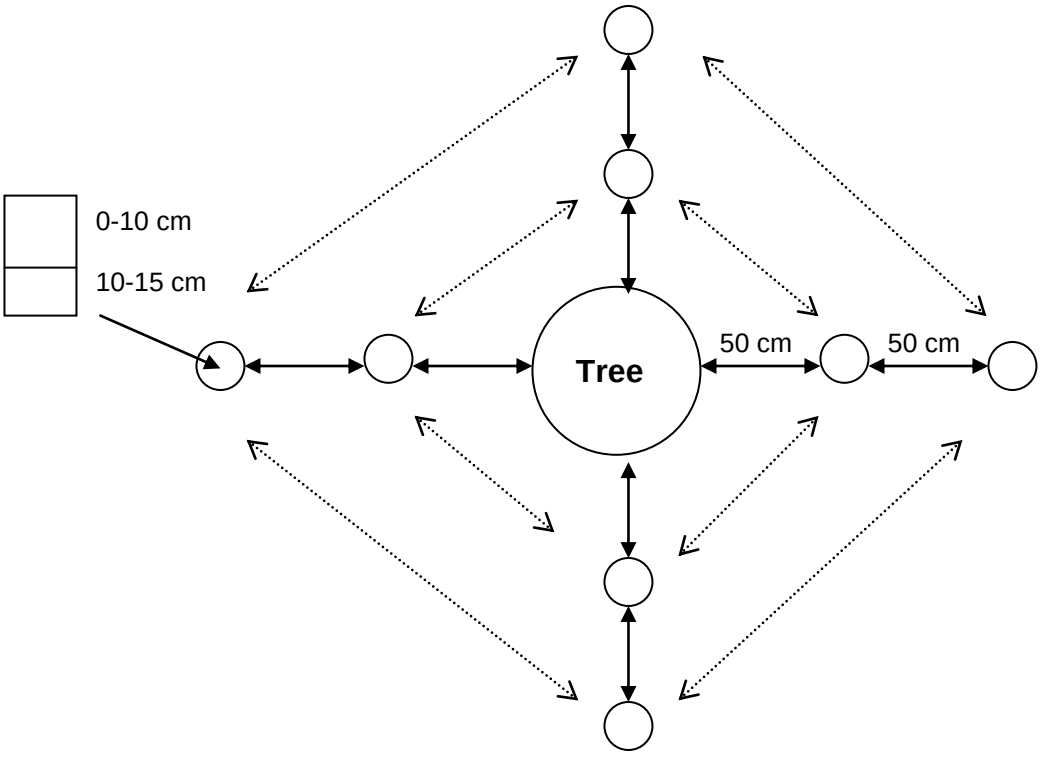

Figure 2.6 Aerial view of the soil sampling method around each tree in the root sampling experiment.

Each sample was then split into rhizosphere and bulk soil using the method described in Badalucco and Kuikman (2001) and Phillips and Fahey (2006). Firstly, all fine roots (<2 mm) were removed from the samples, shaken lightly and put aside. Remaining soil was classed as non rhizosphere soil. Soil adhering to the fine roots was then carefully scraped from the roots with fine forceps. Care was taken to leave roots intact but where this was not possible, root fragments and hairs were removed with tweezers. Root adhering soil was classed as rhizosphere soil. This seemingly crude methodology remains the only technique to differentiate between rhizosphere and non rhizosphere

soil in the field and gain a sufficient amount of rhizosphere soil for analysis. Litter samples were taken randomly from the four radiata pine plots as it was only radiata pine that had a significant litter layer.

2.2.4 Laboratory Analysis

Soils from the root sleeve experiment were analyzed for pH, acid and alkaline phosphomonoesterase activity, total organic P and total P concentration. Roots of radiata pine and eucalypt were analyzed for ectomycorrhizal colonization. Roots could not be sufficiently cleared for arbuscular mycorrhizal colonization assessment of eucalypt and macrocarpa.

Soils in the root sampling experiment were analyzed for organic matter content, pH, dehydrogenase (microbial) activity, acid phosphomonoesterase activity, phosphodiesterase activity, microbial biomass P, resin extractable P, total organic P and total P. Fine root biomass was also assessed in these cores.

Litter from radiata pine plots was sampled in the root sampling experiment for acid phosphomonoesterase activity, phosphodiesterase activity, microbial activity, resin extractable P and microbial biomass P.

Sample preparation

Samples were sieved to 2 mm to remove roots and stored at 4^{o}C. All fresh soil and litter analysis was carried out within 24-72 hours of sampling. A subsample of each soil was taken, air dried with forced air in a drying cabinet at room temperature for 10 days and then finely ground using a pestle and mortar prior to organic matter, organic P and total P analyses. Roots from each root sampling experiment sample were sorted by hand and all those less than 2 mm in diameter were kept, washed free of any remaining soil particles, dried at 70^{o}C for 72 hours and weighed. Litter was cut into <2mm pieces before analysis.

Soil and litter analysis

pH, moisture and organic matter

Soil moisture content was determined gravimetrically using an appropriate amount of soil (10-20 g) dried at 105^{o}C for 24 hours (Blakemore et al., 1987). Soil moisture was calculated as the weight lost per gram after drying. A 10 g (dry weight equivalent) sample of moist soil was dispersed in 20 ml of deionized water and the pH was determined after 30 minutes (Blakemore et al., 1987). Soil organic matter was determined using the loss on ignition method (Blakemore et al., 1987) whereby 0.6 g samples of air dried soil were ignited at 550^{o}C for one hour.

Phosphorus

Inorganic P in extracts was determined colormetrically using the Murphy Riley technique (Murphy and Riley, 1962; Tiessen and Moir, 1993). Suitable aliquots (determined by prior dilution testing) of the extracts were pipetted into 50 ml volumetric flasks with approximately 15 ml deionized water. Samples with high humic material, were precipitated before undergoing colorimetry. Three ml of extract was pipetted into a centrifuge rube with 0.5 ml 0.9M sulfuric acid (H_2SO_4), centrifuged for five minutes at 8000 x *g* and all or an aliquot was used for analysis. The sample was then neutralized using phenolphthalein indicator (1%), 5M sodium hydroxide (NaOH) and 2M H_2SO_4. Following this, 4 ml of colour developing solution was added and the solution was made to up 50 ml with deionized water. After 1 hour (to allow for full colour development) the colour was assessed by absorbance at 880 nm with a UV spectrophotometer. Phosphorus content was then determined using a standard curve ranging from 0-0.5 μg P/mL.

Total soil P was determined using the ignition and acid extraction method of Saunders and Williams (1955). A 0.6 g sample of air dried soil was placed in a muffle furnace and heated for 1 hour at 550^{o}C. The soil was then transferred to a centrifuge tube with 30 ml of 1M H_2SO_4 and shaken for 16 hours. After centrifugation for 15 minutes at 8000 x *g*, a subsample of each supernatant was diluted and analyzed using colorimetry.

An NaOH-EDTA extraction solution was used to determine total organic P. The NaOH solubilizes organic P associated with aluminum and iron oxides and the EDTA chelates metal cations to increase the efficiency of the extraction (Turner et al., 2005a). This solution was determined as the most suitable for quantitative organic P extraction, extracting comparable amounts of organic P to sequential fractionation by Bowman and Moir (1993). It is therefore considered a reasonably accurate estimate of total soil organic P. A 1.5 g sample of air dried, ground soil was shaken for 16 hours with 30 ml of NaOH-EDTA extraction solution (0.25M NaOH – 50mM EDTA) and then centrifuged for 30 minutes at 8000 x *g*. A subsample was then diluted 20 fold for total P analysis using ICP. Another subsample then underwent analysis using colorimetry as described above with the Murphy Reilly method to determine inorganic P in the extract. Organic P was determined my subtracting inorganic P values from colorimetry analysis from total P values from ICP analysis of the NaOH-EDTA extract.

Anion exchange resin has been shown to be more effective at isolating readily available P than chemical extraction (Rubaek et al., 1999). Therefore available P was determined by anion exchange membrane strips using a modified method from Tiessen and Moir (1993). The strips act as a sink for solution P, thus drawing readily soluble P into solution for replenishment. The readily soluble P is also absorbed by the resin and therefore P absorbed into the resin exchange strips is a measure of readily available P. A 5 g (dry weight equivalent) sample was shaken with 80 ml of deionized water and either one 6 x 4 cm anion exchange membrane strip or five 1.5 x 4 cm strips for 24 hours. The strip(s) were then removed and rinsed free of soil and shaken for 1 hour with 50 ml 0.25M sulfuric acid to desorb the P from the anion exchange strip. Phosphorus in the acid was determined using colorimetry as described above.

Microbial biomass P was determined using the same method as described for resin extractable P but with an additional 1 ml of hexanol added to the deionized water for fumigation. This extraction is a modified method from that described in Myers et al. (1999) to release P held in the microbial biomass through lysis of the microbial cells. It was determined by subtracting values for resin extractable P from fumigation released P values.

Phosphatase enzyme activity

Phosphomonoesterase (PME) activity was measured using the method of Tabatabai and Bremner (1969) as modified by Adams (1992). A 1 g (dry weight equivalent) sample of fresh soil was incubated at 37^{o}C for with 0.25 ml of toluene, 4 ml of buffer solution (pH 6.5 for acid and pH 11 for alkaline) and 1 ml of *p*-nitrophenyl solution. The reaction was terminated after 1 hour by the addition of 1 ml 0.5M calcium chloride ($CaCl_2$) and 4 ml 0.5M NaOH. Samples were then centrifuged for 5 minutes at 1366 x *g* and the supernatant was diluted until within the standard range of 0-50 μg *p*- nitrophenyl (usually 25 fold for acid PME and 10 fold for alkaline PME) and analyzed using a UV spectrophotometer at a fixed wavelength of 410 nm. Phosphodiesterase (PDE) activity was measured using the method of Tabatabai (1994). A 1 g (dry weight equivalent) sample of fresh soil was incubated at 37^{o}C with 4 ml of 0.05M THAM buffer (pH 8) and 1 ml *bis- p*-nitrophenyl solution. The reaction was terminated after 1 hour with 4 ml 0.5M NaOH and 1 ml 0.5M $CaCl_2$. Samples were then centrifuged for 5 minutes at 1366 x *g*, diluted with 0.1M of THAM dilutant (pH 10) until they were within the standard range of 0-50 μg *p*- nitrophenyl. These were then analyzed using a spectrophotometer at 410 nm.

Microbial activity

Soil microbial activity was assessed by dehydrogenase (DHE) enzyme activity using the method of Alef (1995). A 5 g (dry weight equivalent) sample of soil was incubated at 30^{o}C for 24 hours with 5 ml Triphenyltetrazolium chloride (TTC). The reaction was then terminated with 40 ml acetone, samples mixed thoroughly and incubated for a further 2 hours. Following incubation, samples were filtered and reduction to Triphenyltetrazolium formazan (TPF) was compared colormetrically to standards ranging from 0-50 μg TPF/mL using a UV spectrophotometer at 546 nm.

Mycorrhizal colonization

Ectomycorrhizal (ECM) colonization was determined using the gridline intersection method described in Sylvia (1994). A sample (approximately 20%) of fine roots from each sleeve was selected, cleaned and placed in a Petri dish marked with a 1 x 1 cm grid. Moving along each grid line, whenever a root crosses a line it was noted until the whole grid was observed. Each line crossing represents a centimeter of root length. This

process was then repeated but only mycorrhizal root tips that cross a line were noted. This was then expressed as mycorrhizal tips per cm of root. Arbuscular mycorrhizal (AM) colonization was attempted but not successful due to insufficient clearing of the roots.

2.2.5 Statistical Analysis

All statistical analyses were performed using GenStat 10 (Lawes Agricultural Trust, Rothamstead Experimental Station, UK). For the root sleeve data, where *F*- ratios were significant ($P < 0.05$), treatment means were compared by least significant differences (LSD) in a one way analysis of variance (ANOVA). Complete ANOVA results are presented in Appendix 2.1.

For the root sampling experiment data, distance from tree was omitted from the statistical analysis as it had no significant effect. Where *F*- ratios were significant ($P < 0.05$), treatment and interaction means were compared by least significant differences (LSD) in a general ANOVA. Pairs of means and LSDs are given in a separate table to highlight the significant interactions described in the results. Complete ANOVA results are presented in Appendix 2.2. Relationships between phosphatase enzyme activity and microbial activity or P types were determined by fitting the data to linear regression functions. Relationships were significant at $P < 0.05$ and importance was determined by regression coefficient ($R^2 > 0.65$).

2.3 Results

2.3.1 Root Sleeve Experiment

Results for soil analysis carried out on root sleeve and control soils after seven months are shown in Table 2.2. There was a consistent small decline in soil pH from 5.5 in the control to 5.2 in the rhizosphere soils. Concentrations of total P and organic P did not differ significantly between rhizospheres or control. Acid phosphomonoesterase activities determined in rhizosphere and control soils were markedly higher than the corresponding alkaline phosphomonoesterase activities. Rates of phosphomonoesterase activity were significantly higher in the rhizosphere of radiata pine (325 μmol/g/hr) and

eucalypt (354 μmol/g/hr) compared with the control soil (255 μmol/g/hr) and the macrocarpa rhizosphere soil (191 μmol/g/hr). On the other hand, alkaline phosphomonoesterase activities were significantly higher in the radiata pine and macrocarpa rhizosphere soils (44-47 μmol/g/hr) compared with the control and eucalypt rhizosphere soils (34-36 μmol/g/hr).

Table 2.3 shows the ectomycorrhizal colonization of eucalypt and radiata pine roots in each sleeve. There was no EM colonization of macrocarpa roots. Eucalypt consistently had more EM tips than radiata pine but colonization of both species varied greatly. Tip numbers varied from approximately 0-765 EM tips per sleeve in radiata pine with 0 tips on older, not actively growing roots and 604-1561 EM tips per sleeve in eucalypt. Only one EM morphotype was found on each species. Figure 2.7a shows the radiata pine morphotype to be smooth and brown to dark brown with a matte luster and irregular branching while Figure 2.7b shows the eucalypt morphotype to be smoother, thinner, a lighter brown, unramified with straight ends and a matte luster. Roots of all three species were assessed for arbuscular mycorrhizal colonization but could not be sufficiently cleared for accurate observations to be made.

Table 2.3 Mean values for soil pH, total P, organic P and phosphomonoesterase enzyme activities determined in the root sleeve experiment. Values in parenthesis are standard errors of mean, n = 8.

Soil Property	Radiata pine	Eucalypt	Macrocarpa	Control	significance
pH	5.2	5.2	5.2	5.5	$P<0.001$
	(0.01)	(0.01)	(0.01)	(0.01)	LSD = 0.02
Total P mg/kg	602	602	599	573	NSD
	(5.4)	(11.1)	(8.1)	(8.5)	
Total organic P mg/kg	518	523	536	513	NSD
	(6.2)	(10.7)	(7.9)	(8.3)	
Acid PME μmol/g/hr	325	354	191	255	$P<0.001$
	(6.6)	(10.1)	(4.9)	(2.3)	LSD = 19.1
Alkaline PME μmol/g/hr	43.6	36.4	47.4	34.4	$P<0.001$
	(1.93)	(0.52)	(1.88)	(0.63)	LSD = 4.08

PME = phosphomonoesterase
LSD = Least Significant Difference (5%), NSD = No significant difference

Table 2.4 Ectomycorrhizal (ECM) roots tips observed per cm of radiata pine and eucalypt root and approximate number of ECM tips in each sleeve in the root sleeve study.

Species	ECM tips/cm root	Approx ECM tips/sleeve
Radiata pine	2.0	270
	5.1	765
	0.0*	0
	0.0*	0
	1.6	149
	4.3	602
	3.4	357
	2.8	440
Eucalypt	18.1	1087
	11.6	1006
	9.1	983
	13.4	1073
	10.1	1459
	5.8	1199
	6.8	1033
	11.0	1561

*** no ECM root tips due to roots being older and not actively growing.**

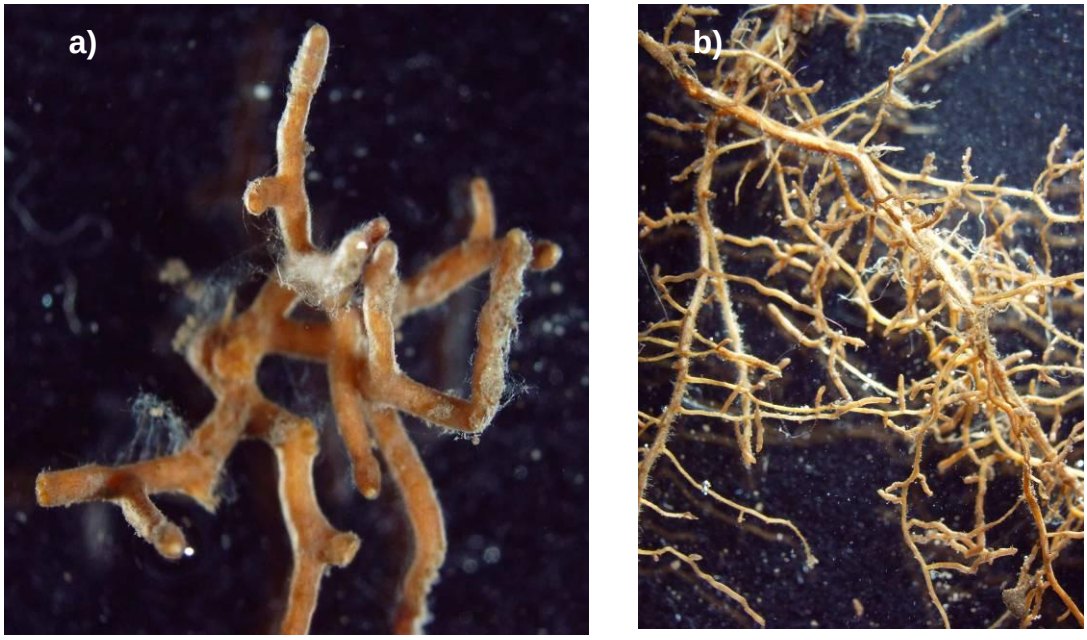

Figure 2.7 Ectomycorrhizal root tip morphotypes of (a) radiata pine and (b) eucalypt found in the root sleeve study.

There was a significant positive correlation between acid phosphomonoesterase activity and ECM colonization in radiata pine and eucalypt (Figure 2.8). The correlation was more significant (P<0.001) and accounted for a larger proportion of acid phosphomonoesterase activity variation (R^2 = 0.80) in radiata pine rhizosphere which had a wider range of tips per sleeve although it appears to be a causal relationship in both species (P = 0.002; R^2 = 0.64 for eucalypt). There was no such correlation with alkaline phosphomonoesterase activity.

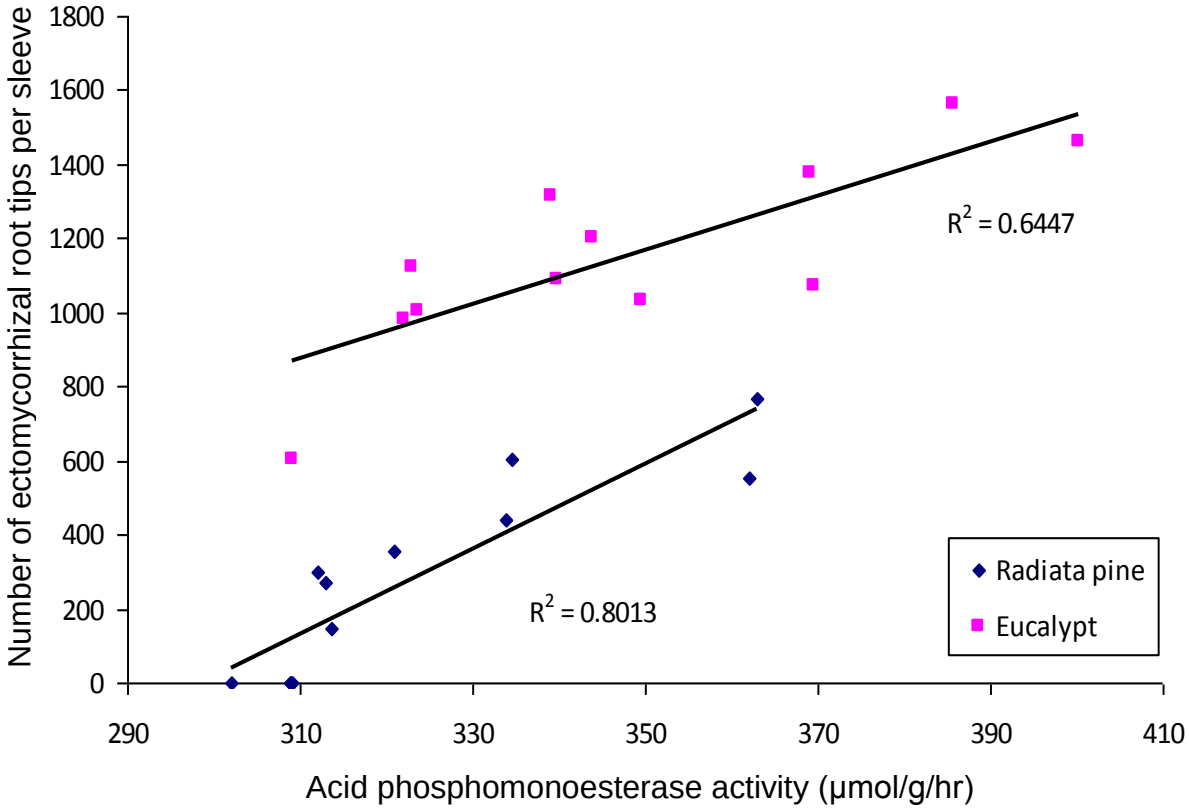

Figure 2.8 Correlations of acid phosphomonoesterase activity with the number of ectomycorrhizal root tips per sleeve found in radiata pine and eucalypt in the root sleeve experiment.

2.3.2 Root Sampling Experiment

Fine root biomass for all three species at both depths and distances are presented in Table 2.4. Distance from tree did not have a large effect on fine root biomass but there was a significantly higher biomass at 0-10 cm compared with 10-15 cm for all species with 63%, 66% and 76% of total fine roots found at this depth for radiata pine, eucalypt and macrocarpa, respectively. At 0-10 cm macrocarpa fine root biomass (8.6 kg/m^3) was significantly (P<0.001) greater than eucalypt (5-5.6 kg/m^3) which in turn was significantly greater than radiata pine (4.4 kg/m^3). Values did not differ significantly between species at 10-15 cm.

Table 2.5 Mean fine root biomass (<2mm) determined for radiata pine, eucalypt and macrocarpa at 50 cm and 100 cm from tree base and 0-10 cm and 10-15 cm depths in the root sampling experiment. Values in parenthesis are standard errors of means, n = 3.

Species	Distance (cm)	Root biomass (dw, kg/m^3)		
		0 – 10 cm	10-15 cm	Total
Radiata pine	50	4.4 (0.02)	2.7 (0.09)	7.1 (0.10)
	100	4.4 (0.03)	2.4 (0.03)	6.8 (0.05)
Eucalypt	50	5.6 (0.19)	2.8 (0.06)	8.4 (0.24)
	100	5.0 (0.29)	2.6 (0.09)	7.6 (0.24)
Macrocarpa	50	8.6 (0.07)	2.8 (0.35)	11.4 (0.33)
	100	8.6 (0.03)	2.7 (0.17)	11.3 (0.20)

Soil properties of rhizosphere and bulk soils are shown in Table 2.5. Eucalypt soils have a slightly lower pH (5.1) than radiata pine (5.4) which in turn has a slightly lower pH than macrocarpa (5.7). Only the rhizosphere of macrocarpa consistently acidified the soil compared to non rhizosphere soil and this was by an average of 0.2 units. Organic matter concentration was greater in soil under macrocarpa compared with radiata pine and eucalypt, although concentrations were generally similar in rhizosphere and non rhizosphere soil under each species.

Table 2.6 Mean pH, moisture and organic matter determined in rhizosphere and non rhizosphere (NR) soil taken from the root sampling experiment. Values in parenthesis are standard errors of mean, n = 3. Those without are values from bulked replicates.

Species	Distance from tree (cm)	Soil depth (cm)	Soil type	pH	Soil moisture (%)	Organic matter (%)
Radiata pine	50	0-10	Rhizo	5.4	9.0	11.7 (0.88)
	50		NR	5.4	9.4	11.7 (0.88)
	100		Rhizo	5.4	9.5	10.7 (0.67)
	100		NR	5.5	8.2	10.7 (0.67)
	50	10-15	Rhizo	5.4	8.2	11.7 (0.88)
	50		NR	5.4	7.5	11.7 (0.88)
	100		Rhizo	5.4	6.8	10.7 (0.67)
	100		NR	5.5	7.6	10.7 (0.67)
Eucalypt	50	0-10	Rhizo	4.9	10.0	9.7 (1.67)
	50		NR	5.1	11.0	9.7 (1.67)
	100		Rhizo	4.9	9.1	11.3 (0.67)
	100		NR	5.1	10.1	11.3 (0.67)
	50	10-15	Rhizo	5.1	8.0	9.7 (1.67)
	50		NR	5.1	8.7	9.7 (1.67)
	100		Rhizo	5.2	9.1	11.3 (0.67)
	100		NR	5.2	8.8	11.3 (0.67)
Macrocarpa	50	0-10	Rhizo	5.5	9.3	12.7 (0.33)
	50		NR	5.8	9.9	12.0 (0.00)
	100		Rhizo	5.7	9.1	14.3 (1.33)
	100		NR	5.9	10.1	13.0 (0.00)
	50	10-15	Rhizo	5.6	8.8	12.7 (0.33)
	50		NR	5.7	8.2	12.3 (0.33)
	100		Rhizo	5.7	8.9	13.0 (0.00)
	100		NR	5.9	8.5	12.3 (0.33)

Table 2.6 displays the concentrations of all P types in rhizosphere and non rhizosphere soils from all species, depths and distances. Distance from the tree had no significant effect on P types therefore Table 2.7 gives mean concentrations from both distances of all P types in rhizosphere and non rhizosphere soil at both depths and also gives P concentrations in the litter layer of radiata pine. Statistical analysis is performed without

distance. Means and LSDs for the significant effects and interactions of each parameter are presented separately in Table 2.8.

All three species soils contained significantly different concentrations of total P with radiata pine containing the most (610 mg/kg) followed by macrocarpa (538 mg/kg) and then eucalypt (469 mg/kg) There was no significant difference in total P between rhizosphere and non rhizosphere soils. Concentration of total P in both radiata pine and macrocarpa soils declined significantly at 10-15 cm whereas there was no such decline with depth in eucalypt soils (Figure 2.9). Depth was the only influencing factor on the concentration of total organic P in this system with significantly less at 0-10 cm than 10-15 cm.

Between species differences in resin P concentration were small but significant (Figure 2.10; Table 2.8). Resin P significantly decreased at 10-15 cm compared to 0-10 cm but this difference was only apparent in rhizosphere soils as non rhizosphere soil values did not differ between depths. The difference between rhizosphere depth values is small at 3 mg/kg. Resin P was markedly higher in radiata pine litter at 131.1 mg/kg compared to that in the underlying soil with an average of 9.8 mg/kg.

Figure 2.10 shows that radiata pine soil had a significantly, and almost 2-fold, higher concentration of microbial P (18 mg/kg) than macrocarpa (11 mg/kg) and eucalypt (10 mg/kg) and significantly higher concentrations were in both rhizosphere and non rhizosphere soils. This makes the radiata pine soil microbial:resin P ratio 2 fold higher (1.9) than eucalypt and macrocarpa (0.9). Rhizosphere soils had a consistently higher microbial P concentration compared to non rhizosphere soils at both depths and for all species. Radiata pine rhizosphere soil had an average of 6.1 mg/kg more microbial biomass P than non rhizosphere soil, eucalypt rhizosphere 7.7 mg/kg more and macrocarpa rhizosphere, 6.7 mg/kg more. At 10-15 cm there was significantly less microbial P compared to 0-10 cm but this difference was only in rhizosphere soils as bulk soil values were not affected by depth. Rhizosphere microbial P concentration declined by an average of 7 mg/kg at 10-15 cm. Microbial biomass P was markedly higher in radiata pine litter than the underlying soils.

Table 2.7 Mean concentrations of resin, microbial, organic and total P determined in rhizosphere and non rhizosphere (NR) soils taken from the root sampling experiment. Values in parenthesis are standard errors of means, n = 3.

Species	Distance From tree (cm)	Soil depth (cm)	Soil type	Resin P mg/kg	Microbial P mg/kg	Organic P mg/kg	Total P mg/kg
Radiata pine	50	0-10	Rhizo	13.9 (1.5)	20.1 (4.1)	270 (44.0)	692.2 (17.5)
	50		NR	8.2 (0.7)	14.6 (3.8)	305 (37.7)	726.9 (5.4)
	100		Rhizo	11.7 (0.2)	26.7 (4.7)	300 (37.6)	667.6 (8.2)
	100		NR	8.4 (0.2)	14.0 (1.1)	335 (48.1)	688.1 (30.3)
	50	10-15	Rhizo	11.1 (0.1)	22.3 (6.3)	317 (34.1)	497.7 (3.6)
	50		NR	8.5 (0.3)	18.3 (4.8)	331 (20.7)	516.1 (7.1)
	100		Rhizo	8.6 (0.1)	15.5 (2.4)	346 (7.3)	550.0 (11.4)
	100		NR	7.1 (0.4)	13.3 (0.9)	345 (9.0)	538.7 19.5)
Eucalypt	50	0-10	Rhizo	13.9 (0.5)	19.1 (3.1)	222 (16.6)	448.6 (19.7)
	50		NR	10.4 (0.7)	9.2 (1.7)	269 (1.8)	491.6 (3.6)
	100		Rhizo	14.1 (0.7)	16.4 (4.2)	270 (54.2)	469.1 (25.2)
	100		NR	11.6 (0.9)	5.0 (2.5)	350 (57.9)	528.4 (16.3)
	50	10-15	Rhizo	11.1 (0.5)	10.5 (0.5)	317 (17.8)	475.2 (45.2)
	50		NR	9.6 (0.6)	5.9 (1.5)	314 (25.7)	454.7 (21.6)
	100		Rhizo	10.2 (0.5)	9.5 (0.8)	285 (15.4)	471.1 (17.5)
	100		NR	9.76 (0.7)	4.6 (0.7)	308 (15.3)	413.8 (22.8)
Macrocarpa	50	0-10	Rhizo	15.5 (0.2)	17.0 (2.4)	266 (15.2)	526.4 (27.5)
	50		NR	11.8 (0.6)	4.8 (2.1)	274 (15.7)	567.3 (31.0)
	100		Rhizo	15.0 (0.7)	20.3 (1.8)	274 (11.6)	628.7 (74.9)
	100		NR	10.1 (0.2)	12.0 (4.9)	272 (7.2)	679.9 (60.4)
	50	10-15	Rhizo	13.0 (0.6)	9.3 (1.4)	350 (2.3)	487.5 (5.4)
	50		NR	12.1 (0.4)	6.0 (0.6)	299 (13.6)	510.0 (3.6)
	100		Rhizo	12.2 (0.4)	10.4 (1.1)	283 (24.9)	448.6 (16.3)
	100		NR	10.2 (0.5)	6.7 (2.3)	309 (14.7)	456.8 (25.2)

Table 2.8 Mean concentrations of resin, microbial, organic and total phosphorus determined in radiata pine litter and rhizosphere and non rhizosphere (NR) soil of radiata pine, eucalypt and macrocarpa. Values are the mean of both distances in the root sampling experiment. Values in parenthesis are standard errors of means, n = 6 and n = 4 for litter.

Species	Depth	Type	Resin P (mg/kg)	Microbial P (mg/kg)	Organic P (mg/kg)	Total P (mg/kg)
Radiata pine	0-10cm	Rhizo	12.8 (0.83)	23.4 (3.17)	285 (26.8)	680 (10.2)
		NR	8.3 (0.32)	14.3 (1.78)	320 (28.2)	708 (16.3)
	10-15cm	Rhizo	9.9 (0.57)	18.9 (3.38)	332 (17.0)	524 (13.0)
		NR	8.3 (0.38)	15.8 (2.46)	338 (10.6)	527 (10.6)
		litter	*131.1 (7.03)*	*88.8 (4.1)*	-	*575 (16.8)*
Eucalypt	0-10cm	Rhizo	14.0 (0.39)	17.7 (2.41)	246 (27.5)	459 (15.0)
		NR	11.0 (0.39)	7.1 (1.64)	309 (31.6)	510 (11.1)
	10-15cm	Rhizo	9.9 (0.37)	10.0 (0.48)	301 (12.6)	473 (21.7)
		NR	7.8 (0.40)	5.2 (0.79)	311 (13.5)	434 (16.8)
Macrocarpa	0-10cm	Rhizo	15.2 (0.35)	18.6 (1.54)	270 (8.8)	578 (42.3)
		NR	11.0 (0.48)	8.4 (2.88)	273 (7.7)	624 (39.4)
	10-15cm	Rhizo	12.6 (0.38)	9.8 (0.83)	316 (18.8)	468 (11.6)
		NR	11.1 (0.50)	6.4 (1.09)	304 (9.2)	483 (16.5)

Table 2.9 Means and 5% LSDs of important significant effects and interactions for each soil P factor in the root sampling experiment.

	Interaction	significance	Means (mg/kg)				LSD
Total P	Species*depth	$P<0.001$		*Radiata*	*Eucalypt*	*Macro*	42.9
			0-10 cm	694	484	601	
			10-15cm	524	454	476	
Organic P	Depth	$P = 0.005$	*0-10 cm*	284			22.6
			10-15 cm	317			
Resin P	Species	$P<0.001$	*Radiata*	*Eucalypt*	*Macro*		0.68
			9.7	11.3	12.5		
	Soil type*depth	$P<0.001$		*Rhizo*	*Non rhizo*		0.79
			0-10cm	14.0	10.1		
			10-15 cm	11.0	9.5		
Microbial P	Species	$P<0.001$	*Radiata*	*Eucalypt*	*Macro*		3.0
			18.1	10.0	10.8		
	Soil type*depth	$P = 0.013$		*Rhizo*	*Non rhizo*		3.4
			0-10 cm	19.9	9.9		
			10-15 cm	12.9	9.1		

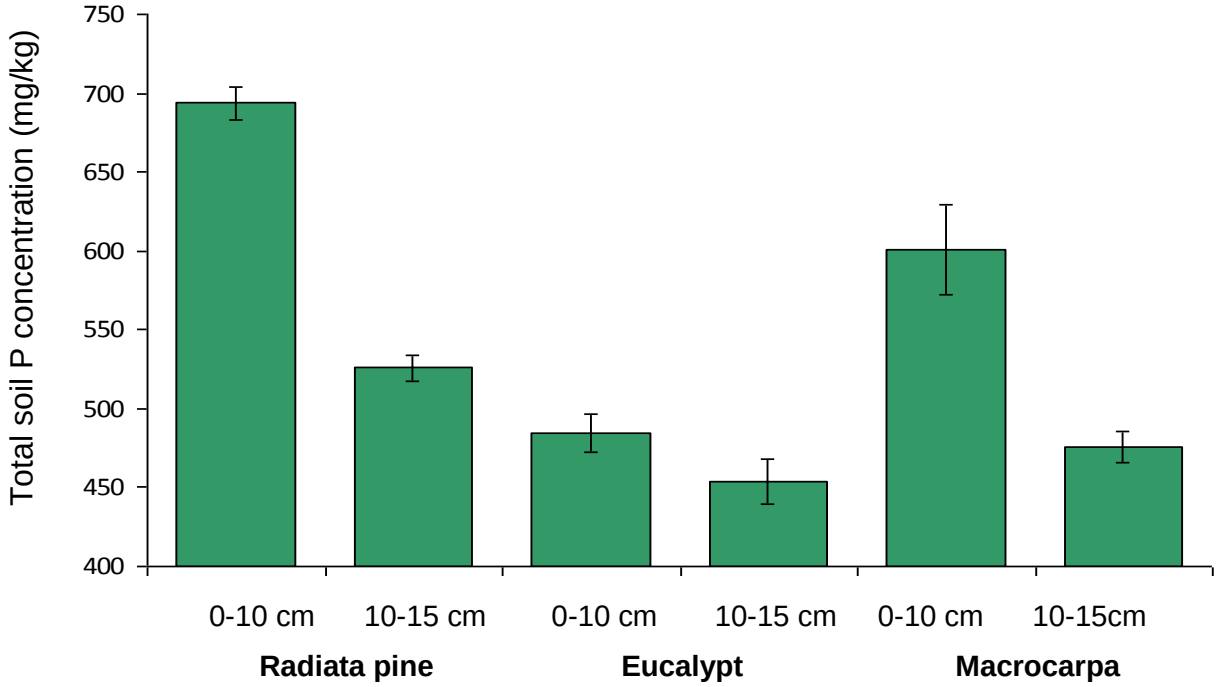

Figure 2.9 Mean concentrations of total soil P determined at 0-10 cm and 10-15 cm for all species in the root sampling experiment. Bars represent standard errors of means, n = 12 for soil.

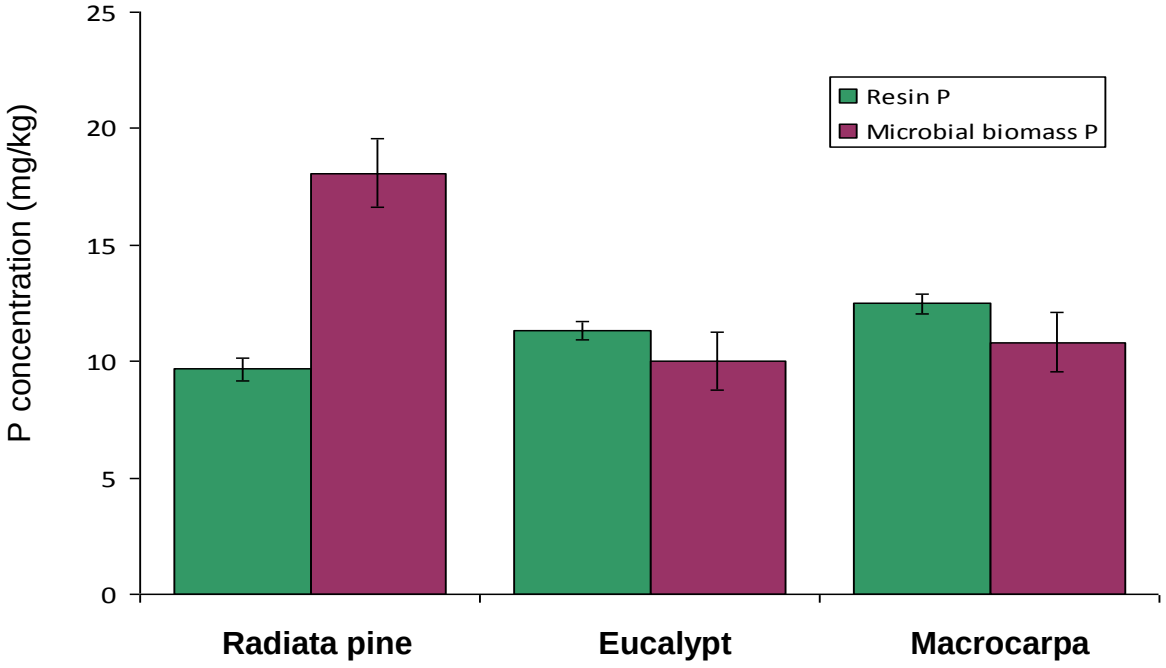

Figure 2.10 Mean microbial biomass and resin P amounts determined for radiata pine, eucalypt and macrocarpa soil in the root sampling experiment. Bars represent standard error of means, n = 24.

Table 2.9 shows mean microbial activity and phosphatase enzyme activities for rhizosphere and non rhizosphere soil of all three species at both distances and depths. Due to lack of rhizosphere soil for analysis, only one sample, containing an even mixture of all replicates was analyzed for microbial activity. Distance from tree bole did not have a significant effect on any of the variables tested and so Table 2.10 gives soil values bulked across distances and values from the radiata pine litter layer for comparison. Means and LSDs for important significant effects and interactions are presented separately for each variate in Table 2.11.

At 0-10 cm radiata pine (4.0 μgTPF/g/hr) and eucalypt soil activity (4.4 μgTPF/g/hr) was significantly lower than macrocarpa soil activity (5.3 μgTPF/g/hr) accounting for the significantly higher macrocarpa activity overall but at 10-15 cm only radiata pine activity was significantly lower accounting for its lower microbial activity overall than eucalypt. Species differences did not interact with soil type showing that the differences between species were observed in rhizosphere and non rhizosphere soil. Activity was significantly decreased at 10-15 cm but only in macrocarpa and radiata pine soils (Figure 2.11) and this difference was only in rhizosphere soil values as non rhizosphere soil activity levels were not affected by depth.

Table 2.10 Mean microbial activity, acid phosphomonoesterase activity and phosphodiesterase activity determined in rhizosphere and non rhizosphere (NR) soil in the root sampling experiment. Values in parenthesis are standard errors of means, n = 3. Those without are values from bulked replicates.

Species	Distance from tree (cm)	Soil depth (cm)	Soil type	Microbial Activity (µgTPF/g/hr)	PME (µmol/ g/hr)	PDE (µmol/g/hr)
Radiata pine	50	0-10	Rhizo	4.4	410 (16.9)	5.4 (0.5)
	50		NR	3.4	283 (12.9)	2.6 (0.1)
	100		Rhizo	4.8	395 (4.7)	4.7 (0.1)
	100		NR	3.3	283 (4.8)	2.9 (0.1)
	50	10-15	Rhizo	3.7	335 (20.2)	3.9 (0.2)
	50		NR	3.5	296 (22.7)	2.1 (0.1)
	100		Rhizo	3.6	355 (17.1)	3.8 (0.1)
	100		NR	3.2	295 (10.1)	2.1 (0.1)
Eucalypt	50	0-10	Rhizo	5.1	469 (11.2)	5.6 (1.1)
	50		NR	3.5	324 (32.8)	3.7 (0.4)
	100		Rhizo	5.1	454 (4.6)	4.8 (0.7)
	100		NR	3.7	302 (50.3)	3.6 (0.2)
	50	10-15	Rhizo	4.8	347 (3.3)	3.7 (0.2)
	50		NR	3.7	285 (6.8)	2.3 (0.2)
	100		Rhizo	4.4	343 (2.7)	4.4 (0.4)
	100		NR	3.5	285 (13.6)	2.2 (0.1)
Macrocarpa	50	0-10	Rhizo	6.0	462 (7.4)	5.5 (0.1)
	50		NR	3.9	286 (21.0)	3.3 (0.2)
	100		Rhizo	6.3	463 (12.6)	5.8 (0.6)
	100		NR	4.9	292 (32.8)	3.8 (0.4)
	50	10-15	Rhizo	4.9	355 (3.8)	4.2 (0.2)
	50		NR	4.8	288 (8.4)	2.3 (0.1)
	100		Rhizo	4.4	356 (4.7)	4.4 (0.4)
	100		NR	3.7	284 (4.1)	2.7 (0.4)

DHE = dehydrogenase, PME = phosphomonoesterase, PDE = phosphodiesterase

Table 2.11 Mean microbial activity, acid phosphomonoesterase activity and phosphodiesterase activity determined in radiata pine litter, rhizosphere and non rhizosphere (NR) soil. Values are means from both distances in the root sampling experiment. Values in parenthesis are standard errors of means, n = 6 except microbial activity where n = 2.

Species	Soil depth	Soil type	Microbial Activity (µgTPF/g/hr)	PME (µmol/ g/hr)	PDE (µmol/g/hr)
Radiata pine	0-10cm	Rhizo	4.6 (0.20)	402 (8.5)	5.1 (0.28)
		NR	3.3 (0.08)	283 (6.2)	2.7 (0.11)
	10-15cm	Rhizo	3.6 (0.07)	345 (12.6)	3.8 (0.16)
		NR	3.4 (0.16)	295 (11.1)	2.1 (0.03)
	litter		*29.9 (2.21)*	*751 (29.2)*	*15.5 (0.67)*
Eucalypt	0-10cm	Rhizo	5.1 (0.01)	462 (6.3)	5.2 (0.64)
		NR	3.6 (0.10)	313 (27.3)	3.7 (0.19)
	10-15cm	Rhizo	4.6 (0.16)	345 (2.1)	4.0 (0.25)
		NR	3.6 (0.07)	285 (11.6)	2.2 (0.10)
Macrocarpa	0-10cm	Rhizo	6.2 (0.14)	462 (6.5)	5.6 (0.29)
		NR	4.4 (0.49)	289 (17.4)	3.6 (0.24)
	10-15cm	Rhizo	4.7 (0.27)	355 (2.7)	4.3 (0.19)
		NR	4.2 (0.56)	286 (4.3)	2.5 (0.20)

DHE = dehydrogenase, PME = phosphomonoesterase, PDE = phosphodiesterase

Rhizosphere soils consistently had a higher acid phosphomonoesterase activity than non rhizosphere soils regardless of depth and species. Across both soil types and depths, radiata pine had a significantly lower acid phosphomonoesterase activity (332 µmol/g/hr) than eucalypt (351 µmol/g/hr) and macrocarpa (348 µmol/g/hr) but Figure 2.12 shows that this difference was only apparent at 0-10 cm as activity did not vary with species at 10-15 cm. Radiata pine soil activity was not affected by depth in soil whereas both eucalypt and macrocarpa activity decreased significantly at 10-15 cm but these depth differences were only apparent in rhizosphere soil as non rhizosphere soil activity did not differ between 0-10 cm and 10-15 cm. Differences observed between species were present in both rhizosphere and non rhizosphere soil.

Table 2.12 Means and LSDs of important significant effects and interactions for each soil enzyme activity in the root sampling experiment.

	Interaction	Significance	Means				LSD
Microbial activity (µgTPF/g/hr)	Species*depth	0.028		*Radiata*	*Eucalypt*	*Macro*	0.301
			0-10 cm	3.95	4.32	5.28	
			10-15cm	3.49	4.10	4.45	
	Soil type*depth	<0.001		*Rhizo*	*Non rhizo*		0.246
			0-10 cm	5.27	3.76		
			10-15 cm	4.30	3.72		
Acid PME (µmol/g/hr)	Species*depth	0.011		*Radiata*	*Eucalypt*	*Macro*	23.1
			0-10 cm	343	387	376	
			10-15 cm	320	315	321	
	Soil type*depth	<0.001		*Rhizo*	*Non rhizo*		18.8
			0-10cm	442	295		
			10-15 cm	349	289		
PDE (µmol/g/hr)	Species	0.014	*Radiata*	*Eucalypt*	*Macro*		0.375
			3.43	*3.77*	*3.99*		
	Depth	<0.001	*0-10 cm*	4.30			0.306
			10-15 cm	3.16			
	Soil type	<0.001	*Rhizo*		*Non rhizo*		0.306
			4.67		2.79		

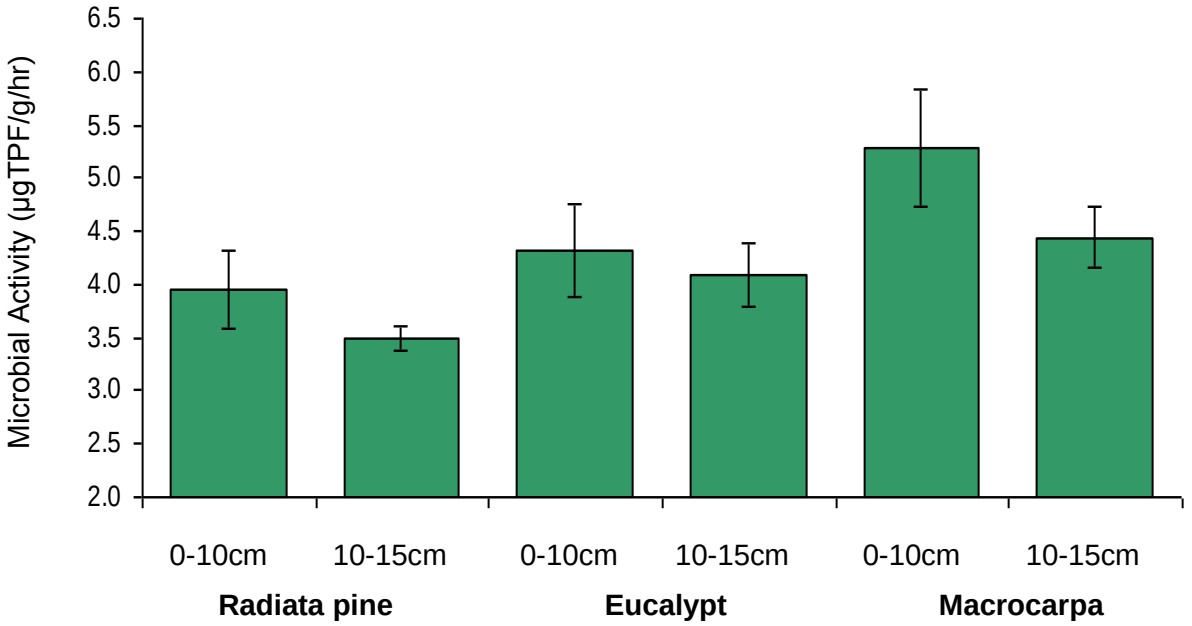

Figure 2.11 Microbial activity determined at 0-10 cm and 10-15 cm for all species in the root sleeve experiment. Bars represent standard errors of means, n = 12.

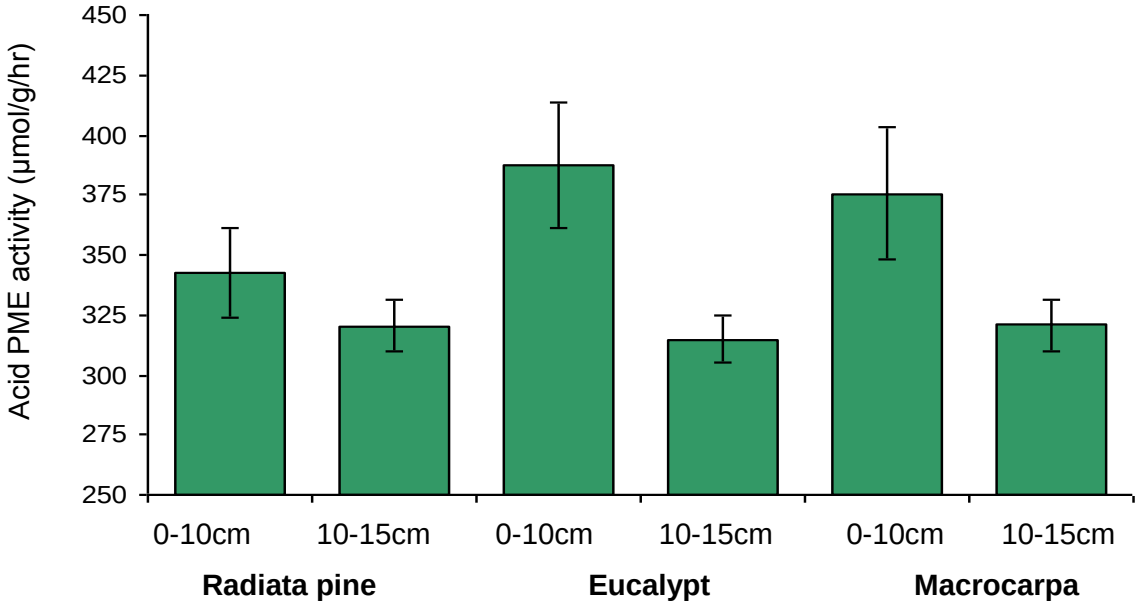

Figure 2.12 Mean acid phosphomonoesterase activity determined at 0-10cm and 10-15cm for all species in the root sampling experiment. Bars represent standard errors of means, n = 12.

Phosphodiesterase activity was consistently much lower than the corresponding phosphomonoesterase activity and all three species soil activity followed the same trends. There was almost a 2 fold increase in rhizosphere soil activities compared to the corresponding non rhizosphere soil activity and a significant drop in activity at 10-15 cm compared to 0-10 cm for all three species. Across both depths and soil types, radiata pine soil (3.4 µmol/g/hr) had a significantly lower phosphodiesterase lower activity than eucalypt (3.8 µmol/g/hr) and macrocarpa soil (4.0 µmol/g/hr) but this difference was very minor although statistically significant.

2.4 Discussion

2.4.1 Root Sleeve Experiment

The purpose of this experiment was to investigate short term rhizosphere P dynamics of seven year old radiata pine, eucalyptus and macrocarpa trees. An artificial rhizosphere was created with the same fresh pasture soil, placed at the same depth but with differing tree species roots forming the rhizosphere therefore it is reasonable to assume that all differences in P concentrations and rhizosphere processes are related to the tree.

Total P and organic P concentrations in rhizosphere soils were at similar levels between species and in the control, hence in the short term, these tree species did not influence total P or total organic P concentrations in rhizosphere soil. This is to be expected as total soil P and soil organic P are large pools, comprising many P forms and as a result, measurement of these is not a useful measure for mineralization (Condron et al., 2005). Phosphatase enzymes are thought to control the hydrolysis of organic P (McGill and Cole, 1981; Magid et al., 1996) and so are better measures of mineralization rates and organic P utilization in a system than simply measuring changes in soil P concentration.

As expected, significant differences in phosphomonoesterase activity were observed between species in this experiment. The short term nature of the experiment indicates that the phosphatase differences between species are a result of differing responses of the trees to organic P. Previous afforestation studies indicate that the pasture soil would have contained more organic P and less inorganic P than the soil in which the trees were previously growing in (Condron et al., 1996; Chen et al., 2000a) and this study was designed to measure the response of the different trees to this. The *P* nitrophenol substrate enzyme assay used in this experiment detects all soil phosphatases: residual, such as those attached to debris and those immobilized by clay and/or humic colloids (Burns, 1986) and response i.e. those actively produced as a response to P demand (Speir and Ross, 1978). It is reasonable to assume residual phosphatase levels in the soil were the same upon rhizosphere establishment as the soil did not differ in any of the factors that could affect residual enzyme levels such as plant species, clay content, organic matter content (Sinsabaugh, 1994), thus any difference in activity is likely a

result of the rhizosphere organisms response to the P amounts and forms in the new rhizosphere.

Mycorrhizal colonization is likely to be a major factor in the variability of phosphatase enzyme production between species. Eucalypt and radiata pine rhizosphere soil had the highest acid phosphomonoesterase activity. Radiata pine has an ectomycorrhizal association (Figure 2.7a), eucalypt can form associations with both types but appears to be predominantly ectomycorrhizal in this system (Figure 2.7b) and macrocarpa has an arbuscular mycorrhizal association. Ectomycorrhizal trees have been demonstrated to have a higher rhizosphere phosphatase activity than arbuscular mycorrhizal trees (Antibus et al., 1997; Philips and Fahey, 2006) and ectomycorrhizae have been demonstrated to increase plant P uptake compared to arbuscular mycorrhizae on the same host (Jones et al., 1998). Häussling and Marschner (1989) and Firsching and Claasen (1996) found phosphatase activity increases with degree of ectomycorrhizal colonization in Norway spruce. Similarly in this experiment, acid phosphatase activity correlated significantly with the number of ectomycorrhizal root tips per sleeve, a measure of ectomycorrhizal colonization, in both radiata pine and eucalypt (Figure 2.8). This correlation indicated the clear influence of ectomycorrhizae on P dynamics in these species. It is likely that the eucalypt mycorrhizal colonization was predominantly ectomycorrhizae as studies involving other eucalypt species have found that arbuscular mycorrhizae dominate initially with ectomycorrhizae succeeding over time (Lapeyrie and Chilvers, 1985; Chilvers et al., 1987; Lodge, 2000). Once ectomycorrhizae have established, new colonization of arbuscular mycorrhizae is made difficult with the additional ectomycorrhizal mantle to penetrate (Chilvers et al., 1987) and fine root production is decreased following ectomycorrhizal establishment thus decreasing new root surface for arbuscular colonization (Lodge, 2000).

Although arbuscular mycorrhizal colonization was not assessed, and so colonization of it and phosphatase enzyme activity could not be correlated, it can be safely said that the arbuscular mycorrhizal macrocarpa rhizosphere has much lower phosphatase activity at acid pH than the ectomycorrhizal eucalypt and radiata pine rhizospheres. However, it does along with radiata pine sleeves, have the highest activity at alkaline pH but it is unlikely that an alkaline enzyme has a significant effect in this more acidic soil. Acid

phosphomonoesterase has the highest activity, likely due to the optimum pH of the assay (6.5) being closer to the pH of the soil (5.2) than the alkaline assay (11) and the pH optima of soil phosphatase activities is often closely related the soil pH (Speir and Ross, 1978). This accounts for the lack of correlation between alkaline phosphomonoesterase and ectomycorrhizal colonization.

Neither phosphatase enzyme activity correlated with total organic P in this experiment which is to be expected due to the large, total organic P pool and the short term nature of the experiment. Chemically and physically protected forms of organic P such as inositol phosphates, protected by association with metal ions or precipitation with calcium, can form up to 90% of total organic P (Condron et al., 2005) and a solubilizing agent such as organic acids is needed to release the P from binding sites before hydrolysis can occur and this maybe the limiting factor on organic P utilization. The total organic P pool will also include phosphodiesters such as DNA, RNA and phospholipids which require phosphodiesterases to convert them to phosphomonoesters before phosphomonoesterase can mineralize them (Celi and Barberis, 2005). It is more likely that a redistribution of P types within the organic P pool occurred. Therefore specific measurement of the more labile organic P pool including sugar phosphates or of plant available solution P would probably provide values that better correlate with phosphomonoesterase activity in the soil although it is unlikely that a relationship would be observed in seven months. The decomposition of organic C can also release P as a byproduct (Speir and Ross, 1978) and so a lack of relationship between organic P and phosphatase enzymes may also mean that decomposition of organic C is a controlling factor in organic P mineralization.

The involvement of phosphomonoesterases in organic P mineralization is well established (Quiquampoix and Mousain, 2005) and so species differences in enzyme production warrant further investigation along with corresponding changes in available P on a long term basis to determine organic P use by the three species. The clear species differences in phosphomonoesterase activity demonstrate there are differences in potential organic P utilization and dynamics between the three species and the correlation between ectomycorrhizal colonization and activity of this enzyme shows that ectomycorrhizae play a big part in this. This study was essentially an artificial

rhizosphere and some of the soil in the sleeve was more akin to bulk soil due to the growth of roots being less than anticipated and so masked some of the rhizosphere effects and the short term nature of the experiment meant no changes were found in the large P pools investigated.

2.4.2 Root Sampling Experiment

Investigation involving small, dynamic P pools more indicative of changes in P cycling is necessary to determine species differences in rhizosphere processes that influence P utilization. Organic P mineralization is essentially microbially driven (Jakobsen et al., 2005) and so microbial activity and biomass P together with a measure of available P will demonstrate the importance of organic P to plant nutrition. Phosphodiesterase is potentially of great importance in a forest system as much of the organic P types in forests have been identified as phosphodiesters (Condron et al., 1996). To further the information gained from the short term root sleeve investigation, a root sampling experiment was conducted on field tree rhizospheres including the additional variables mentioned above to give a detailed picture of P dynamics in the rhizosphere of radiata pine, eucalypt and macrocarpa.

The primary aim of this study was to assess and quantify differences in rhizosphere P dynamics of radiata pine, eucalypt and macrocarpa trees by sampling rhizosphere and non rhizosphere soil around established trees to reveal the cumulative effects of nine years tree growth on these processes. Root distribution has been shown to vary spatially (e.g. Bouillet et al., 2002; Gautam et al., 2002; O'Grady et al., 2005) as a response to differing soil conditions and so it is hypothesized that rhizosphere processes will vary spatially also but little attention has been paid to the spatial distribution of rhizosphere processes in the field (Watt et al., 2006). Therefore this study compared horizontal and vertical distributions of rhizosphere processes and P dynamics under eucalypt, macrocarpa and radiata pine.

2.4.2.1 Spatial distribution of rhizosphere effects

The rhizosphere had an effect on all of the variables investigated except for total P and total organic P. This was to be expected and is consistent with many other studies that employed a similar method of rhizosphere soil collection (Häussling and Marschner,

1989; Scott and Condron, 2003; Philips and Fahey, 2006) and so it appears that this method of categorizing rhizosphere and non rhizosphere soil is realistic. The spatial distribution of rhizosphere effects followed similar trends across all species. Differences between rhizosphere soil values and corresponding non rhizosphere soil values decreased below 10 cm and were not largely affected by distance from the tree. Therefore between species differences in rhizosphere processes were usually only apparent in the top 10 cm of soil, a trend also observed by Chen et al. (2000a) in a comparison of P dynamics under pasture and radiata pine at different depths.

The spatial distribution of the rhizosphere effects can largely be explained by fine root distribution. Fine root biomass decreased significantly with depth (Table 2.4). This is not surprising as fine tree roots in forests are normally concentrated in litter layers and surface soil (Vogt et al., 1981; Sparling et al., 1994). Decreases in depth have been found by other studies involving similar species. For example, Nambier (1990) demonstrated in young pine plantations that fine root density declined rapidly with depth. Gautam et al. (2002) found a significant decrease in fine root density with depth in a radiata pine silvopastoral system in New Zealand. Chen et al. (2000a) found a marked decrease in fine root biomass of radiata pine with depth in an afforested system in New Zealand. The same trends have been found with *Eucalyptus globulus* in Tasmania (O'Grady et al., 2005) and *Eucalyptus* clones in Congo (Bouillet et al., 2002). Such a marked decline in root biomass with depth is perhaps surprising in macrocarpa plots due to competition from the pasture understory. Gautam (1998) found that trees grown with a ryegrass-clover pasture understory concentrated roots at 10-20 cm to avoid competition with the shallower pasture roots but this is not occurring in this trial as macrocarpa fine root biomass declined markedly below 10 cm and did not differ significantly from the other two species at this depth. Another mechanism must therefore be in place to avoid competition. This maybe an increase in roots at the tree bole at 0-10 cm where pasture competition is less intensive, a trend also observed by Gautam (1998), and so not detected in this experiment. Resource partitioning is also a likely mechanism to avoid competition between these vegetation types at this depth (Turner, 2008) where macrocarpa roots are adapted to efficiently utilize different forms of P to pasture roots and so can avoid competition without spatial separation.

Fine root biomass was not greatly influenced by distance from the tree. Distance had been shown to influence tree fine root biomass in the experiments described above but it was shown to decrease in importance with stand age in young *Eucalyptus globulus* forests up to 14 months old (O'Grady et al., 2005) or to not be of any significance in nine year old eucalyptus clone stands other than an increase in fine roots directly under the tree stump (Bouillet et al., 2002). It appeared that the main differences were found in soil next to and at distance from the tree bole and this may account for the lack of distance effect on fine root distribution in the present experiment. Lateral roots at tree boles for all species at the Orton Bradley Park site were extremely thick. Therefore a soil corer could not be used to sample at the tree bole efficiently thus tree bole fine roots were not measured in this study. The lack of distance effect on all of the soil parameters under investigation was initially a surprising result. Scott (2002) found that soil containing radiata pine trees grown with a ryegrass understory had higher levels of extractable organic P with increasing distance from the tree and when grown with a bare ground understory extractable organic P decreased with increasing distance from the tree. Extractable inorganic P had the opposite effect, demonstrating a change in P dynamics with tree distance. In this experiment macrocarpa trees had a pasture understory, eucalypt trees had a bare ground understory and so P spatial variability would be expected to be similar to that observed by Scott (2002). However, the distances measured in the Scott (2002) study were tree line and 3.5m from the tree line – a much greater distance than measured in this experiment.

2.4.2.2 Soil P concentrations

Concentrations of total P were not expected to differ between species, just the distribution of the P pools within that total. However, radiata pine soil had a significantly higher concentration of total P than macrocarpa soil which had a significantly higher concentration than eucalypt soils. This is likely explained by a combination of factors including differences in tree growth and understory composition between the three species after nine years growth.

The trees were planted into an established pasture; therefore during the initial stages of forest development the understory would have been vigorously growing pasture. The radiata pine trees, through thinning, pruning and litterfall, have shed a large amount of

slowly decomposing litter that, along with canopy closure, quickly shaded out the pasture. Figure 2.13 demonstrates that almost all of the pasture has now been replaced with a litter layer. Phosphorus contained in the pasture and also some contained in the pine litter would have been broken down and incorporated into the soil by the abundant microbes in litter layers (Attiwill and Adams, 1993; Saggar et al.,1998) and thus contributes to the higher amount of P in these soils. Pine needles contain a great deal of P compared to other forest litters (Conn and Dighton, 2000) and in this study the litter layer had a concentration of 583 mg/kg. Leaching of mainly organic types of P from litter to the underlying soil can account for a major proportion of the annual litterfall nutrient input of forest soils and Cortina et al. (1995) found that 6% of total P in the forest floor was leached into the underlying soil annually in a mature radiata pine stand. The significantly higher amount at 0-10 cm may simply be a result of the input from leaching through and decomposition of litter at these surface layers. The rooting characteristics of radiata pine when grown with pasture may accentuate this. Early in the Orton Bradley trial establishment, whilst the pasture persisted, much of the radiata pine roots may have been at 10-20 cm to avoid competition with the pasture (Gautam, 1998) and consequently would deplete P at this depth. Now the pasture is decomposed and the trees are growing alone, radiata pine rooting density is highest at 0-10 cm as this is where the labile P is continually being added from litter. Phosphorus input from litter is likely not penetrating past the surface soil layers due to its immobility (Holford, 1997) and so levels remain low at 10-15 cm.

Macrocarpa are slow growing trees with a lower biomass in this trial. The average volume of macrocarpa trees in this trial is 109 m^3/ha compared to 165 m^3/ha and 212 m^3/ha of eucalypt and radiata pine respectively. As a consequence, macrocarpa have not achieved complete canopy closure and pasture still forms the majority of this species understory even after nine years growth, despite some thinning debris (Figure 2.14). Pasture root litter decomposition is a faster process, therefore nutrients are released more rapidly from it into the soil than from leaf litter (Chen et al., 2002; Davis and Condron, 2002; Chen et al., 2008). Much of the P in the macrocarpa system will be contained within the pasture and this P, coupled with that from the thinning debris is continually being added to the soil thus contributing to the higher total P level in macrocarpa soils than the eucalypt soils which have almost no understory and also

explaining the significantly higher amount at 0-10 cm compared to 10-15 cm. Also, only 81 m^3/ha of this volume is live trees, therefore many of the trees will not be up taking P from the soil.

After nine years of growth the eucalypt trees eventually dominated the pasture and the understory is now predominantly bare earth with a small amount of eucalypt litter and some sparse pasture (Figure 2.15). Most of the pasture understory died, not due to shading as the canopy is not closed, but perhaps due to the fast growth and extensive moisture uptake of the trees, out-competing the pasture for this resource. Decreased soil moisture also lowers microbial activity (Diaz-Raviña et al., 1995; Chen et al., 2003a; Jakobsen et al., 2005) resulting in slower pasture decomposition. This would make the dead pasture susceptible to wind removal and slow incorporation of its nutrients into the soil – explaining why total P concentrations in eucalypt soil do not vary with depth. The eucalypt trees in this trial have a 34% higher biomass then macrocarpa trees and are also subject to a lower thinning rate than macrocarpa demonstrating the return of P from the tree to the soil is much lower. This low return rate and higher tree uptake, accounts for the lower P concentration in eucalypt soils compared to radiata pine and macrocarpa. Much of the P in eucalypt plots of this system is likely to be contained within the tree biomass.

Figure 2.13 The changing understory of radiata pine plots at the Orton Bradley Park afforestation trial site. a) 2004 where thinning evidence is present and a small amount of pasture remains. b) 2009 where pasture has completely gone and has been replaced by a litter layer.

Figure 2.14 The pasture understory of macrocarpa plots at the Orton Bradley Park afforestation trial site. a) 2004 where some litter is present but pasture is dominating the understory. b) 2009, where pasture is still dominant but not so tall and more thinning debris is present.

Figure 2.15 The changing eucalypt understory at the Orton Bradley Park afforestation trial site. a) 2004 where pasture understory was still present. b) 2009, where the pasture understory has gone and bare ground with some eucalypt litter forms the understory.

2.4.2.3 Soil biological and biochemical properties

Litter layer nutrient inputs should result in a higher concentration of organic P at 0-10 cm and the different and changing state of the understories of the species discussed above should result in different concentrations of organic P between soils under each species. However, soil under all three species did not differ significantly in organic P concentrations and all species have significantly lower concentrations at 0-10 cm indicating utilization of organic P by each species but to different extents. Organic forms of P have been shown to be depleted by trees in many studies (e.g. Turner and Lambert, 1985; Adams et al., 1989; Davis and Lang, 1991; Magid, 1993; Condron et al., 1996; Chen et al., 2000a).

Much of the organic matter and associated nutrients released from decomposition of pasture has been shown to be labile organic forms (Condron and Newman, 1998) and have a lower C:N and C:P ratio (Chen et al., 2000a) making them more bioavailable substrates supporting a larger and more active microbial biomass (Yeates et al., 1997). The turnover of organic P is essentially microbially mediated (Jakobsen et al., 2005; Oberson and Joner, 2005) so the increased microbial biomass and activity resulting from decomposing pasture under radiata pine, coupled with the high P demand and uptake of the trees before canopy closure (Chen et al., 2000a) would have driven a higher rate of organic P mineralization in earlier stages under this species. The P in these soils had likely been redistributed from labile organic pools to more inorganic and recalcitrant forms as found in other studies investigating effects of radiata pine on pasture soils (e.g. Davis and Lang, 1991; Condron et al., 1996; Chen et al., 2000a).

Now the radiata pine plots have a typical plantation floor with a substantial litter layer of dead material and green material from thinning practices (Girisha et al., 2003; Figure 2.13 and 2.16). The litter layer ranges from 2-11 cm in thickness, depending on slope and thinning debris. There was no obvious segregation of litter into L, F and H layers but there was a significant change between the litter at the surface and litter at the interface with the soil (Figure 2.16). Litter layers of *Pinus* species are known to contain a significant amount of P and to act as a source and a sink of P in the closed P cycle of a forest system (Comerford and De Barros, 2005). Resin P in the radiata pine litter was 10-15 times higher than that in the underlying soil, a finding similar to than of Chen et

al. (2000a) who found resin extractable P to be 3-10 times higher in the litter layer of radiata pine than the underlying mineral soil in another New Zealand study. Therefore most of the labile P in the radiata pine system is in the litter layer. The lowest microbial and phosphatase enzyme activity levels in radiata pine soil are also likely attributed to the litter layer influence over these soils. It is well known that forest floor material supports high levels of microbial activity involved in the decomposition and release of nutrients from it (Attiwill and Adams, 1993; Saggar et al., 1998) and microbial activity in pine litter from this study was approximately seven times higher than that in the underlying soil. In addition, organic matter proportion of the soil did not differ with depth despite inputs to the surface layers from pasture indicating high rates of decomposition at the surface layers. Chen et al. (2000a) also found that soils of radiata pine have a low microbial activity and phosphatase activity in the mineral soil compared to that in the litter layer above.

Resin P was significantly correlated with acid phosphomonoesterase (P = 0.005; R^2 = 0.75) and phosphodiesterase (P = 0.001; R^2 = 0.85) activity in radiata pine demonstrating the importance of these enzymes for increasing levels of available P in radiata pine soils and that the low level in radiata pine soils was not a result of negative feedback (Speir and Ross, 1978; Fox and Comerford, 1992). Some P can be directly taken up from the litter layer (Attiwill and Adams, 1993), effectively bypassing some of the need for rhizosphere processes in radiata pine soil. Ectomycorrhizae have been shown to increase levels of phosphatase enzymes (Häussling and Marschner, 1989; Adams, 1992; Pasqualini et al., 1992; Liu et al., 2004a, b; 2005) and are considered to be more effective at doing so than arbuscular mycorrhizae (Joner and Jakobsen, 1995; Joner et al., 1995). Consequently, for ectomycorrhizal radiata pine to have had a lower soil phosphatase activity level than arbuscular mycorrhizal macrocarpa and arbuscular and ectomycorrhizal eucalypt it is likely that the active hyphae were in the litter layer where the labile P is concentrated. Perez Moreno and Read (2000) demonstrated that mycorrhizal hyphae utilize P direct from the litter layer and this maybe an important pathway for plant availability in a forest system and accounts for the 2-5 times higher phosphatase activity in the litter layer compared to the underlying soil. Mycorrhizal hyphae were not directly measured in this experiment but inspection of the soil-litter interface revealed a large presence of fungi (Figure 2.16b and c). There was also a

concentration of ectomycorrhizal root tips in the soil immediately below the soil-litter interface, thus it seems reasonable to conclude that there was an abundance of mycorrhizal hyphae in the litter layer. It is the litter layer that had the largest influence on distribution of rhizosphere processes in radiata pine plots.

At 18 mg/kg, microbial biomass P comprised 3% of total P and was nearly 2 fold higher in radiata pine than the other two species and higher than levels previously recorded by Chen et al. (2000a) for radiata pine in other New Zealand soils. This is a surprising result given the low microbial activity in soils of radiata pine and that previously, microbial biomass P had been recorded as 30-50 times lower in soils than the overlying litter layer (Chen et al., 2000a). Chen at al (2000a) also found that microbial biomass P under radiata pine was low but activity was high thus indicating a fast flux of P through the microbial biomass under forest. The opposite result was found in the present study. Microbial biomass P was still seven times higher in pine litter in this study but it was still high compared to soil under the other tree species.

It has been shown that microbial biomass C:P ratios are higher for fungi than bacteria (Anderson and Domsch, 1980) indicating that bacteria immobilize proportionally more P than fungi and so it is possible that radiata pine selects for a more bacterial dominated microbial community. This is unlikely though as it is usual that in acid coniferous soils, fungi dominates over bacteria (Anderson and Domsch, 1975; Berg et al., 1998). Phosphorus is released from the microbial biomass by freeze-thawing and drying-rewetting (Pulleman and Tietema, 1999): processes that the pine litter layer may protect soils from to an extent. It is also released by grazing of microfauna (Cole et al., 1978). Jentschke et al. (1995) found that ectomycorrhizal fungi colonization increased the abundance of microfauna which feed on the hyphae. In this experiment it was speculated that much of the ectomycorrhizal hyphae were in the litter layer and bypass the underlying mineral soil and thus the grazing microfauna (amoebae and protozoa) were located in the litter layer also and thus not grazing on and releasing P from, the microbial biomass in the underlying soils, resulting in a higher microbial P pool in these soils.

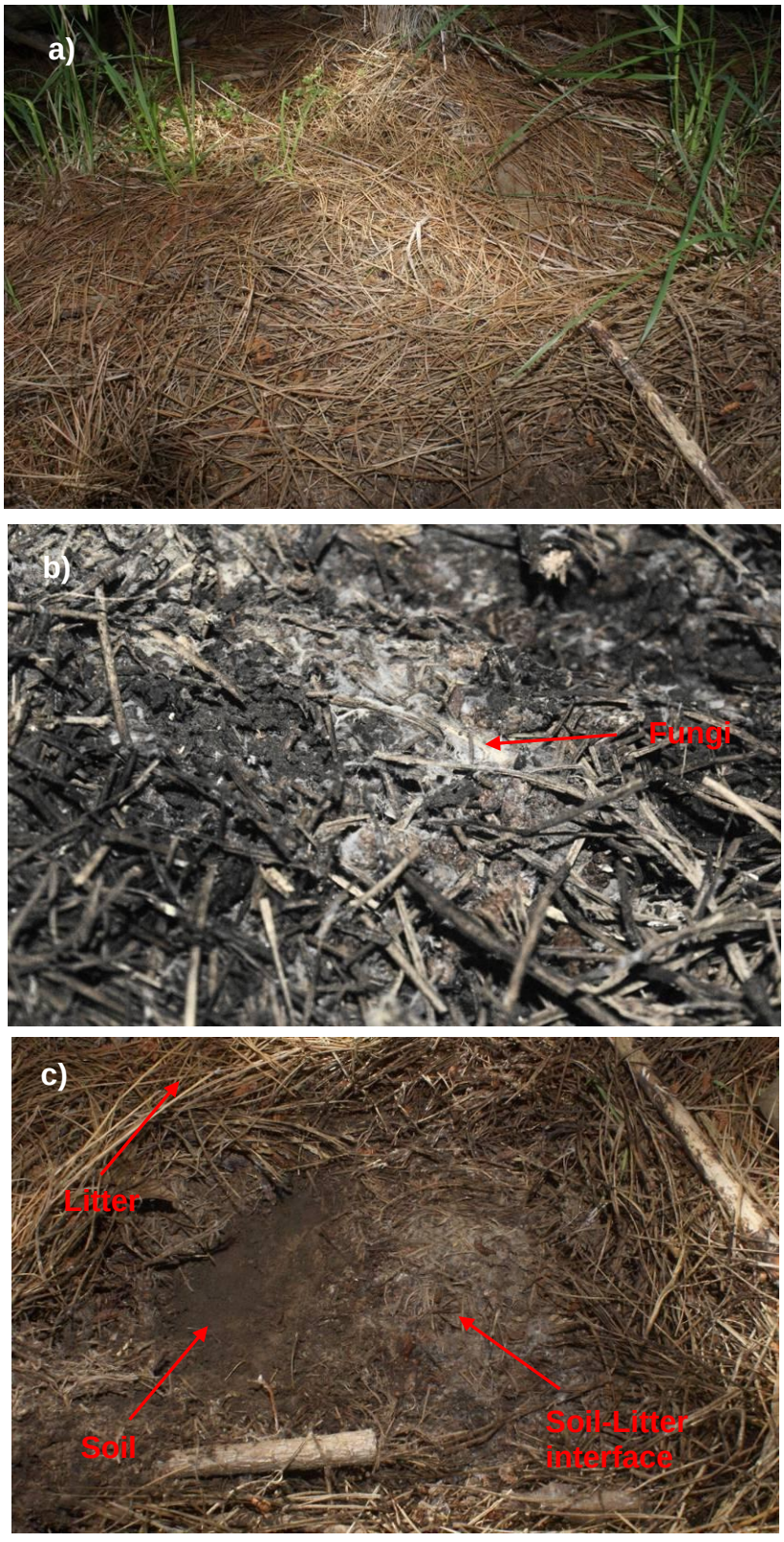

Figure 2.16 The litter layer from radiata pine plot 9 at the Orton Bradley Park field site. a) undisturbed litter layer, b) the soil-litter interface showing the presence of fungi, c) a comparison of soil, litter and the soil-litter interface.

A more likely explanation for the significantly higher microbial biomass P in radiata pine soils is that microbial turnover is slower. There may be a large microbial biomass but not an active one thus immobilizing P within it. The chemical composition of the litter layer influences microbial activity. Kanerva and Smolander (2007) found pine needle forest floors to have a lower microbial activity than deciduous forest floors. Pine needle litter has been demonstrated to contain more phenolics, tannins and terpenes than deciduous leaf litter (Kanerva et al., 2008). Phenolics and terpenes are a C source for microbes and were found to correlate positively with microbial biomass C but not N mineralization by Kanerva et al. (2008) possibly indicating they cause an immobilization of nutrients within microbes. Terpenes can also inhibit enzyme activity which may account for the lower acid phosphomonoesterase in radiata pine soils.

Berg et al. (1998) found that bacterial and fungal activity in Scots pine needle litter was higher than that in the underlying soil but that the activity decreased markedly in the H layer. This was attributed to C limitation caused by easily degradable C compounds, such as carbohydrates being decomposed quickly in the litter layer by young, microbial communities with a fast turnover and so later in the decomposition process, i.e. in the H layer, C is now more stable and more lignin based (Don and Kalbitz, 2005). It is the H layer that forms the litter/soil interface therefore microbes at this zone will be less active due to the stable substrate for them to work on. Organic matter inputs in radiata pine soils are closer to those of macrocarpa with a pasture understory than those of eucalypt which has no understory. Organic carbon inputs from later decomposition stages have been shown to be greater from pine needle litter than deciduous tree litter (Don and Kalbitz, 2005), and this pine C input was demonstrated to be of greater aromacity and complexity. Condron and Newman (1998) demonstrated that organic matter in soils under conifer trees was more recalcitrant and less from fresh plant matter than that under pasture. This will cause a slower microbial turnover in soil and a high P immobilization within the biomass. The immobilization of P in the microbial biomass may be an important means of protecting P from adsorption and precipitation reactions in the soil (Magid et al., 1996; Jakobsen et al., 2005).

Soil under macrocarpa trees had the highest microbial and phosphatase activity and the pasture understory likely had the strongest influence on this. Organic matter proportion

was highest in macrocarpa soils and was greater at 0-10 cm, probably due to the higher pasture root turnover. A greater release of substrates such as sugars, organic acids and amino acids from roots in a pasture soil is thought to be partly responsible for the higher microbial biomass and activity in pasture soils compared to forest soils (Yeates et al., 1997; Yeates and Saggar, 1998; Chen et al., 2000a). The strong positive correlation between microbial activity and acid phosphomonoesterase activity (P <0.01; R^2 = 0.77) and phosphodiesterase activity (P = 0.02; R^2 = 0.66) suggests the microbial origin of some of the enzymes and thus explains the higher activity in these soils. The correlation between resin P amount and acid phosphomonoesterase activity (P<0.01; R^2 = 0.86) and phosphodiesterase activity (P = 0.02; R^2 = 0.63) indicates the importance of phosphatase enzymes in increasing available P in this species soils. Microbial biomass P is a very important labile pool of soil P because the P continuously released during microbial turnover is largely labile organic forms such as nucleic acids and phospholipids (Jakobsen et al., 2005) and the high activity of the microbial biomass under macrocarpa means the pool is very dynamic and that P is continuously being released from it and contributing to the resin P pool (Guggenberger et al., 2000). The large and active microbial biomass in these soils is likely driving the mineralization of organic P and accounts for the significantly lower concentration of organic P at 0-10 cm. Tree utilization will decrease soil P and so drive mineralization, resulting in phosphatase enzyme production (McGill and Cole, 1981; Magid et al., 1996) as well as a by product of C decomposition, a function that the microbes are performing anyway.

There was no definite understory pasture and less litter from reduced thinning in the eucalypt plot. Organic matter inputs from this species were therefore lower than the other species (Table 2.5) and will be mainly a result of root turnover. Tree organic matter inputs are less a labile substrate for microorganisms (Condron and Newman, 1998) and so microbial activity was lower than the pasture influenced macrocarpa soils but, without a litter layer, activity was concentrated in the soil thus making soil activity higher than radiata pine. Labile organic P forms have previously been shown to correlate with the productivity of *Eucalyptus* spp (Adams et al., 1989) and organic P utilization was evident in eucalypt despite the lack of an active pasture microbial biomass. The phosphatase activity of these plots was not significantly different to that of the pasture influenced macrocarpa soils, indicating the high potential for organic P

mineralization by the trees in these soils. It is possible that biochemical P mineralization was a more important pathway in eucalypt soils due to the lower rate of decomposition from less litter in and the high P demand of the trees, further enhanced by the lack of litter to replace P lost from soil by tree uptake.

Macrocarpa and eucalypt had similar acid phosphomonoesterase and diesterase activity levels despite differences in fine root biomass and microbial activity indicating that eucalypt roots were more efficient at increasing soil phosphatase activity than the roots in macrocarpa soils and the lower microbial activity yet similar phosphatase activity indicated less of a microbial origin of these enzymes in eucalypt. The lower phosphatase activity was surprising though as pasture has been shown to increase soil phosphatase activity compared to trees in other New Zealand soils (Chen et al., 2000a; Scott and Condron, 2003). These were comparisons between rye grass and radiata pine therefore it may be that eucalypt species do produce comparable amounts of phosphatases to grasses. The roots of eucalypt had a similar morphology to pasture roots, being much finer than those of radiata pine and macrocarpa, a factor aiding in increased surface area for root phosphatase secretion and for increased mycorrhizal colonization (Lodge, 2000). Mycorrhizal associations probably play a significant role in organic P dynamics in eucalypt plots. Eucalypts form associations with both ectomycorrhizae and arbuscular mycorrhizae, whereas macrocarpa and pasture are solely arbuscular mycorrhizal. Evidence suggests that ectomycorrhizal trees are more efficient at producing phosphatase enzymes than arbuscular mycorrhizal trees (Antibus et al., 1997; Jones et al., 1998; Philips and Fahey, 2006 and root sleeve data). It is likely that the eucalypt mycorrhizal colonization was predominantly ectomycorrhizal as a significant level of ectomycorrhizal colonization was found on eucalypt roots in the root sleeve experiment. So it is likely that eucalypts ectomycorrhizal association had a strong influence on soil phosphatase levels making them comparable to macrocarpa and pasture despite a lower overall fine root biomass.

It should be noted that in comparing rhizosphere with non rhizosphere soil using this technique, the mycorrhizosphere is not accounted for. The method employed, involves the scraping off of soil which adheres to the roots and classing this as rhizosphere soil. The fine extraradical fungal mycelium will be removed with the shaking as well as the

scraping of this method and so are likely influencing the non rhizosphere soil. Further to this, the non rhizosphere soil may have been rhizosphere soil at some point in the nine years of tree growth due to root turnover. This accounts for the between species differences occurring in rhizosphere and non rhizosphere soil that should, in theory, be away from tree influence.

2.5 Conclusions

Results from this chapter indicate that the three species at Orton Bradley Park are essentially in three different P cycles, confined to the top 10 cm and influenced by differences in the understory composition. In radiata pine, P is taken up by trees with some returned to the soil through litterfall with additional return through thinning and pruning and that which was contained within the pasture is now returned to the soil. In macrocarpa plots, much of the P is contained within the pasture understory with less in the slower growing tree than radiata pine and eucalypt. Small amounts of P are continually returned to soil through pasture root turnover along with some thinning debris decomposition, a process accelerated by the active pasture microbial biomass. Much of the P in the fast growing, high biomass, eucalypt system is held within the tree biomass with little returned to the soil as litter and thinning debris.

Organic P is much lower in the surface soil layer under all three species. For all three species, levels of available P in soil are similar and correlate with acid phosphomonoesterase and diesterase activity which are of microbial and plant root origin. Microbial biomass P may be of greater importance to trees with a litter layer, such as *Pinus* species as this layer may protect the microbial biomass from physical processes and trophic interactions which release P from it. Microbial activity and phosphatase enzyme activity are lower in radiata pine soils – likely due to rhizosphere processes being more important in the litter layer and at the litter-soil interface. Levels are comparable in macrocarpa and eucalypt despite the pasture understory under macrocarpa, accounted for by the ectomycorrhizal colonization of eucalypt. Evidence from the root sleeves showed that acid phosphomonoesterase activity increases with ectomycorrhizal colonization and that arbuscular mycorrhizal macrocarpa roots alone

produce significantly less phosphatase than pine and eucalypt and so it seems likely that these ectomycorrhizal species enhance mineralization of organic P to a greater extent than macrocarpa. Accordingly, it is possible that without understory vegetation, eucalypt and radiata pine would enhance soil organic P mineralization to a much greater extent than macrocarpa which will be investigated further in Chapters 3 and 4.

Chapter 3

Rhizosphere Compartment Study of Phosphorus Dynamics under Different Tree Species

3.1 Introduction

Results presented in Chapter 2 demonstrate that the rhizosphere of radiata pine, eucalypt and macrocarpa all exert different influences on soil P dynamics. Although tree rhizosphere effects were partly masked by understory factors, eucalypt and radiata pine rhizospheres were shown to increase acid phosphomonoesterase activity compared to macrocarpa. Evidence suggests that this finding is linked to the differing mycorrhizal associations of the trees. What remains unknown and difficult to answer using field studies is what proportion of these rhizosphere effects can be attributed to roots, mycorrhizae and the microbial biomass.

Most rhizosphere studies have not investigated the proportion of rhizosphere effects linked to roots, mycorrhizae or microbial biomass and so the importance of plant and microbially mediated P mobilization in the rhizosphere is poorly understood (Jakobsen et al., 2005). This chapter aims to assess the specific separate influences of roots, mycorrhizal hyphae and microorganisms on P dynamics associated with radiata pine, eucalypt and macrocarpa - three temperate tree species with contrasting mycorrhizal associations. A short-term glasshouse experiment was established using mesh of different pore sizes to separate roots, mycorrhizal hyphae and microorganisms to confine and enhance rhizosphere processes in distinct measurable compartments. The objectives of this study were to determine the relative contribution of mycorrhizae to P dynamics within the rhizosphere of radiata pine, eucalypt and macrocarpa and to compare this contribution between these trees of contrasting mycorrhizal associations.

3.2 Materials and Methods

3.2.1 Root Study Container Experiment

In August 2008, a three compartment rhizosphere study container experiment was set up using radiata pine, eucalypt and macrocarpa. It was based on the three compartment rhizosphere study container used by Li et al. (1991) for arbuscular mycorrhizal and non mycorrhizal clover.

Three 100 mm diameter compartments made from cut PVC piping were packed with 4 mm sieved soil from 0-10 cm depth and bulked from plots 9, 8 and 7 of the Orton Bradley Park field site described in section 2.2.1. Table 3.1 shows the properties of the soil prior to planting. Six 30 cm high seedlings (supplied by Southern Woods Nursery Ltd, New Zealand) of each species were used, roots cleaned and planted in top compartments and monitored for root growth. The top compartments therefore contained roots, fungal hyphae and microorganisms (RHM) and was separated from the compartments below by a 25 μm nylon mesh. After one month, a root mat had formed at the 25 μm mesh and so this upper compartment was placed on two lower compartments and the experiment was continued under the same conditions.

The two lower compartments were each 15 mm high to eliminate the need for microtome cutting which severs mycorrhizal hyphae and liberates any internal phosphatases and thus alters soil activity levels (Joner et al., 1995). The middle compartment contained fungal hyphae and microorganisms (HM) as roots cannot penetrate 25 μm. This was separated from the compartment below by a 1 μm nylon mesh resulting in the bottom compartment containing only microorganisms (M) as hyphae cannot penetrate 1 μm and is therefore similar to non-rhizosphere or 'bulk' soil. A 100 mm mesh was placed over the bottom end of this compartment to allow water drainage (Figure 3.1). The addition of a third compartment isolated the mycorrhizal hyphae effects from those of the microorganisms with a 'hyphoplane' forming and influencing P dynamics at the 1 μm mesh as the rhizoplane would at the 25 μm mesh. Compartments were held together using duct tape tightly placed around the outside of the two adjoining compartments. This system was maintained in a randomized design in a glasshouse between 15 and 19^{o}C and watered daily (Figure 3.2). After one month the

soil from all three compartments was harvested and stored at 4^{o}C before analysis in October 2008. All soil from within the HM and M compartments was used for analysis. For RHM soil compartment analysis, the tree was removed, shaken vigorously and soil adhering to roots was scraped off where possible and all soil within the compartment was used for analysis. It should be noted that RHM compartments maintained tree growth for two months due to the time taken for a root mat to form prior to the addition of HM and M compartments which were maintained for one month.

Table 3.1 Selected characteristics of the soil used in the root study container experiment prior to planting.

Soil characteristic	Value
Total P (mg/kg)	601
Total organic P (mg/kg)	423
Microbial biomass P (mg/kg)	15
Resin P (mg/kg)	14
pH	6.1
Microbial Activity (μgTPF/g/hr)	4.1
Acid PME (μmol/g/hr)	365
Alkaline PME (μmol/g/hr)	131
PDE (μmol/g/hr)	4.0

DHE = dehydrogenase, PME = phosphomonoesterase, PDE = phosphodiesterase

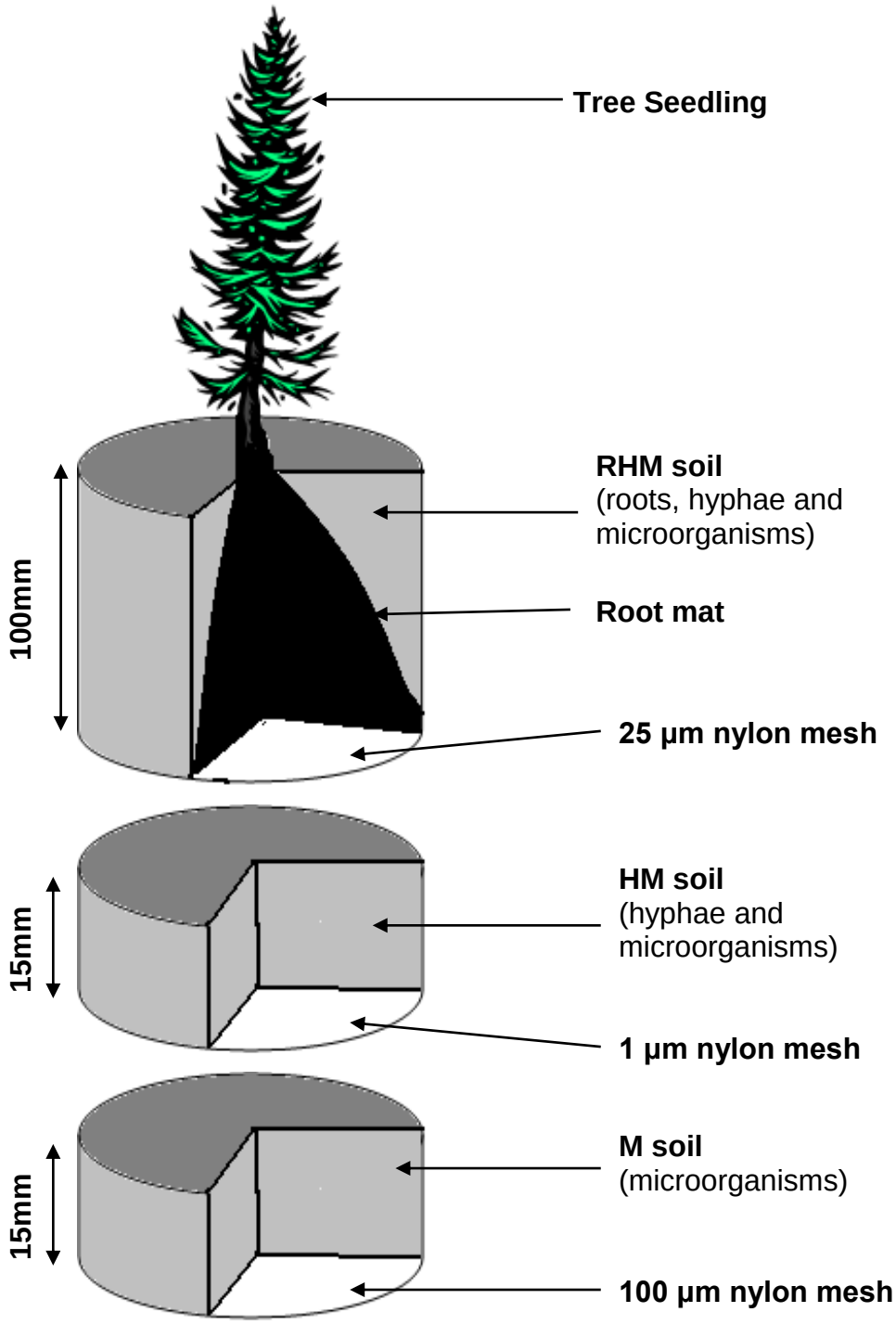

Figure 3.1 Diagram of Rhizosphere Study set up.

Figure 3.2 The root compartment study at experiment termination. By this time the trees were approximately 60 cm high. a) Shows all replicates plus spares of each species. b) Shows one replicate of each species on the mesh table in the glasshouse where they were maintained in a randomized design for the duration of the experiment.

A preliminary 'proof of concept' experiment was carried out in April 2008 to ensure the validity of this method. This was set up in the same way except soil was mixed with potting mix (supplied by Southern Woods Nursery, Ltd) in a 1:4 ratio on a volume basis to ensure fast tree growth. Results of this experiment are presented in Appendix 3.1.

3.2.2 Laboratory Analyses

The original soil and samples from the experiment were analyzed for pH, moisture content, acid and alkaline phosphomonoesterase activity, phosphodiesterase activity, microbial activity, resin extractable P, microbial biomass P, organic P and total P using the methods described in Chapter 2. All fresh soil analysis was carried out within 24-72 hours of sampling. A subsample of each soil was taken and air dried at 20^{o}C and finely ground prior to organic and total P analysis.

Low molecular weight organic anions exuded by plant roots were determined with a method based on that of Schefe et al. (2008) using anion exchange membrane strips (VWR International, Poole, England). At experiment termination, root mats were exposed at the bottom of the RHM compartment by removal of the mesh and thoroughly moistened. A 6 x 4 cm anion exchange membrane strip was placed against the roots and left for one hour. This strip was then removed with tweezers, washed in deionized water and shaken in 10 ml 0.5M HCl at 4^{o}C for three hours. This acid extract was then immediately frozen at -20^{o}C prior to analysis using HPLC (Schefe et al., 2008).

3.2.3 Statistical Analysis

Statistical analysis was conducted using GenStat 10 (Lawes Agricultural Trust, Rothamstead Experimental Station, UK). Whole trial data was analyzed using a one way analysis of variance (ANOVA) to compare inter-species differences in soil P types and enzyme activities between corresponding compartments. Intra-species comparisons between all three compartments were obtained by separate, species specific one way ANOVAs. Where *F*- ratios were significant ($P<0.05$), treatment means were compared by least significant differences (LSD) at 5%. Complete ANOVA results are presented in Appendix 3.2.

3.3 Results

Results are presented in two sections. The first is an inter-species comparison between corresponding compartments to compare rhizospheres of different composition between radiata pine, eucalypt and macrocarpa. The second is an intra-species comparison between the three compartments to assess the contribution of all rhizosphere components to P dynamics within each species. Table 3.2 contains the entire experimental data set and gives values from all compartments of all three species.

3.3.1 Inter-Species Comparison

3.3.1.1 RHM compartments

Radiata pine soil had the lowest concentrations of all P forms except resin P whereas macrocarpa soil had the highest concentrations of all P forms except microbial biomass P. All three species soils differed in total P and organic P concentration with radiata pine containing significantly less than eucalypt, which in turn contained significantly less than macrocarpa. Macrocarpa soil also had a higher concentration of resin P (19 mg/kg) than radiata pine (17 mg/kg), which in turn had a significantly higher concentration than eucalypt (14 mg/kg). Both eucalypt and macrocarpa soils had a significantly higher concentration of microbial biomass P (23 and 21 mg/kg, respectively) than radiata pine (15 mg/kg).

Microbial activity in macrocarpa soil was significantly higher than in eucalypt soil and the eucalypt microbial activity was significantly higher than activity in radiata pine soil. Acid phosphomonoesterase activity was approximately 4-fold higher than alkaline phosphomonoesterase and 40-fold higher than phosphodiesterase activity. Eucalypt soil had a significantly higher acid phosphomonoesterase activity (484 μmol/g/hr) than radiata pine (451 μmol/g/hr), which in turn had a significantly higher activity than macrocarpa soil (413 μmol/g/hr). On the other hand, trends were different for the lower activity level phosphatase enzymes. Macrocarpa soils had the highest alkaline phosphomonoesterase and phosphodiesterase activities compared to eucalypt which in turn were significantly higher than those in radiata pine soil.

Collection of LMWOAs

Organic acids exuded by roots of all three species and collected at the base of the RHM compartment upon experiment termination are presented in Table 3.3. A total of eight organic acids were consistently detected under eucalypt and the highest concentrations of most organic acids were collected from this species with a range of 0.008 $\mu g/cm^2$ (fumaric and shikimic acid) to 2.942 $\mu g/cm^2$ (quinic acid). Eight organic acids were detected under radiata pine and concentrations ranged from 0.013 $\mu g/cm^2$ (maleic acid) to 0.575 $\mu g/cm^2$ (lactic acid). Nine organic acids were detected under macrocarpa although one of these (fumaric acid) was present at a low concentration and found in only one replicate. Concentrations of organic acids ranged from 0.008 $\mu g/cm^2$ (maleic acid) to 0.779 $\mu g/cm^2$ (lactic acid).

Most organic acids were not detected consistently across all six replicates of the three species. Lactic acid was found in exudates from all three species and in the highest concentration, whereas maleic and shikimic acids were also found in exudates from all three species but in much lower concentrations. Tartaric and quinic acid concentrations were too low for detection from radiata pine, but were detected in eucalypt (0.683 and 2.942 $\mu g/cm^2$ for tartaric and quinic, respectively) and macrocarpa (0.117 and 0.463 $\mu g/cm^2$ for tartaric and quinic, respectively). Formic and malic acid were also present in exudates from all three species, with highest quantities determined for eucalypt (0.771 and 0.479 $\mu g/cm^2$ for formic and malic acid, respectively), compared with radiata pine (0.275 and 0.192 $\mu g/cm^2$ for formic and malic acid, respectively) and macrocarpa (0.350 and 0.454 $\mu g/cm^2$ for formic and malic acid, respectively). Melanic acid was only detected in radiata pine (average 0.042 $\mu g/cm^2$), whereas fumaric acid was only exuded by eucalypt (and one replicate of macrocarpa) and at very low concentrations (0.008 $\mu g/cm^2$). Acetic acid was detected in radiata pine and macrocarpa at 0296-0.404 $\mu g/cm^2$.

Table 3.2 Mean values of all variables determined in each compartment of radiata pine, eucalypt and macrocarpa rhizosphere in the root study container experiment. Values in parenthesis are standard errors of mean, n = 6.

	RHM			HM			M		
	Pine	Eucalypt	Macro	Pine	Eucalypt	Macro	Pine	Eucalypt	Macro
pH	5.9 b (0.02)	5.7 a (0.05)	5.7 a (0.03)	6.1 a (0.03)	6.0 a (0.03)	6.0 a (0.01)	6.2 b (0.02)	6.1 a (0.02)	6.1 a (0.03)
Total P (mg/kg)	518 a (5.5)	531 b (3.0)	561 c (2.5)	536 a (0.8)	542 a (2.7)	569 b (5.7)	563 b (4.6)	535 a (2.3)	556 b (7.5)
Organic P (mg/kg)	364 a (2.9)	380 b (3.0)	407 c (2.5)	387 a (0.9)	391 a (2.2)	413 b (2.8)	415 c (4.0)	393 a (3.5)	408 b (4.8)
Resin P (mg/kg)	16.9 b (0.2)	14.3 a (0.8)	18.8 c (0.2)	19.9 b (0.3)	18.4 a (0.2)	19.9 b (0.4)	21.2 b (0.4)	19.2 a (0.2)	21.1 b (0.2)
Microbial P (mg/kg)	15.4 a (1.5)	23.4 b (0.7)	20.5 b (1.7)	16.5 b (1.3)	24.1 a (1.0)	17.1 b (1.1)	14.7 a (1.0)	18.9 b (1.0)	16.4 b (0.7)
Mic Activity (µgTPF/g/hr)	5.7 a (0.1)	6.2 b (0.3)	7.1 c (0.2)	5.0 ab (0.1)	5.4 b (0.1)	4.7 a (0.2)	4.8 a (0.2)	5.5 b (0.1)	4.9 a (0.1)
Acid PME (µmol/g/hr)	451 b (5.6)	484 c (4.8)	413 a (3.7)	411 a (1.7)	480 b (6.0)	400 a (2.8)	399 b (1.5)	482 a (5.2)	382 c (7.6)
Alk PME (µmol/g/hr)	116 a (1.9)	151 b (5.5)	182 c (5.7)	122 a (6.0)	133 a (4.6)	177 b (2.3)	140 b (5.5)	106 a (4.6)	156 c (10.4)
PDE (µmol/g/hr)	9.9 a (0.2)	11.5 b (0.9)	13.1 c (0.5)	10.8 a (0.4)	12.2 b (0.9)	14.8 c (0.2)	11.6 a (0.5)	11.0 a (1.2)	11.0 a (0.8)

PME = phosphomonoesterase, PDE = phosphodiesterase
Within each compartment, means with different letters are significantly different ($P<0.05$) between species

Table 3.3 Average concentrations of low molecular weight organic acids (LMWOAs) exuded by roots of radiata pine, eucalypt and macrocarpa in the root study container experiment. Values in parenthesis are the number of reps each acid was detected in.

LMWOA (µg/cm^2)	Tartaric	Quinic	Formic	Malic	Melanic	Shikimic	Lactic	Acetic	Maleic	Fumaric
Pine	-	0.026	0.275	0.192	0.042	0.026	0.575	0.296	0.013	-
	-	(1)	(5)	(3)	(2)	(5)	(5)	(3)	(6)	-
Eucalypt	0.683	2.942	0.771	0.479	-	0.004	1.21	-	0.008	0.008
	(3)	(2)	(5)	(4)	-	(5)	(6)	-	(6)	(5)
Macrocarpa	0.117	0.463	0.350	0.454	-	0.192	0.779	0.404	0.008	0.008
	(5)	(4)	(6)	(5)	-	(6)	(6)	(3)	(6)	(1)

3.3.1.2 HM compartments

Eucalypt soil contained the lowest concentrations of all P types, except microbial biomass P where it contained a markedly higher concentration than the other two species. Total P and total organic P followed the same patterns with significantly less in both eucalypt and radiata pine compared with macrocarpa. Eucalypt soil had a significantly lower resin P concentration compared with radiata pine and macrocarpa, but significantly higher concentrations of microbial biomass P were determined under eucalypt (24 mg/kg) compared with radiata pine and macrocarpa (17 mg/kg).

Eucalypt soil microbial activity was significantly higher than macrocarpa, but neither of these differed significantly from radiata pine. Acid phosphomonoesterase activity was again, 4-fold higher than alkaline phosphomonoesterase and 40-fold higher than phosphodiesterase activity and under eucalypt (480 μmol/g/hr), activity was significantly higher than under both radiata pine and macrocarpa (411 and 400 μmol/g/hr). Macrocarpa soils had significantly higher alkaline phosphomonoesterase and phosphodiesterase activity levels than those determined under both eucalypt and radiata pine.

3.3.1.3 M compartments

Differences between species in soil P concentrations were smaller in this compartment but eucalypt soil contained the lowest concentrations of all P types, except microbial biomass P. Both radiata pine and macrocarpa soil had a significantly higher concentration of total P than eucalypt soil. Total organic P in eucalypt soil was significantly lower than macrocarpa which in turn was significantly lower than radiata pine. Resin P was also significantly lower in eucalypt soils than both radiata pine and macrocarpa soil but microbial biomass P was significantly higher in eucalypt soil.

Eucalypt soil had a significantly higher microbial activity than both radiata pine and macrocarpa soil. Eucalypt soil also had a markedly higher acid phosphomonoesterase activity (482 μmol/g/hr) than radiata pine (399 μmol/g/hr) and macrocarpa (382 μmol/g/hr) activity. On the other hand, macrocarpa soil alkaline phosphomonoesterase activity was significantly higher than radiata pine activity which was significantly

higher than eucalypt activity. Phosphodiesterase activity did not differ between species in this compartment.

3.3.2 Intra-Species Comparison

Results of this section are presented in Figures (3.3-3.5) to highlight trends in descending order from root compartments.

3.3.2.1 Radiata pine

All forms of P, except microbial biomass P, had significantly (P<0.05) different concentrations in each compartment, being consistently lowest in the RHM and highest in the M compartment. In contrast, microbial biomass P was much more variable and not significantly affected by compartment (Figure 3.3a and b). Figure 3.3c shows that microbial activity was significantly higher in the RHM compartment compared to both the HM and M compartments. Acid phosphomonoesterase activity decreased significantly with distance from the root compartment whereas alkaline phosphomonoesterase and phosphodiesterase increased with distance from root compartment (Figure 3.3d). Phosphodiesterase activity was low and between compartment differences were small.

3.3.2.2 Eucalypt

The RHM compartments contained significantly (P<0.05) lower concentrations of all P types, except microbial biomass P, than both HM and M compartments which did not differ significantly from each other. The differences between the RHM and the other compartments in total P (3-10 mg/kg) and organic P (12-14 mg/kg) were small. The difference in resin P was however more pronounced (Figure 3.4a). Conversely, Figure 3.4a also shows that microbial biomass P concentration was significantly higher in the RHM (23 mg/kg) and HM (24 mg/kg) compartments compared to the M compartment (19 mg/kg).

Microbial activity was significantly higher in the RHM compartment than the HM and M compartments (Figure 3.4b). Acid phosphomonoesterase activity did not differ significantly between compartments but alkaline phosphomonoesterase activity was significantly different in all compartments where it was highest in RHM and lowest in

M compartments (Figure 3.4c). Phosphodiesterase was lower than that of alkaline phosphomonoesterase and activity differences were small.

3.3.2.3 Macrocarpa

Neither total P nor total organic P differed significantly between compartments. Resin P was the only P type significantly (P<0.05) influenced by all compartments and Figure 3.5a shows that differences were small with 19 mg/kg in RHM, 20 mg/kg in HM and 21 mg/kg in M compartments. Microbial biomass P was much more variable than resin P and the RHM compartment (20 mg/kg) contained significantly more than the M compartment (16 mg/kg) but the HM compartment (17 mg/kg) did not differ significantly from either the RHM or M compartment (Figure 3.5a).

Figure 3.5b shows that microbial activity was significantly higher in the RHM compartment (7.1 μgTPF/g/hr) than the HM and M compartments (4.7 and 4.9 μgTPF/g/hr, respectively). All phosphatase enzyme activities were affected by the same compartments in that there was no significant difference between the RHM and HM compartments and these had a significantly (P<0.05) higher activity than the M compartment (Figure 3.5c).

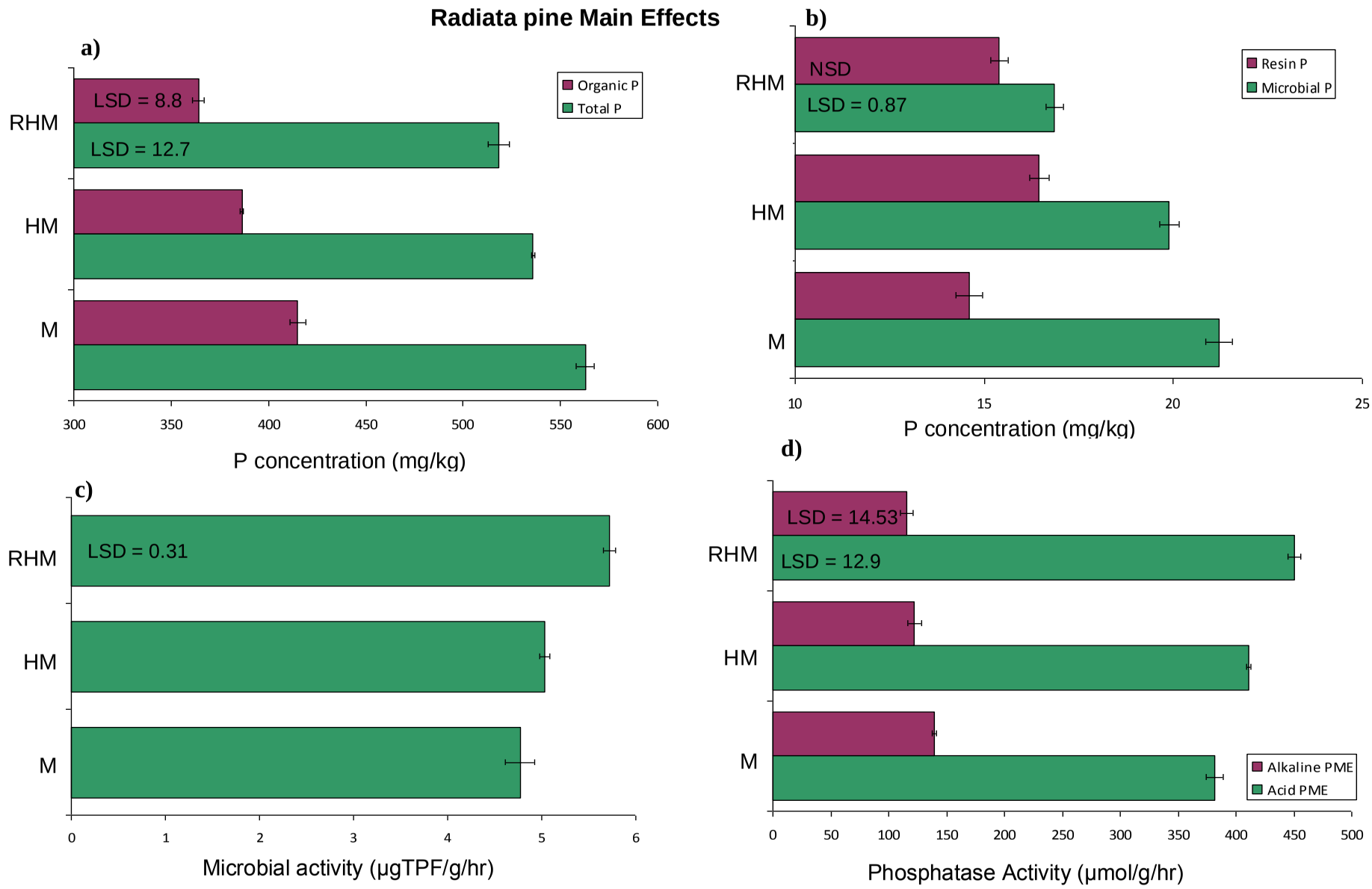

Figure 3.3 Radiata pine: Means of a) total and organic P concentrations, b) microbial biomass and resin P concentrations, c) microbial activity and d) acid and alkaline phosphomonoesterase activity determined in each compartment in the root study container experiment. Bars represent standard errors of means, n = 6.

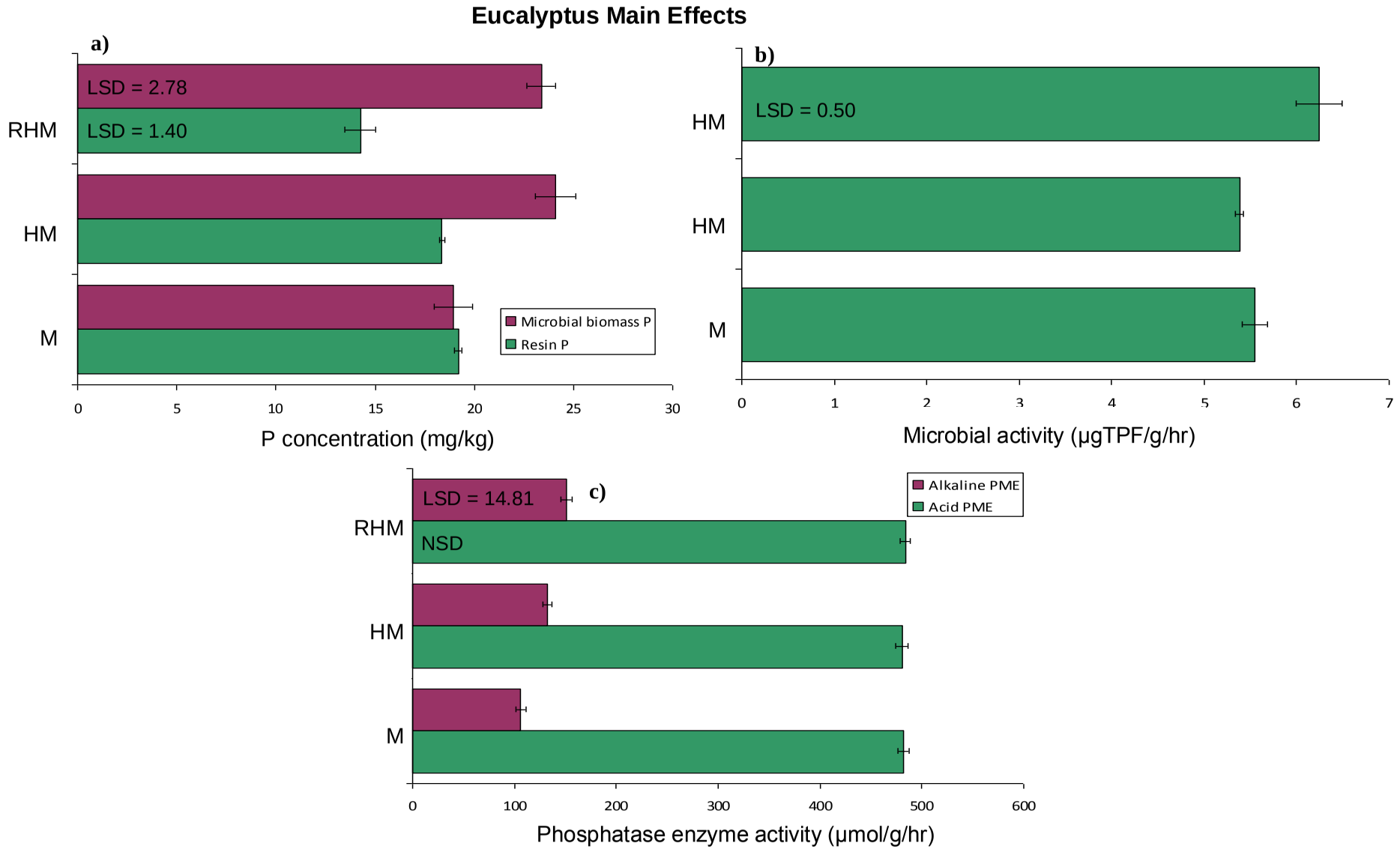

Figure 3.4 Eucalypt: Means of a) microbial biomass and resin P concentrations, b) microbial activity and d) acid and alkaline phosphomonoesterase activity determined in each compartment in the root study container experiment. Bars represent standard errors of means, n = 6.

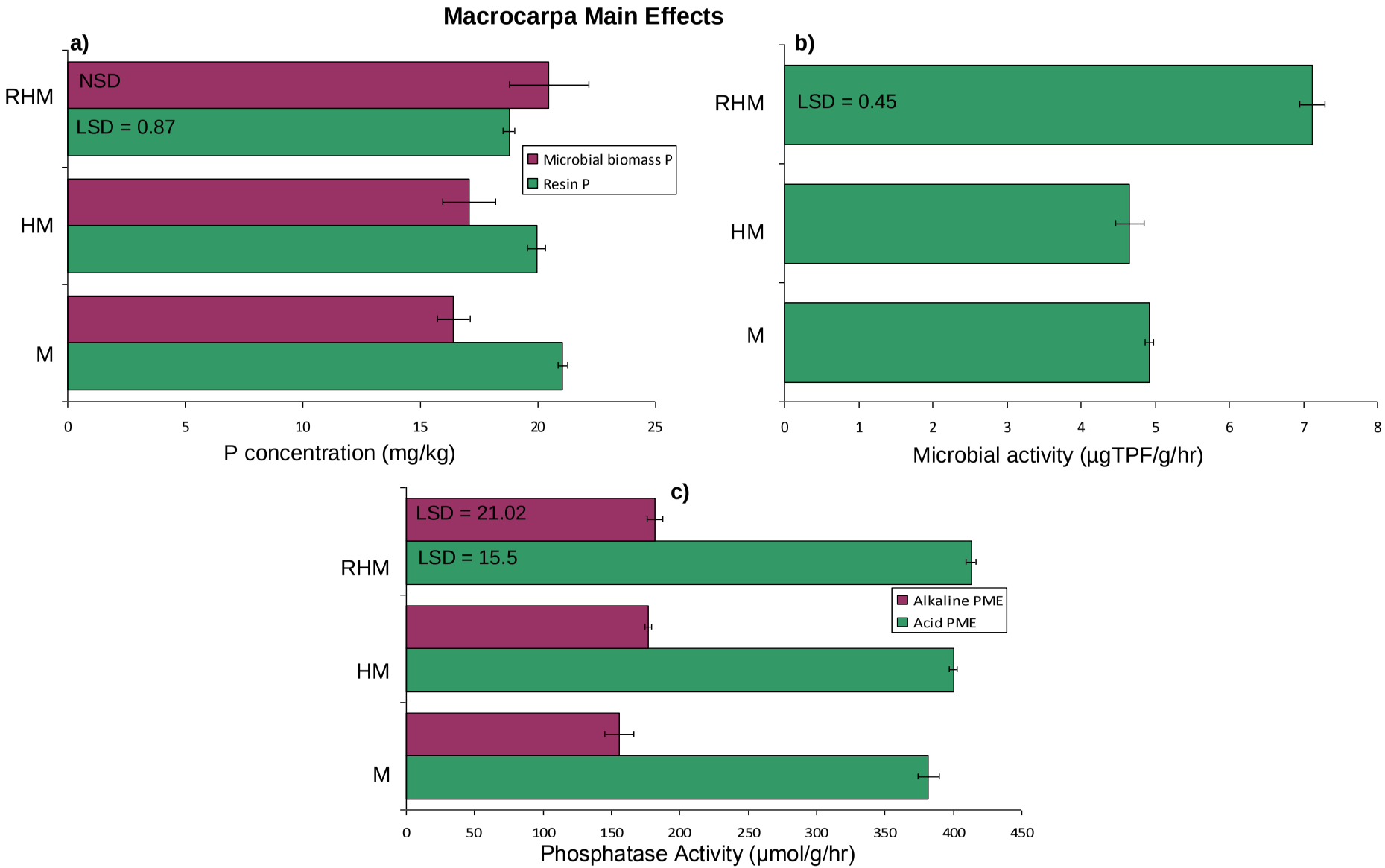

Figure 3.5 Macrocarpa: Means of a) microbial biomass and resin P concentrations, b) microbial activity and d) acid and alkaline phosphomonoesterase activity determined in each compartment in the root study container experiment. Bars represent standard errors of means, n = 6.

3.4 Discussion

The previous experiments in Chapter 2 compared rhizosphere effects and P dynamics of radiata pine, eucalypt and macrocarpa and found striking differences especially in acid phosphomonoesterase activity. This experiment was designed to determine and compare the relative contribution of mycorrhizae to the rhizosphere effects and P dynamics under these three species. For most parameters, significant differences between species in each compartment as well as between compartments within each species were observed. As expected, rhizosphere effects were greatest in RHM compartments, the complete mycorrhizosphere, and generally lowest in M compartments, the compartment most akin to bulk soil. Note that the RHM compartment maintained plant growth for two months compared to the one month of the HM and M compartments and contained the main drivers of rhizosphere dynamics – the roots. The M compartment contained neither roots nor hyphae and is at some distance from the roots which leads to a decline in microbial activity (Chen et al., 2002; Manoharachary and Mukerji, 2006). This demonstrates that the technique was successful and elucidated mechanisms by which tree roots and mycorrhizal hyphae influence rhizosphere processes of radiata pine, eucalypt and macrocarpa and the relative contribution of these components to rhizosphere processes within each species.

3.4.1 Radiata Pine

All parameters measured under radiata pine, except for microbial biomass P and microbial activity, were significantly different in HM than M compartments and it is only the HM compartment of radiata pine that has a lower concentration of total P and organic P compared to non rhizosphere soil (M compartment). Root hairs and roots cannot penetrate the 25 μm mesh but fungal hyphae can, meaning the HM compartment is essentially a hyphosphere. However, root surface influence extends a few mm into soil (Lynch, 1982; Martin et al., 2001) and so will influence some soil but the hyphosphere is known to extend more than 10 cm from the root surface (Timonen and Marschner, 2006; Cavagnaro et al., 2001; Martin et al., 2001) and so will be the main driving force of P dynamics in the HM compartments. Therefore the differences in soil P concentrations and phosphatase enzyme activities of this compartment compared to

that of non rhizosphere soil indicate that mycorrhizae have a significant role in P dynamics under radiata pine.

Radiata pine is an ectomycorrhizal species (Chu-Chou and Grace, 1984) and this mycorrhizal type is documented to be more efficient than arbuscular mycorrhizae at soil P utilization (Lodge, 2000) and in the hyphal zones of this study, ectomycorrhizal radiata pine has significantly lower levels of total P and organic P than the arbuscular mycorrhizal macrocarpa. Abundant hyphae were clearly seen in ectomycorrhizal eucalypt and radiata pine HM soils but not in the arbuscular mycorrhizal macrocarpa, indicating this was likely due to the higher hyphae biomass and length of ectomycorrhizae compared to arbuscular mycorrhizae exploring larger volumes of soil (Smith and Read, 1997; Jones et al., 1998). Not only are ectomycorrhizal hyphae more abundant, they have also been demonstrated to be more efficient at P solubilization through organic anions release and mineralization through phosphatase enzyme activity than arbuscular mycorrhizae. Low molecular weight organic anion presence was not measured in these compartments but many studies demonstrate that ectomycorrhizae can exude LMWOAs, especially oxalate, which solubilize various P forms, thus increasing soil P availability (Lapeyrie et al., 1987, 1991; Lapeyrie, 1988; Liu et al., 2004a, b; 2005).

There is considerable evidence for ectomycorrhizal production of phosphatases (Häussling and Marschner, 1989; Antibus et al., 1992; Pasqualini et al., 1992; Liu et al., 2004b; 2005; Nygren and Rosling, 2008) and in this study, acid phosphomonoesterase activity in the hyphal influenced HM soil is higher in the ectomycorrhizal eucalypt and radiata pine compared to arbuscular mycorrhizal macrocarpa, although only significantly so in eucalypt. This may not reflect similar capabilities of radiata pine and macrocarpa mycorrhizae to increase acid phosphomonoesterase but simply the requirement at the current level of resin P which does not differ significantly between them. It has been documented that phosphatases are induced or repressed by the P status of their environment (Quiquampoix and Mousain, 2005). For example, Tadano and Sakai (1991) found that low solution P levels (0.05-0.5 mg/kg) induced phosphatase activity in the roots of many crop species and sufficient solution P levels (3 mg/kg) repressed phosphatase activity. From this experiment it appears that the reaction of the

plants to the P environment is a sensitive one and therefore seems likely that the similar levels of available P in the soil at the time of sampling will account for the lack of significant acid phosphomonoesterase activity difference in radiata pine and macrocarpa HM compartments. The lower levels of total and organic P in radiata pine HM soil likely demonstrate a higher soil P utilization by radiata pine mycorrhizal hyphae which indicates that at some stage acid phosphomonoesterase must have been greater to hydrolyze the organic P for plant use and to keep resin P at a similar level to that of macrocarpa despite possible different utilization levels.

Soil P concentrations, microbial activity and phosphatase enzyme activity were not significantly different between radiata pine and macrocarpa in the M compartments. This is likely because the M compartment lacks the extraradical hyphae (due to the 1μm mesh) which drive radiata pine P dynamics and also the roots (due to the 25 μm mesh) which exude C compounds for the microbial community, which likely drive macrocarpa P dynamics. The resultant higher level of resin P compared to that in the higher compartments means that less phosphatase activity is induced as microbial phosphatases have also been shown to respond to the P status of their environment (Alaksieva and Micheva-Viteva, 2000; Antelmann et al., 2000). Acid phosphomonoesterase is still slightly higher in radiata pine than macrocarpa, possibly due to some ectomycorrhizal influence at the hyphoplane (hyphae surface formed at the 1μm mesh).

3.4.2 Eucalypt

Resin and microbial P as well as acid phosphomonoesterase measured in the HM compartments were significantly different in eucalypt compared to the other two species. The eucalypts in this experiment were likely to be predominately ectomycorrhizal as ectomycorrhizal root tips could be seen with the naked eye and usually once ectomycorrhizal associations are formed, subsequent arbuscular mycorrhizal colonization is hindered by the impenetrable fungal sheath barrier (Chen et al., 2000b; Lodge, 2000). In addition, abundant hyphae were clearly seen in eucalypt and radiata pine HM soils but not in the arbuscular mycorrhizal macrocarpa, further indicating that the mycorrhizal association of eucalypt in this study is ectomycorrhizae as they are known to produce a greater hyphal length and biomass than arbuscular mycorrhizae (Smith and Read, 1997; Jones et al., 1998).

Although both ectomycorrhizal species have a higher acid phosphomonoesterase activity and lower resin P concentration than the arbuscular mycorrhizal species, only eucalypt values are significantly different and also in the microbial parameters measured. This perhaps indicates a more efficient ectomycorrhizal association of eucalypt than radiata pine or the additional greater influence of it on the microbial biomass which then, in turn, further influences rhizosphere processes. The additional large influence of the microbial biomass rhizosphere processes under this species is demonstrated by the lack of significant difference in acid phosphatase activity, resin P and microbial parameters between HM and M compartments. It seems likely that both roots and mycorrhizae drive microbial P dynamics and rapid P cycling under this species and this influence extends some distance.

Eucalypt roots exuded almost all the detected LMWOAs in the largest amounts compared to macrocarpa and radiata pine, especially quinic, formic, lactic and tartaric acid. Release of LMWOAs has long been known to enhance the solubility of sparingly soluble mineral P (Jones, 1998) by competing with phosphate esters for adsorption sites (Lopez-Hernandez et al., 1986; Hinsinger, 2001) thus increasing the availability of P in soil. These exudates have also been shown to increase organic P availability in soil by breaking down organic P in humic matter to a lower molecular weight by breaking the hydrophobic bonds holding the molecules together (Nardi et al., 2000), thus increasing its susceptibility to hydrolysis. Organic anions are also shown to dissolve Al and Fe organic P complexes by chelation and thus release organic P and Tang et al. (2006) found that citrate, oxalate and malate could improve solubility of inositol organic P. It has been suggested that it is the solubility of organic P, and phosphatase activity control organic P mineralization (Adams and Pate, 1992; Tiessen et al., 1999). Furthermore, water soluble organic carbon (WSOC) is thought to be mainly derived from root exudates and residues (McGill et al., 1986) and Scott and Condron (2004) found that plant P uptake was significantly correlated with WSOC in soil planted with combinations of radiata pine, ryegrass and lurcene. Thus inorganic and organic P in the rhizosphere of eucalypt is likely solubilized by root exudates which will lead to a greater mineralization and plant uptake and therefore lower P concentrations under this species.

Exudation of LMWOAs also provides the microbial biomass with a mineralizable C source (Jones et al., 2003; Richardson et al., 2009b) and some acids have been shown to be more readily metabolized by the microbial biomass than others (Ström et al., 2001; 2002). Citric acid was demonstrated by Ström et al. (2001) to be more resistant to microbial degradation but was not collected in detectable amounts from eucalypt roots and malic acid, shown to be less resistant, and therefore a better C source for the microbial biomass, was collected from eucalypt roots. Consequently, it appears that the LMWOAs exuded from eucalypt may provide the microbial biomass with a good, readily metabolizable C source. This, coupled with the lower concentrations of soil P, especially resin P, may render the microbes in the eucalypt rhizosphere P limited, therefore causing P to be immobilized, not released, which is demonstrated by the high microbial biomass P under this species. Microbes are known producers of phosphatases (Richardson, 2001; Oberson and Joner, 2005; Jakobsen et al., 2005; Singh and Walker, 2006) and Chen et al. (2000a) found a direct relationship between microbial activity and phosphatase enzyme activity and so the high concentrations of LMWOAs released by eucalypt may account for the higher acid phosphatase activity in this species compared to the also ectomycorrhizal radiata pine through the influence on the microbial community. Direct evidence for the stimulatory effect of LMWOAs and other root exudates on rhizosphere hydrolases is lacking, however Renella et al. (2007) demonstrated that release of oxalate and citrate in both a sandy and a clay soil increased phosphomonoesterase and diesterase activity of the microbial biomass in the rhizosphere. Eucalypt acid and alkaline phosphomonoesterase activity and also phosphodiesterase activity were significantly higher than those of radiata pine in RHM compartments where C exuding roots are present and so the stimulatory effect of root exudation on the microbial biomass seems a likely explanation for this.

Ectomycorrhizal hyphae have been repeatedly demonstrated to stimulate microbial activity through exudation of organic substances (Timonen and Marschner, 2006; Calvaruso et al., 2007) and it is likely that the microbial activity stimulated by the ectomycorrhizal hyphae is from a selected microbial community with a different functional diversity to that of arbuscular mycorrhizospheres. For example, Calvaruso et al. (2007) found that the ectomycorrhizae associated with a mature oak species stand selected for a particular microbial community efficient at P solubilization through

proton and organic acid release. Also, Frey-Klett et al. (2005) found that ectomycorrhizae associated with nine month old Douglas fir species seedlings selected for *Pseudomonas fluorescens* isolates with greater P mobilization ability when compared to those found in bulk soil. These two studies indicate the mycorrhizosphere effect on microbial functional diversity occurs in nursery and field, old and young trees, different tree and symbiont species and different bacterial groups. Therefore it seems reasonable to assume that a similar effect is occurring in the mycorrhizosphere of eucalypt and radiata pine in this study and so can account for the apparent greater soil organic P utilization under these two species compared to macrocarpa.

The greater P utilization in eucalypt rhizosphere extends past the hyphoplane as concentrations are still significantly lower compared to radiata pine and macrocarpa in the M compartments. Differences between species in the M compartments are smaller, to be expected without roots and hyphae to drive the rhizosphere processes. Lower levels of resin P could be attributed to diffusion and mass flow moving P to the adjacent HM compartments driven by the lower P concentrations, from plant and the hyphae uptake (Jungk, 1996). It appears that it is the microbial community that produces much of the acid phosphomonoesterases in eucalypt rhizosphere as activity does not differ significantly between compartments and is therefore still high when there are no roots and hyphae present to produce them. Clearly something is continually stimulating microbial activity and acid phosphomonoesterase activity even when roots and hyphae are excluded. There could be some stimulatory effect from the hyphoplane at the second mesh, influencing the higher microbial activity in eucalypt M compartment indicating that despite both being ectomycorrhizal, the symbiont associated with eucalypt is a more prominent driver of P dynamics through effect on microbial activity, than that associated with radiata pine.

3.4.3 Macrocarpa

Where it is likely that hyphae of the ectomycorrhizal species are stimulating and selecting for a microbial biomass efficient at P mobilization, only the roots of macrocarpa rhizosphere appear to stimulate microbial activity. Rhizosphere processes do not appear to be as dynamic in hyphal zones of macrocarpa as in eucalypt and radiata pine as many parameters did not differ significantly between HM and M compartments.

Macrocarpa roots, like eucalypt roots, also exude large amounts of LMWOAs compared to those of radiata pine. This may partly explain the higher alkaline phosphomonoesterase and phosphodiesterase activity under this species. Phosphodiesterases are best known for their ability to degrade nucleic acids (Tabatabai, 1994) and the significantly higher activity of these enzymes in macrocarpa soils is likely because turnover of the microbial biomass will release P in the form of nucleic acids continually into the soil (Jakobsen et al., 2005). Phosphomonoesterases work in conjunction with phosphodiesterases to hydrolyze phosphodiesters. Alkaline phosphomonoesterase is primarily of microbial origin (Tabatabai, 1994) thus the highest levels of these enzymes in macrocarpa rhizosphere demonstrate the larger role of the microbial biomass in P cycling in this rhizosphere, both as a P source through release of nucleic acids, and a dynamic P pool through the hydrolysis and incorporation of this P. Higher levels of resin P mean the active microbial biomass (stimulated by LMWOA derived C sources) is not P limited and will be continually releasing P as a readily available water soluble organic P source (Fransson and Jones, 2007).

The capacity for microorganisms to provide P to plants is increased in the rhizosphere where there is a large supply of ready metabolizable C from roots (Kouno et al., 2002; Jakobsen et al., 2005; Renella et al., 2007). The fact that microbial biomass P is not significantly lower than in eucalypt, despite P conditions not inducing immobilization maybe simply be because the microbial biomass is larger – indicated by the significantly higher microbial activity level in this species in root compartments. Turnover of P through the active microbial biomass has been demonstrated to be a major factor in the maintenance of soil organic and inorganic P in solution (Seeling and Zasoski, 1993). Guggenberger et al. (2000) concluded that much resin P is of microbial origin and so the large microbial turnover likely contributes to the higher level of resin P found in macrocarpa soils.

The influence of arbuscular mycorrhizae on phosphatase enzyme activity is thought to be relatively small, possibly due to low biomass in soils (Joner et al., 2000) and this accounts for the lower acid phosphatase and higher P concentrations found in arbuscular mycorrhizal macrocarpa compared to radiata pine and eucalypt HM compartments in this study. Phosphatase enzyme activities and resin P concentrations were however

significantly different between HM and M compartments under macrocarpa but this was more likely due to some LMWOA influence at the rhizoplane contributing to the hyphae influence of HM soil.

The lack of extraradical hyphae and roots in the M compartment mean soil P concentrations, microbial activity and phosphatase enzyme activity of macrocarpa is not significantly different to that of radiata pine. It is also possible that the higher P concentrations in the higher compartments of this species meant that less P is derived from M compartments through diffusion and mass flow. Phosphodiesterase and alkaline phosphomonoesterase activities are still higher in macrocarpa, as in higher compartments, indicating that the microbial community is still important in P dynamics under this species, but at distance from roots, the activity is lower.

3.5 Conclusions

This study separated the rhizosphere components of eucalypt, radiata pine and macrocarpa to investigate their effects on rhizosphere P dynamics. This enabled assessment of the relative contribution of microorganisms, mycorrhizal fungi and roots to rhizosphere P dynamics within each species.

Radiata pine roots do not appear to stimulate much microbial activity compared to eucalypt and macrocarpa as microbial activity was lowest in the radiata pine RHM compartment. Its mycorrhizal association on the other hand does appear to stimulate microbial activity and phosphatase activity and therefore P dynamics. It is possible that the mycorrhizal association supplies enough P to the plant either directly through phosphatase exudation or indirectly though stimulation of microbial activity, without investing excess energy into root exudation other than the C supply for its symbiont.

High phosphatase enzyme activity, microbial activity and LMWOA exudation in eucalypt, coupled with lower concentrations of all P types except microbial P suggests P utilization is greater in this species compared to radiata pine and macrocarpa. This is due to active root exudation of LMWOAs stimulating more microbial activity and a

mycorrhizal association increasing phosphatase activity and further selecting for an efficient P solubilizing microbial community all contributing to rhizosphere processes and P depletion under eucalypt.

The bacterial microbial community appears to be a greater driver of P dynamics under macrocarpa than in the rhizosphere of radiata pine and eucalypt, indicated by the high levels of microbial activity, alkaline phosphomonoesterase and phosphodiesterase and stimulated by a large efflux of LMWOAs. The large and active microbial community of macrocarpa rhizosphere could be a compensation for its less efficient arbuscular mycorrhizal association or macrocarpa may not need a more efficient mycorrhizal symbiont as it would have little benefit because the microbial community drives P dynamics.

What still remains to be answered is what proportion of phosphatase activity and LMWOA production can be directly attributed to mycorrhizal hyphae as opposed to their influence on other rhizosphere components.

Chapter 4

Mycorrhizae and Rhizosphere Processes of Different Tree Species

4.1. Introduction

Mycorrhizal fungi form a symbiotic relationship with 90% of all terrestrial plants involving a bidirectional nutrient flow of carbon to the fungi and nutrients, including P to the plant (Smith and Read, 1997). It is vital to understand linkages between the actions of key mycorrhizal species and ecological functions (Dahlberg, 2001), and the processes responsible for increased organic P mineralization under trees are not fully understood (Condron and Tiessen, 2005). Mycorrhizae have been well documented to boost plant P acquisition from solution P by increasing the volume of soil explored, decreasing the possible pore size for exploration and by increasing the surface area for nutrient absorption (Marschner and Dell, 1994). However much less is understood of the role of mycorrhizae in plant acquisition of soil organic P, including the question of whether mycorrhizae influence rhizosphere phosphatase levels and organic P solubility by organic anion exudation.

The rhizosphere P dynamics of radiata pine, eucalypt and macrocarpa in the field are described in Chapter 2. The separate influence of the rhizosphere components on P dynamics for each species is investigated in Chapter 3. It was revealed that mycorrhizae have proportionally different roles in the P dynamics of each of these species with the mycorrhizal association of eucalypt and radiata pine playing a larger role in P dynamics of these two species than the mycorrhizal association of macrocarpa. This chapter aims to assess what processes were occurring in the hyphal compartment of Chapter 3 and determine the direct contribution of mycorrhizal extraradical hyphae to P dynamics of ectomycorrhizal radiata pine, tripartite eucalypt and arbuscular mycorrhizal macrocarpa. Measurement of phosphatase activity, microbial activity and LMWOA production in

inoculated and non inoculated sterile trees was used to link in these activities with mycorrhizal infection. Following the inoculation experiment, the hyphae of mycorrhizal fungi infecting the three tree species both in the field and in a glasshouse study were extracted from sand The capacity of the hyphae for producing phosphatase enzymes in response to organic P was determined by using sand that was either spiked or not spiked with calcium phytate, a sparingly soluble organic P source and using enzyme labeled fluorescence (ELF).

4.2 Materials and Methods

4.2.1 Inoculation Experiment

This experiment was designed to compare and assess the phosphatase enzymes and LMWOAs exuded in the rhizosphere of eucalypt, macrocarpa and radiata pine trees with either arbuscular mycorrhizal associations, ectomycorrhizal associations or no mycorrhizal association.

4.2.1.1 Seedling Germination

In September 2008, seeds of radiata pine, eucalypt and macrocarpa were surface sterilized by soaking in 5% $NaOCl_2$ for 30 minutes (radiata pine), 10 minutes (eucalypt) or 15 minutes (macrocarpa). They were then germinated in a growth medium of 50% coarse silica sand, 30% pumice and 20% potting mix (80% bark and 20% pumice with 20 g Osmocote extract [16-3.9-10 NPK], 10 g lime and 10 g hydraflo wetting agent per 10 litres) sterilized by autoclaving at 121^{o}C and placed in sealed UV irradiated sun trays with water to 10% volume. These trays were placed on larger plastic trays and kept in a glasshouse (15-19^{o}C) until sufficient growth for transplantation and experimental set up. Deionized water was maintained at 1 cm depth on the larger plastic trays so the mix could draw water up as required during the germination period.

4.2.1.2 Mycorrhizal Inoculum Production

To make the ectomycorrhizal inoculant, two isolates of *Rhizopogon rubescens* (KOS55 and KOS51, both originally isolated from *R. rubescens* sporocarps in the Kaingaroa

forest under five year old pine trees and supplied by K. Walbert, SCION Rotorua, New Zealand) were grown on malt extract agar (Difco, Beetun Dickinson and Company, ML, USA) in the dark at 20^{o}C. To build up an inoculation source, ten 2 L Erlenmeyer flasks were filled to 1 L with 2 mm sieved peat and vermiculite mix (1:4 on a volume basis) and autoclaved for 15 minutes at 121^{o}C. Subsequently, 190 ml malt extract was added to each flask and these were autoclaved a second time for 15 minutes. For both KOS55 and KOS51, five flasks were inoculated with 15, 5 mm plugs taken from the edge of actively growing colonies on malt extract agar. A bung covered with tin foil was placed in the opening to prevent contamination by axenic culture but allow respiratory gas exchange. The flasks were then incubated at 20^{o}C in the dark for two months with periodic shaking, every week, to mix.

To produce an arbuscular mycorrhizal inoculum, arbuscular mycorrhizal spores from the Orton Bradley Park field site were collected using the wet sieving method described in Brundrett et al. (1996). Three kg of soil from plot 12 at the site was collected and soaked in water (10 L water for 1 kg soil) for two hours, stirred vigorously and left to stand for 30 minutes. The weight of soil collected was based on a predetermined spore count of 0.22 spores/g in Orton Bradley Park soil, the results of which are presented in Appendix 4.1. The water was then passed through a series of sieves (700-500-150-50 μm) and everything caught on the 150 and 50 μm sieves was washed into 50 g fine silica sand and mixed.

A crude bacterial filtrate (free of AM fungi) was made by thoroughly mixing 100 g soil (from Orton Bradley Park) in 1 L of water for one hour, letting this stand for 30 minutes and passing the water through a series of filters (11-6 μm) using a vacuum pump. The water was collected after passing through the 6 μm filter and applied in the experimental set up the same day.

4.2.1.3 Experimental set up

After germination and sufficient growth to withstand transplanting (2-3 weeks) in October 2008, the seedlings were transplanted into 224 cm^{3}, UV irradiated pots containing sterile growth media of the same composition as that for germination (section 4.2.1.1), with the appropriate treatment. Five replicates of each species received

the ectomycorrhizal treatment (EM). Here, the fungal inoculum was added to the growth media prepared for these replicates and mixed by hand at a 10% volume (5% of each isolate) rate. Each of these replicates also received 25 ml of the bacterial filtrate pipetted onto the surface of each pot.

Five replicates of each species received the arbuscular mycorrhizal treatment (AM). Here, the 50 g of fine silica sand containing the sieved spores (described in Section 4.2.1.2) was thoroughly mixed into the growth media prepared for these replicates. Each of these replicates also received 25 ml of the bacterial filtrate pipetted onto the surface of each pot. Five replicates of each species received Bacteria treatment (Bac) which consisted of no mycorrhizal inoculum, only 25 ml of bacterial filtrate (described in Section 4.2.1.2) pipetted onto the surface of each pot. There were 15 replicates of each species, five with EM treatment, five with AM treatment and five with bacterial filtrate alone. The pots were placed in sun bags to avoid contamination and incubated in a glasshouse with a minimum temperature of 15^{o}C. Each pot was watered once weekly with 10 ml deionized water using a needle and syringe to pierce the sun bag membrane without compromising the contamination barrier and direct the water into the pot.

4.2.1.4 Harvesting and Analysis

In early April 2009, the experiment was terminated and analyzed. The growth media adhering to the tree root system upon removal was classed as rhizosphere. It was removed from the roots by gentle shaking and scraping with fine forceps and each sample was analyzed for pH, acid and alkaline phosphomonoesterase and phosphodiesterase activity using the methods described in Chapter 2. Low molecular weight organic anions were extracted using the method described by Mimmo et al. (2008) where a 5 g sample of growth medium was extracted with 20 ml 10 mM KH_2PO_4, shaken for four hours and then centrifuged at 15,000 x *g* for 5 minutes. The supernatant was then filtered through a 20 μm syringe filter and frozen at -20^{o}C prior to analysis using high pressure liquid chromatography (HPLC).

The seedlings were removed from the growth media, and soil removed as described in section 4.2.1.4. Above ground parts were then cut to separate from below ground parts and the height and fresh weight of the above ground seedling biomass was determined.

The root system fresh weight was taken before for mycorrhizal colonization analysis. Roots from all samples were analyzed for both ectomycorrhizal and arbuscular mycorrhizal colonization to ensure the treatments had been effective. Approximately 30% of the root system was used for ectomycorrhizal colonization determination using the gridline intersection method described in Chapter 2. Arbuscular mycorrhizal colonization was determined using an INVAM modified clearing and staining method (http://invam.caf.wvu.edu/methods/mycorrhizae/staining.htm). Approximately 30% of the root system was used in this procedure. Roots were thoroughly washed and placed in plastic tissue cassettes (Ted Pella Inc. CA, USA), covered with 10% KOH and autoclaved at 120^{o}C for 10 minutes. This KOH was then washed off and roots were placed in 2% HCl for 20 minutes for acidification. The acid was then poured off and root samples placed in 0.05% trypan blue at room temperature. After 12 hours, samples were rinsed and kept in de stain solution (1:1 water:glycerol) for 2-7 days at 4^{o}C prior to mycorrhizal colonization assessment. Roots were then placed in a Petri dish with a 1 x 1 cm grid and the gridline intersection method was used to assess % colonization. A stereo microscope at 10x magnification was used to count total root crossings and root crossings containing stained fungal structures. Each crossing represented 1 cm of root and the proportion of crossings containing fungal structures was used to calculate % colonization. The remaining 40% of each root system was combined with the corresponding shoots and dried at 70^{o}C for 72 hours and ground prior to total P content analysis by ICP-OES.

4.2.2 Hyphal Phosphatase Enzyme Activity

Detection of phosphatase activity in mycorrhizal hyphae was done using hyphae from both a glasshouse and a field study using enzyme labeled fluorescence (ELF) with an ELF® 97 Endogenous phosphatase detection kit (Molecular probes, Invitrogen Detection Technologies).

4.2.2.1 Glasshouse Study

Mesh bags were created by heat sealing two 12 x 12 cm squares of 25 μm nylon mesh on three sides. In July 2007, six approximately 20 cm high eucalypt, macrocarpa and

radiata pine seedlings (supplied by Southern Woods Nursery Ltd, New Zealand) were placed inside the mesh bags which were then filled with a 1:4 (volume:volume) mixture of potting mix (60% peat, 20% bark and 20% pumice, supplied by Southern Woods Nursery Ltd, New Zealand) and Orton Bradley Park soil (bulked from plots 9,8 and 7 at the Orton Bradley Park field site as described in Chapter 2 and passed through a 4 mm sieve). These bags containing the newly planted seedlings were then placed in pots surrounded by fine, acid washed silica sand (Figure 4.1). Three pots of each species contained just sand, and three contained sand spiked with calcium phytate ($C_6H_6Ca_6O_{24}P_6$) at 100 mgP/kg. Calcium phytate was used due to its insolubility in water and was prepared by adding saturated calcium chloride to a solution of sodium phytate. The pore size of the mesh allows hyphae but not root penetration into the sand and therefore is essentially a hyphosphere. Sand allows for ease of hyphal extraction. In October 2008 the experiment was terminated, mesh bags removed and the hyphae were extracted from the sand.

Figure 4.1 Glasshouse experimental set up for hyphae extraction and ELF staining showing the soil: potting mix filled 25 µm mesh bag surrounded by acid washed silica sand. a) eucalypt, b) macrocarpa.

4.2.2.2 Hyphae Extraction and Staining

Hyphae were extracted from the sand as described in van Aarle et al. (2001). The sand was submerged in deionized water and stirred rapidly which caused the hyphae to float. After 1 hour, to allow the sand to settle, the water was poured through a 50 µm sieve on which the hyphae were collected. The hyphae were then washed into a Petri dish with

deionized water and any sand particles remaining on the hyphae were carefully removed with forceps under a dissecting microscope.

The extracted hyphae were stained for phosphatase enzyme activity using ELF with a modified version of the manufacturer's protocol (Molecular probes, Invitrogen Detection Technologies) and that described by van Aarle et al. (2001).

Hyphae from each replicate rhizosphere were firstly permeabilized by submersion in 0.2% TWEEN (Tween 80 Analar ® ,BDH Chemicals Ltd, Poole, England) in phosphate buffered saline (PBS) for 10 minutes in separate Petri dishes. They were then removed from each dish with tweezers and placed in another containing PBS for a further 10 minutes with occasional gentle agitation. Following this, the samples were removed from the Petri dishes using tweezers and incubated in 50 µl of the chosen detection buffer in a 2 ml microcentrifuge tube. Alkaline phosphatase detection buffer was made by combining component A (phosphatase substrate) and B (detection buffer) of the detection kit and acid phosphatase detection buffer was made by combining component A with 90 mM citrate buffer (Sigma Aldrich, pH 4.8), both at a 1:20 dilution rate.

After 30 minutes (tested to be the optimum incubation time after a series of time trials specific to each species), the reaction was stopped by removing the hyphae from the buffer solution and resubmerging the samples in 0.5 ml of the appropriate stop buffer. Hyphae were removed after five minutes and placed in a new tube of stop buffer for a further five minutes and again until each sample underwent three changes of the stop buffer to ensure all ELF crystals were removed thus reducing background signal. The stop buffer was a solution of PBS with 25 mM EDTA and 2 mM sodium fluoride (NaF) for acid phosphatase or 2 mM potassium cyanide (KCN) for alkaline phosphatase. Both NaF and KCN were predetermined to be suitable enzyme inhibitors by van Aarle et al. (2001). Excess water was then removed from the samples before mounting in component C (mounting medium) of the detection kit on microscope slides and detecting fluorescence at an excitation of 345 nm and emission of 530 nm using an Olympus Systems microscope BX51 with a Hoest/DAPI longpass filter set 345-530 nm.

4.2.2.3 Field Study

Extractable hyphae were also collected from the same species growing in field conditions. To do this, 12 x 12 cm 25 μm nylon mesh bags were filled with fine acid washed silica sand in October 2007, and placed in selected plots at Orton Bradley Park (plot 10 for radiata pine, plot 11 for eucalypt, and plot 12 for macrocarpa). Fungal hyphae but not tree roots are able to penetrate the mesh bag enabling extraction when the bags are removed and therefore making the bag content a hyphosphere. Three bags of plain sand and three spiked with organic P as described for the glasshouse experiment were placed within 60 cm of the randomly chosen tree base and 10 cm below the soil surface. These bags were removed in November 2008 and hyphae were extracted from the sand and then subject to the staining procedure described above for the glasshouse study.

4.2.3 Statistical Analysis

Statistical analysis was completed for the mycorrhizal inoculation experiment using GenStat 10 (Lawes Agricultural Trust, Rothamstead Experimental Station, UK). Interactions between species and treatment on biomass parameters, phosphatase enzyme activities and P uptake were analyzed using two way ANOVAs. Where *F*- ratios were significant ($P<0.05$), treatment means were compared by least significant differences (LSD). Complete ANOVA results are presented in Appendix 4.2. Relationships between phosphatase enzyme activity and mycorrhizal colonization and P uptake were determined by fitting the data to linear regression functions. Relationships were significant at $P<0.05$ and importance was determined by regression coefficient ($R^2> 0.65$).

4.3 Results

4.3.1 Inoculation Experiment

Plant Growth and Mycorrhizal Colonization

Results presented in Table 4.1 are plant biomass at experiment termination with all treatments. Species was the only influencing factor on plant biomass. Eucalypt

consistently had a significantly higher biomass in length and weight of both above ground and below ground parts than radiata pine and macrocarpa which did not significantly differ from each other. Treatment had no influence on any plant biomass parameter.

Table 4.1 Mean length and fresh weight of radiata pine, macrocarpa and eucalypt roots and shoots determined in response to inoculation with bacterial filtrate only (Bac), *R. rubescens* plus bacteria (EM) and arbuscular mycorrhizal spores plus bacteria (AM). Values in parenthesis are standard errors of mean, n = 5.

		Tree height (cm)	Tree FW (g)	Root length (cm)	Root FW (g)
Radiata pine	Bac	21.9 (2.1)	1.6 (0.4)	360 (61.1)	1.6 (0.3)
	EM	20.9 (2.5)	1.7 (0.4)	373 (65.9)	1.8 (0.3)
	AM	22.4 (1.6)	2.1 (0.4)	278 (35.5)	1.3 (0.2)
	Mean	**21.7**	**1.8**	**337**	**1.5**
Macrocarpa	Bac	20.7 (1.7)	1.3 (0.2)	341 (39.2)	1.3 (0.2)
	EM	22.6 (1.4)	1.8 (0.3)	346 (14.5)	1.4 (0.1)
	AM	20.1 (2.3)	1.25 (0.4)	255 (31.9)	0.9 (0.2)
	Mean	**21.1**	**1.5**	**314**	**1.2**
Eucalypt	Bac	27.8 (0.8)	3.2 (0.4)	1656 (177.5)	2.2 (0.3)
	EM	24.9 (1.4)	2.7 (0.4)	1541 (160.3)	2.1 (0.2)
	AM	26.4 (1.7)	2.6 (0.4)	1902 (99.0)	2.6 (0.3)
	Mean	**26.4**	**2.8**	**1700**	**2.3**
Significance		$P = 0.002$	$P<0.001$	$P<0.001$	$P<0.001$
LSD		2.96	0.61	155.5	0.42

Table 4.2 demonstrates that radiata pine roots showed no colonization by arbuscular mycorrhizae with any treatment. No colonization with either mycorrhizal type was found on radiata pine roots with Bac treatment (Figure 4.2a). Ectomycorrhizal colonization of radiata pine roots occurred in both the EM and AM treatment (Figure 4.2b) with a 1.6 fold higher colonization rate from the AM treatment. Ectomycorrhizal tips were light yellowish brown showing mainly dichotomous ramification with some unramified and a few coralloid ramification examples. Ramified ends were straight with

a smooth surface of some luster and appeared to be of the same morphotype with both inoculum treatments.

Macrocarpa showed no ectomycorrhizal colonization with any treatment. Arbuscular mycorrhizal colonization occurred with the AM treatment but not with any other treatment. The arbuscular mycorrhizal structures seen in macrocarpa roots were arbuscles, hyphae, spores and vesicles (Figure 4.3).

Eucalypt showed similar colonization patterns to radiata pine. There was no arbuscular mycorrhizal colonization found in any treatment. No colonization by arbuscular mycorrhizae or ectomycorrhizae mycorrhizae was found in the Bac treatment. Ectomycorrhizal root tips observed from the EM treatment were short, smooth and dark in colour with some luster and mainly monopodial-pinnate ramification (Figure 4.4 a and b). A 1.7 fold higher ectomycorrhizal colonization rate was found with the AM treatment where most tips were longer and paler in colour with less luster and unramified (Figure 4.4 c and d).

Table 4.2 Mean arbuscular (%) and ectomycorrhizal (ECM tips/cm or total ECM tips/ seedling) colonization rate found in roots of radiata pine, macrocarpa and eucalypt inoculated with bacterial filtrate only (Bac), *R. rubescens* plus bacteria (EM) or arbuscular mycorrhizal spores plus bacteria (AM), n = 5.

		EM tips/cm	Total EM tips	AM col %
Radiata pine	Bac	0	0	0
	EM	0.4	159	0
	AM	0.9	261	0
Macrocarpa	Bac	0	0	0
	EM	0	0	0
	AM	0	0	48
Eucalypt	Bac	0	0	0
	EM	0.2	320	0
	AM	0.3	550	0

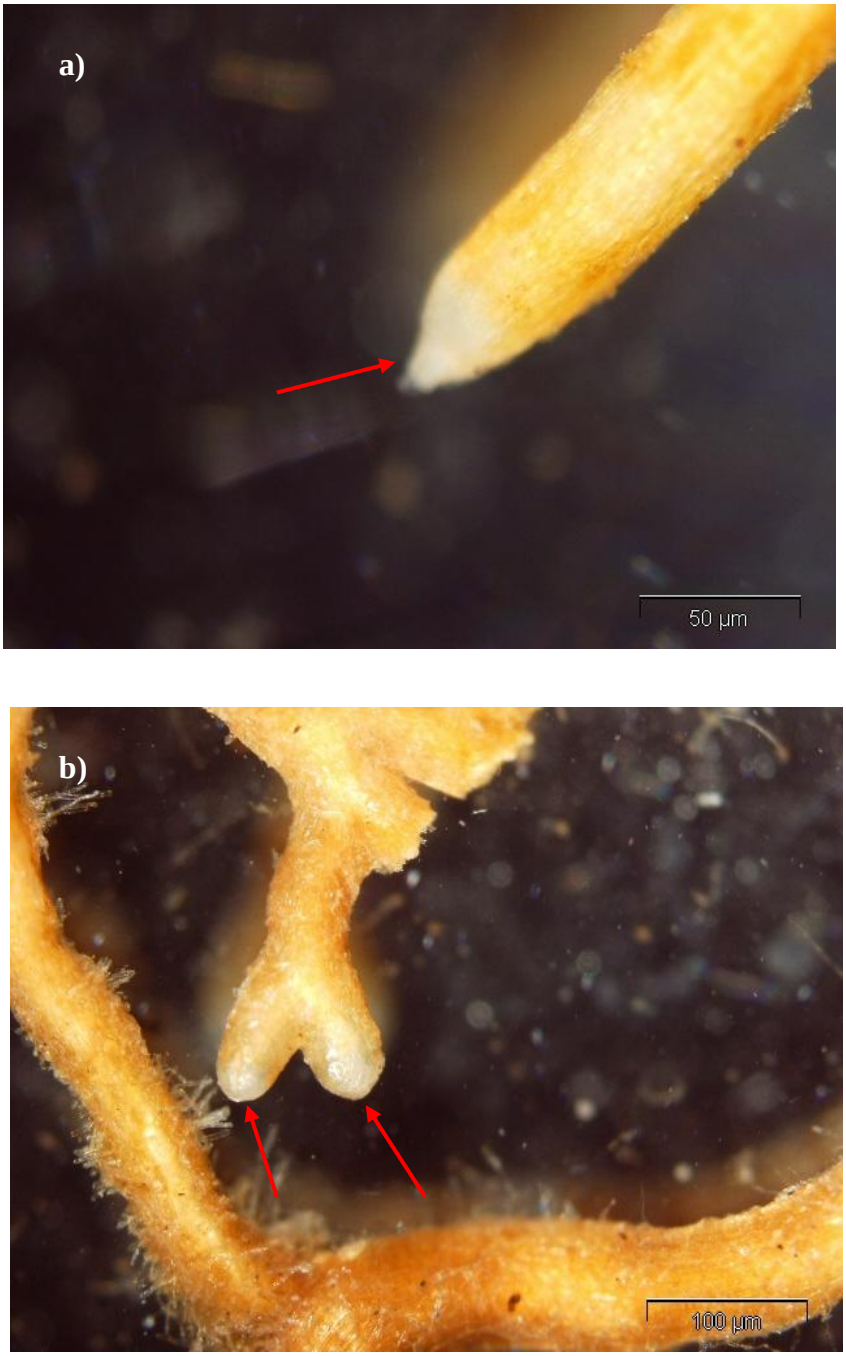

Figure 4.2 a) An uncolonized radiata pine root tip from a seedling with bacterial filtrate only and b) ectomycorrhizal root tips of radiata pine grown with *R. rubescens* plus bacterial filtrate. Tips are indicated with red arrows.

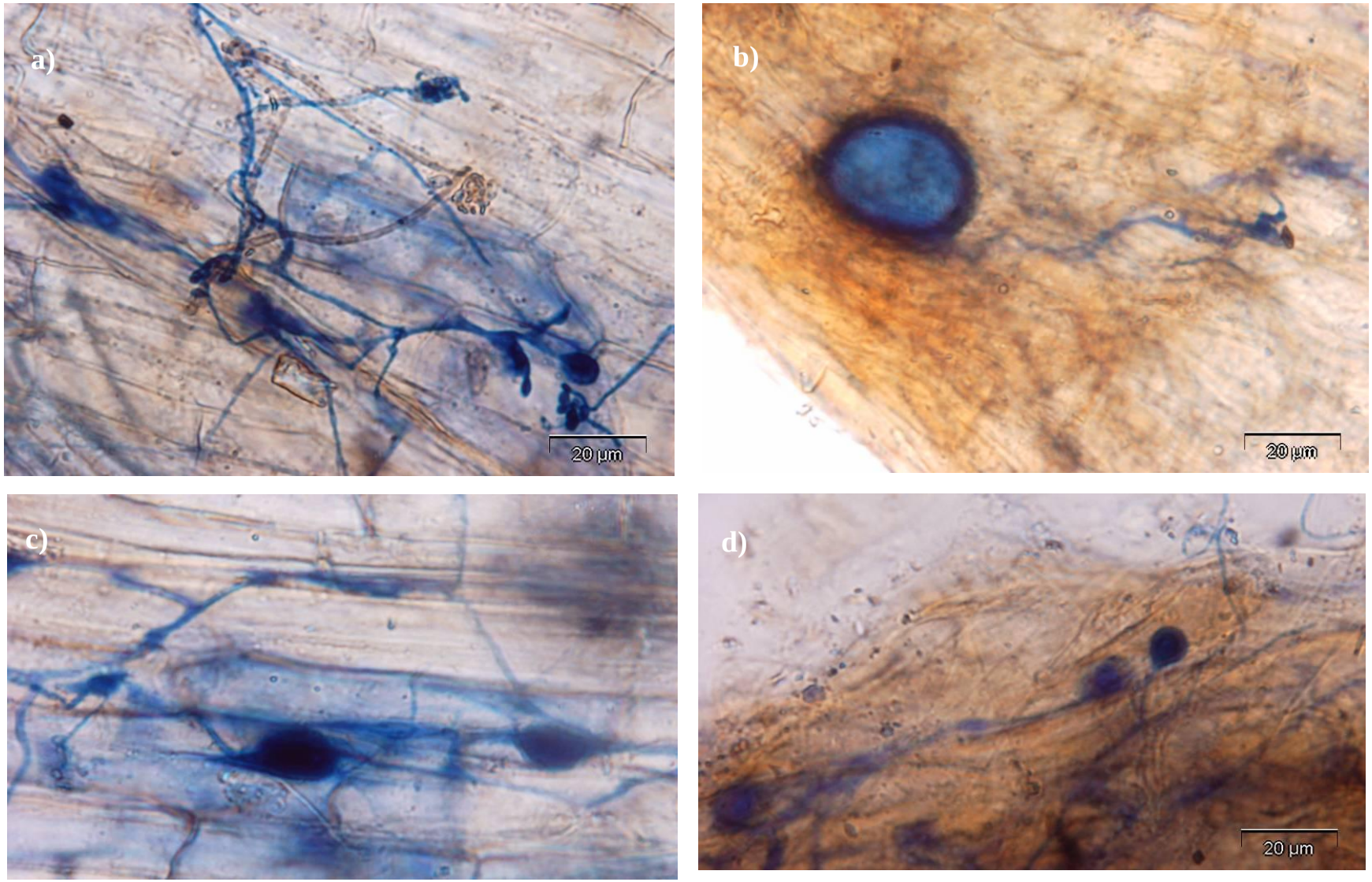

Figure 4.3 Arbuscular mycorrhizal structures observed in roots of macrocarpa seedlings inoculated with arbuscular mycorrhizal spores and a bacterial filtrate.

Figure 4.4 Ectomycorrhizal tips of eucalypt seedlings inoculated with (a, b) *R. rubescens* plus bacteria (EM) and (c, d) arbuscular mycorrhizal spores plus bacteria (AM). Tips are indicated with red arrows.

Phosphatase enzyme levels

Results presented in Table 4.3 are phosphomonoesterase and diesterase activities in the growth media of all three species with all treatments. For radiata pine, a significantly higher acid phosphomonoesterase activity was observed with the AM treatment than with both the EM and Bac treatment and activity with the EM treatment was significantly higher than with the Bac treatment. Significantly higher acid phosphomonoesterase activity was recorded with the AM treatment for macrocarpa and eucalypt with no significant difference observed between the EM and Bac treatments for either species.

With both the AM and Bac treatments, eucalypt had a significantly higher acid phosphomonoesterase activity than radiata pine, which in turn, had a significantly higher activity than macrocarpa, whereas with the EM treatment, eucalypt activity was only significantly higher than that of macrocarpa and radiata pine activity did not differ significantly from either of these species.

Alkaline phosphomonoesterase activity was much lower than that of acid phosphomonoesterase and differences between treatments were relatively small. Treatment had no significant effect on alkaline phosphomonoesterase activity within any species. Only species had a significant effect in alkaline phosphomonoesterase with eucalypt and radiata pine having a significantly higher activity than macrocarpa.

Phosphodiesterase activity was much lower than either phosphomonoesterase activity. Both species and treatment had a significant influence on phosphodiesterase activity but this did not significantly interact. Eucalypt and radiata pine both had a significantly higher activity than macrocarpa and the EM treatment resulted in a significantly higher activity than both the AM and Bac treatment.

Table 4.3 Mean acid and alkaline phosphomonoesterase and phosphodiesterase activity determined in growth media of eucalypt, radiata pine and macrocarpa inoculated with bacterial filtrate alone (Bac), *R. rubescens* plus bacteria (EM) or arbuscular mycorrhizal spores plus bacteria (AM). Values in parenthesis are standard errors of means, n = 5.

		Eucalypt	Radiata pine	Macrocarpa
Acid PME (μmol/g/hr)	Bac	11.5 (0.21)	8.3 (0.14)	6.5 (0.39)
	EM	11.7 (0.15)	10.5 (0.65)	7.5 (0.50)
	AM	17.6 (0.84)	13.8 (1.02)	10.3 (0.64)
Alkaline PME (μmol/g/hr)	Bac	0.5 (0.05)	0.5 (0.06)	0.4 (0.04)
	EM	0.5 (0.02)	0.5 (0.05)	0.4 (0.04)
	AM	0.4 (0.03)	0.5 (0.06)	0.3 (0.02)
PDE (μmol/g/hr)	Bac	0.08 (0.008)	0.10 (0.007)	0.06 (0.011)
	EM	0.13 (0.011)	0.12 (0.008)	0.08 (0.010)
	AM	0.09 (0.005)	0.07 (0.010)	0.06 (0.009)

PME = phosphomonoesterase, PDE = phosphodiesterase

Table 4.4 Means and 5% LSDs for important significant influences and interactions on phosphatase enzyme activity in the mycorrhizal inoculation experiment.

Enzyme	Interaction	Significance		Means			LSD
Acid PME	Species*Treatment	$P = 0.048$		*AM*	*EM*	*Bac*	1.67
			Radiata	13.8	10.5	8.3	
			Eucalypt	17.6	11.7	11.5	
			Macro	10.3	7.5	6.5	
Alkaline PME	Species	$P = 0.002$	*Radiata*	0.5			0.08
			Eucalypt	0.4			
			Macro	0.4			
PDE	Species	$P<0.001$	*Radiata*	0.10			0.014
			Eucalypt	0.10			
			Macro	0.07			
	Treatment	$P<0.001$		*AM*	*EM*	*Bac*	
				0.07	0.11	0.08	

PME = phosphomonoesterase, PDE = phosphodiesterase

Plant P uptake

Plant P content data taken from total plant dry matter is displayed in Table 4.5. Eucalypt seedlings (average 2.47 mg) always contained significantly more P than radiata pine (average 1.28 mg) and macrocarpa (average 0.98 mg) across all treatments. Radiata pine and macrocarpa P content did not differ significantly from each other within any treatment. Within each species, plant P content was not significantly different with any treatment.

Plant P concentration (mg/g) was lowest with EM treatment for eucalypt and macrocarpa but lowest with Bac treatment for radiata pine. The AM treatments resulted in the highest P concentration between treatments for all species.

Table 4.5 Mean total plant P content and plant P concentration determined in eucalypt, radiata pine and macrocarpa inoculated with bacterial filtrate alone (Bac), *R. rubescens* plus bacteria (EM) or arbuscular mycorrhizal spores plus bacteria (AM). Values in parenthesis are standard errors of means, n = 5.

		Eucalypt	Radiata pine	Macrocarpa
Plant P content (mg)	Bac	2.34(0.396)	1.26 (0.336)	1.12 (0.146)
	EM	2.39 (0.164)	1.11 (0.128)	0.87 (0.130)
	AM	2.69 (0.396)	1.48 (0.290)	0.95 (0.203)
	Mean	**2.47**	**1.28**	**0.98**
			$P<0.001$; LSD = 0.410	
Plant P concentration (mg/g)	Bac	2.46	2.39	2.75
	EM	2.32	2.68	2.61
	AM	2.80	2.81	2.86

LMWOAs

Results presented in Table 4.6 show the organic acids detected in the growth media containing each species under each treatment. Only four organic acids (tartaric, formic, maleic and citric acid) were detected in the growth media from this experiment. Formic acid was detected in relatively high concentrations (1.188-2.400 mg/kg) and usually in 2-3 replicates. Maleic acid was produced with the most regularity (3-5 replicates) but at smaller concentrations (0.028-0.092 mg/kg). Both citric and tartaric acid were detected at sometimes high concentrations but it was not consistent across replicates (1-4 replicates) or in the concentration detected (0.800-2.868 mg/kg and 1.520-3.520 mg/kg for tartaric and citric acid, respectively).

All four organic acids were detected in eucalypt growth media with EM treatment where formic acid was detected at the highest concentration (2.188 mg/kg). Only tartaric and maleic were detected with Bac treatment and this was inconsistent across replicates (one replicate for tartaric) or in low concentrations (0.036 mg/kg for maleic). Only maleic and citric were detected with AM treatment and citric was at a high concentration (3.520 mg/kg) but only in one replicate and maleic was at a low concentration (0.044 mg/kg).

With Bac treatment, all organic acids were detected in radiata pine growth media at consistent concentrations (0.188-1.544 mg/kg) and numbers of replicates (3-5) except tartaric acid which was only detected in one replicate. Formic, maleic and citric acids were detected with EM and AM treatments. Formic (2.256 mg/kg) and citric (2.488 mg/kg) acid concentrations were highest with EM treatment but only in one replicate whereas they were lower in the AM treatment (1.188 mg/kg and 1.728 mg/kg for formic and citric acid, respectively) but found in two replicates.

All organic acids were detected in macrocarpa growth media with all treatments, except citric acid under EM treatment. AM treatment resulted in the most organic acids being detected at higher concentrations (0.092-2.904 mg/kg) and in 2-4 replicates whereas Bac and EM treatments produced varying levels of each acid with varying regularity (1-5 replicates).

Table 4.6 Low molecular weight organic acids detected in growth media inoculated with bacterial filtrate only (Bac) *R. rubescens* plus bacteria (EM) or arbuscular mycorrhizal spores plus bacteria (AM) and planted with eucalypt, radiata pine or macrocarpa in the inoculation experiment. Values in parenthesis are the number of replicates the acid was detected in.

Species	Treatment	Tartaric acid mg/kg	Formic acid mg/kg	Maleic acid mg/kg	Citric acid mg/kg
Eucalypt	**Bac**	1.736 (1)	-	0.036 (3)	-
	EM	1.332 (2)	2.188 (2)	0.048 (3)	1.520 (1)
	AM	-	-	0.044 (5)	3.520(1)
Radiata pine	**Bac**	1.300 (1)	1.388 (3)	0.188 (5)	1.544(3)
	EM	-	2.256 (1)	0.028 (3)	2.480 (1)
	AM	-	1.188 (2)	0.080 (3)	1.728 (2)
Macrocarpa	**Bac**	0.800 (1)	2.400 (3)	0.100 (5)	2.172 (2)
	EM	2.868 (1)	2.276 (2)	0.028 (3)	-
	AM	2.544 (2)	2.292 (3)	0.092 (4)	2.904 (4)

Both acid phosphomonoesterase and phosphodiesterase activity across all species correlated significantly with the total root length but with R^2 values of 0.34 and 0.11, respectively, this relationship did not explain all of the data. Alkaline phosphomonoesterase did not correlate with root length. For each species individually, total root length did not correlate with any of the phosphatase enzyme activity levels.

Table 4.7 gives significance levels and R^2 values for significant relationships between mycorrhizal colonization and acid phosphomonoesterase activity. There was a significant positive correlation between ectomycorrhizal colonization (both ECM tips/cm root and total ECM tips) of both radiata pine and eucalypt and acid phosphomonoesterase activity. The correlations in radiata pine had slightly higher significance levels and higher R^2 values (0.65 and 0.57 for radiata pine and 0.47 and 0.54 for eucalypt tips/cm root and total tips, respectively). There were no such correlations between ectomycorrhizal colonization and alkaline phosphomonoesterase or phosphodiesterase activity. Percentage arbuscular mycorrhizae colonization of roots was also seen to correlate significantly with rhizosphere acid phosphomonoesterase (R^2 = 0.52) but not alkaline phosphomonoesterase or phosphodiesterase activity in macrocarpa (Table 4.7).

Table 4.7 Significant relationships determined between rhizosphere acid phosphomonoesterase (PME) activity and species specific mycorrhizal colonization in the inoculation experiment.

Relationship with acid PME	Significance	R^2
Radiata pine EM tips/cm	0.001	0.65
Radiata pine total EM tips	0.001	0.57
Eucalypt EM tips/cm	0.005	0.47
Eucalypt total EM tips	0.002	0.54
Macrocarpa % AM colonization	0.002	0.52

Plant P content correlated significantly with acid phosphomonoesterase activity across all species and treatments but with an R^2 of 0.34, this did not explain all of the data. Alkaline phosphomonoesterase and phosphodiesterase activity did not correlate with plant P content. Within each species separately, plant P content did not correlate with activity levels of any enzyme or with mycorrhizal colonization.

4.3.2 Hyphal Phosphatase Activity

The aim of this experiment was to detect internal phosphatase activity or that on walls of mycorrhizal hyphae which is seen as fluorescent crystals. The data is given in two ways. First the proportion of hyphae present that are showing some degree of phosphatase activity (fluorescent crystals) is stated and then the brightness of the crystals is given using the scale described in Tables 4.8 and 4.9.

4.3.2.1 Glasshouse Study

Results presented in Table 4.8 give acid and alkaline phosphatase activity levels shown by fluorescent staining of the hyphae associated with the three tree species when spiked or not spiked with calcium phytate P in the glasshouse study. Activity shown by % of hyphae fluorescing and the brightness of this fluorescence, of all three species was greater at alkaline pH although hyphae also showed activity at acid pH. Hyphae in the macrocarpa hyphosphere had the greatest phosphatase activity at both acid and alkaline

pH when given an organic P source (spiked). This high activity was evident in both the percentage of hyphae fluorescing and the amount of fluorescence within each hypha (given by the brightness value). The hyphal phosphatase activity in macrocarpa was not greatly affected by the presence of an organic P source as the amount of hyphae showing activity did not change when spiked with organic P (Figure 4.5 a-d). Activity was always greater at alkaline pH for macrocarpa either in amount of hyphae showing activity or the brightness of the activity. Some young arbuscular mycorrhizal spores were present on these hyphae and were seen to fluoresce (Figure 4.6). A small number of fine roots were also present in some samples from mnacrocarpa and were shown to fluoresce at acid but not alkaline pH.

The phosphatase activity of hyphae from the radiata pine hyphosphere was not greatly affected by the presence of an organic P source (Table 4.8; Figure 4.7). Activity was slightly higher under alkaline pH both in amount of hyphae fluorescing (78% versus 70-75%) and brightness of the fluorescence (2.3 versus 1.3). Radiata pine activity was consistently lower than that of macrocarpa associated hyphae under both pH levels. Hyphal phosphatase activity of radiata pine was generally higher than that of eucalypt associated hyphae when given an organic P source but lower when not supplied with an organic P source across both pH values.

Hyphae from the hyphosphere of eucalypt were very dense and formed large clumps, especially in P spiked sand (Figure 4.8a). The phosphatase activity varied between spiked and non spiked treatments. When an organic P source was present, eucalypt activity was much lower than for the other two tree species. Less than half the hyphae present showed activity (42-43%) and brightness values were lower (1.6-1.8; see Figure 4.9 a and b). When an organic P source was not present, all hyphae showed activity but brightness values were still low (1.3-1.5; see Figure 4.9 c and d).

Table 4.8 Average percentage of hyphae showing phosphatase activity and average brightness value (in parenthesis) at both acid and alkaline pH when given an organic P source (spiked) or not (non spiked) in the glasshouse ELF staining study, n = 6.

	P spiked		Non spiked	
	Acid	Alkaline	Acid	Alkaline
Eucalypt	43%	42%	100%	100%
	(1.6)	(1.8)	(1.3)	(1.5)
Radiata pine	70%	78%	75%	78%
	(1.3)	(2.3)	(1.3)	(2.3)
Macrocarpa	95%	95%	95%	98%
	(2)	(2.8)	(2)	(2.3)

Brightness values: 1 = some small fluorescing crystals, 2 = some large and small fluorescing crystals, 3 = many large fluorescing crystals.

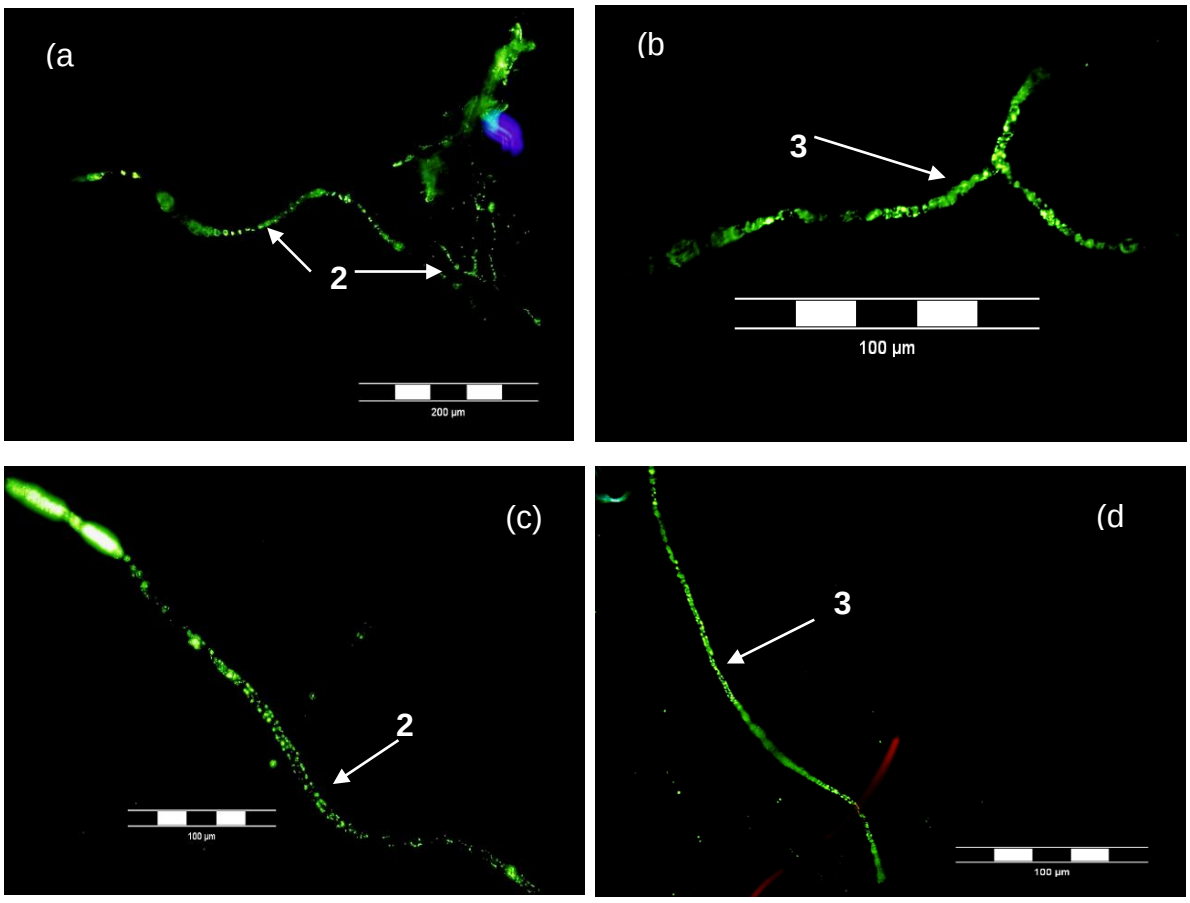

Figure 4.5 Phosphatase activity of hyphae in macrocarpa hyphosphere as shown by ELF staining from the glasshouse study. (a,b) From P spiked and (c,d) non P spiked sand showing activity at acid pH with brightness value of 2 or 3. Examples are indicated with white arrows.

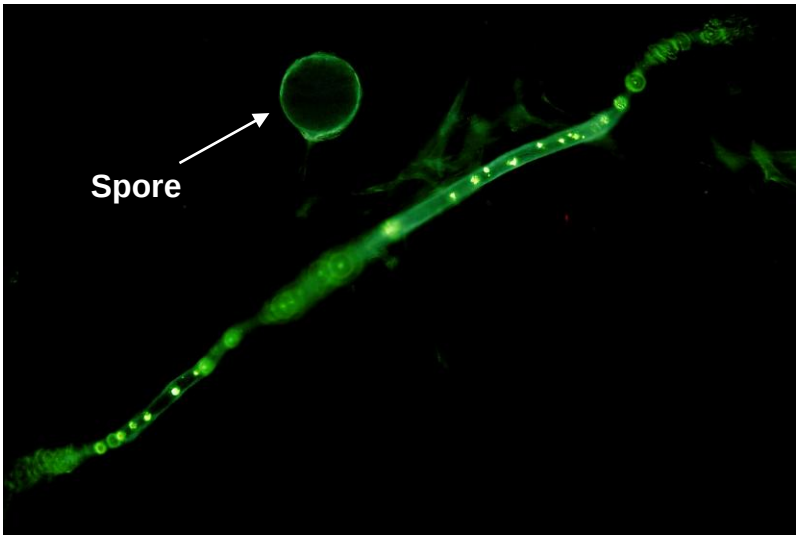

Figure 4.6 Phosphatase activity of AM hyphae and young intact spore as shown by ELF staining at acid pH in the hyphosphere of macrocarpa.

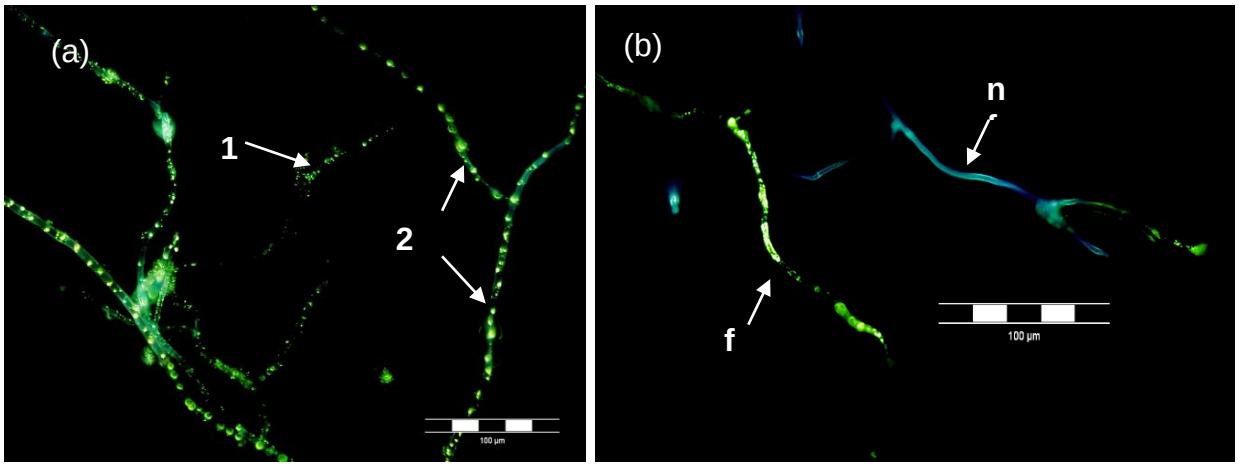

Figure 4.7 Phosphatase activity of hyphae in radiata pine hyphosphere as shown by ELF staining in the glasshouse study. a) Showing activity with brightness value of 1 and 2 and b) a comparison of hyphae with (f) and without (nf) activity in P spiked sand. Examples are indicated with white arrows.

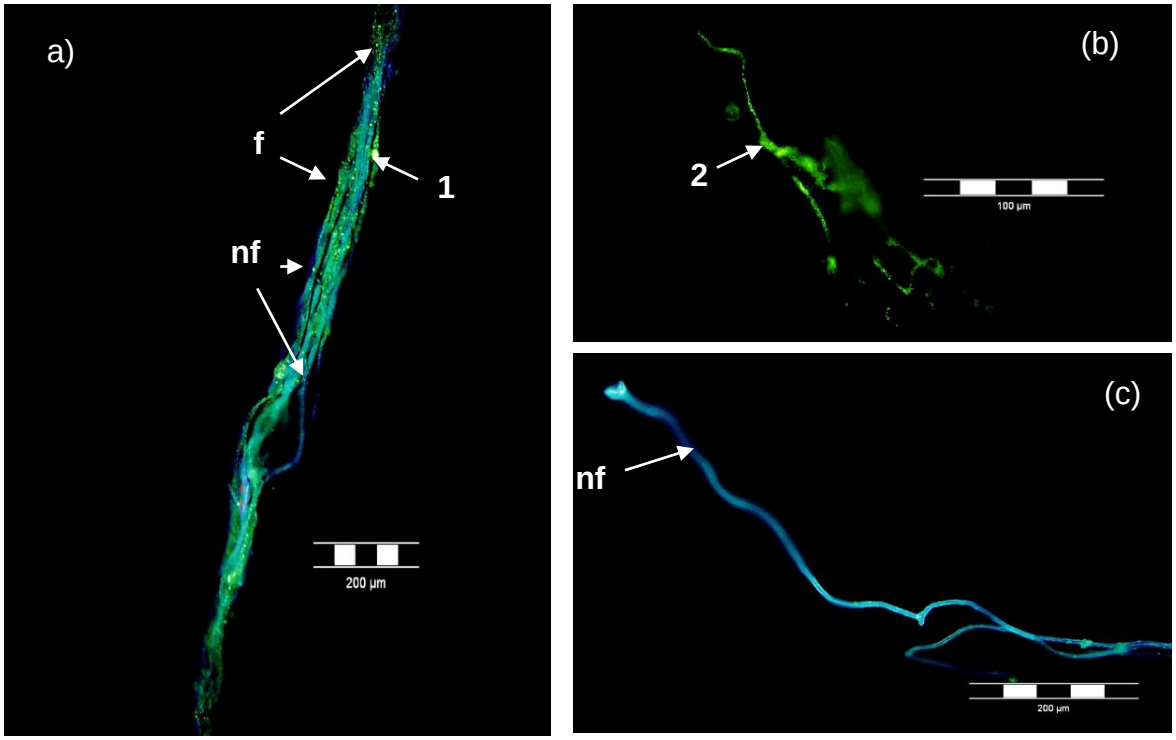

Figure 4.8 Phosphatase activity of hyphae in eucalypt hyphosphere in P spiked sand as shown by ELF staining from the glasshouse study. (a) Hyphae with (f) and without (nf) activity with brightness value of 1 at acid pH, (b) hyphae activity with brightness value of 2 at acid pH and (c) hyphae without activity (nf). Examples are indicated with white arrows.

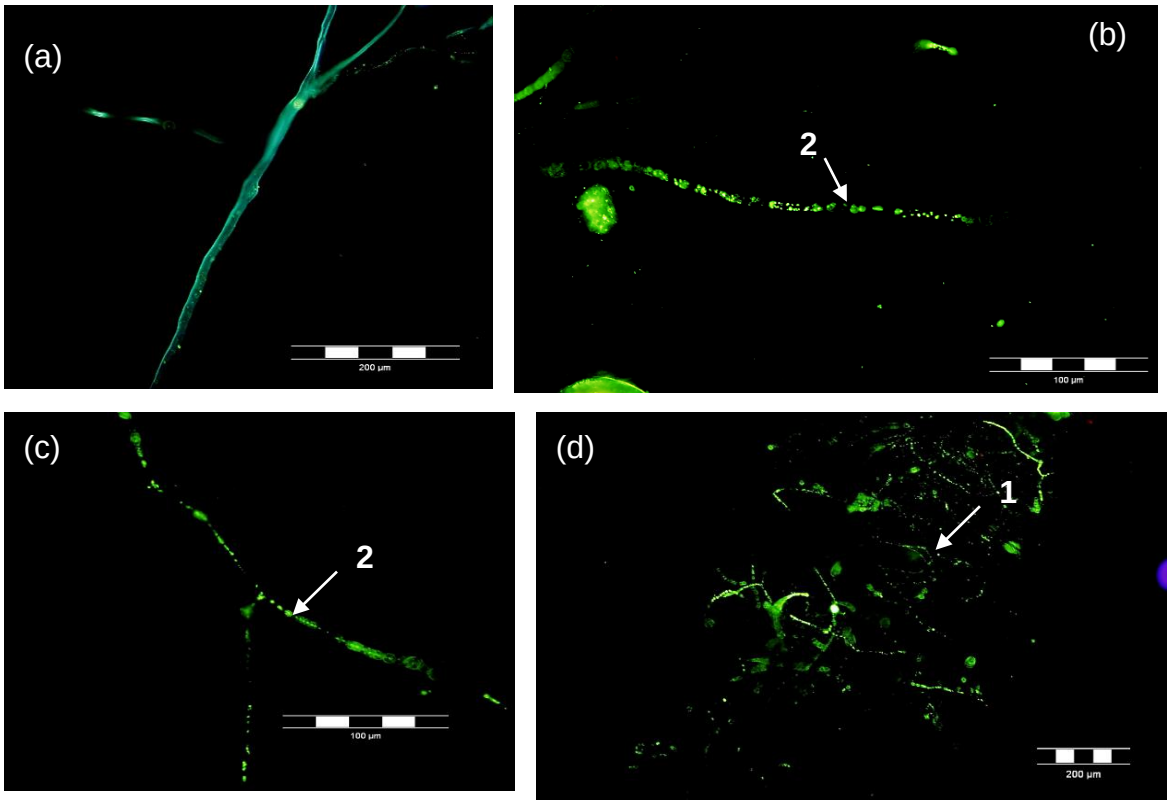

Figure 4.9 Phosphatase activity of hyphae in eucalypt hyphosphere as shown by ELF staining in the glasshouse study at alkaline pH. (a) without, (b) with activity of brightness value 2 in P spiked sand. (c) Brightness value of 2 and 1 (d) from sand without P spiking. Examples are indicated with white arrows.

4.3.2.2 Field Study

Results presented in Table 4.9 give acid and alkaline phosphatase activity levels shown by fluorescent staining of the hyphae associated with the three tree species when spiked or not spiked with P in the field study. Species differences in hyphae phosphatase activity were much smaller in the field than in the glasshouse study. A slightly lower percentage of hyphae of each species showed fluorescence at alkaline pH but overall a higher brightness value was achieved. Hyphae from both radiata pine and macrocarpa hyphospheres had decreased activity in the absence of P spiking. Eucalypt associated hyphae also had slightly decreased activity without P spiking but the difference was much smaller than for the other two species.

Macrocarpa associated hyphae had the greatest activity at both pHs (Figure 4.10) evident in number of hyphae fluorescing and the brightness value of the fluorescence. This activity was slightly decreased in non spiked sand. Overall, less hyphae fluoresced and the fluorescence within each hypha was less in the field samples than glasshouse samples. When organic P was present eucalypt hyphae showed the least phosphatase activity and when organic P was not present, radiata pine hyphae showed the least activity.

Table 4.9 Average percentage of hyphae showing phosphatase activity and average brightness value (in parenthesis) at both acid and alkaline pH when given an organic P source (spiked) or not (non spiked) in the field ELF staining study, n = 6.

	P spiked		**Non spiked**	
	Acid	Alkaline	Acid	Alkaline
Eucalypt	73%	63%	72%	63%
	(1.7)	(1.8)	(1.5)	(1.6)
Radiata pine	76%	68%	65%	42%
	(2)	(1.6)	(1.2)	(2.2)
Macrocarpa	87%	82%	80%	77%
	(1.8)	(2.2)	(1.7)	(2.2)

Brightness values: 1 = some small fluorescing crystals, 2 = some large and small fluorescing crystals, 3 = many large fluorescing crystals.

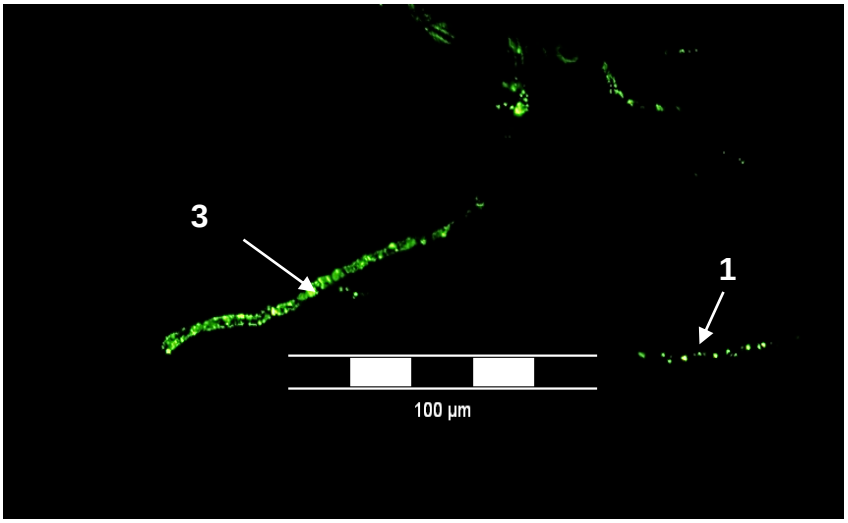

Figure 4.10 Phosphatase activity of hyphae in macrocarpa hyphosphere from the field experiment as shown by ELF staining. Showing activity in P spiked sand with brightness values of 3 and 1 at alkaline pH. Examples are indicated by white arrows.

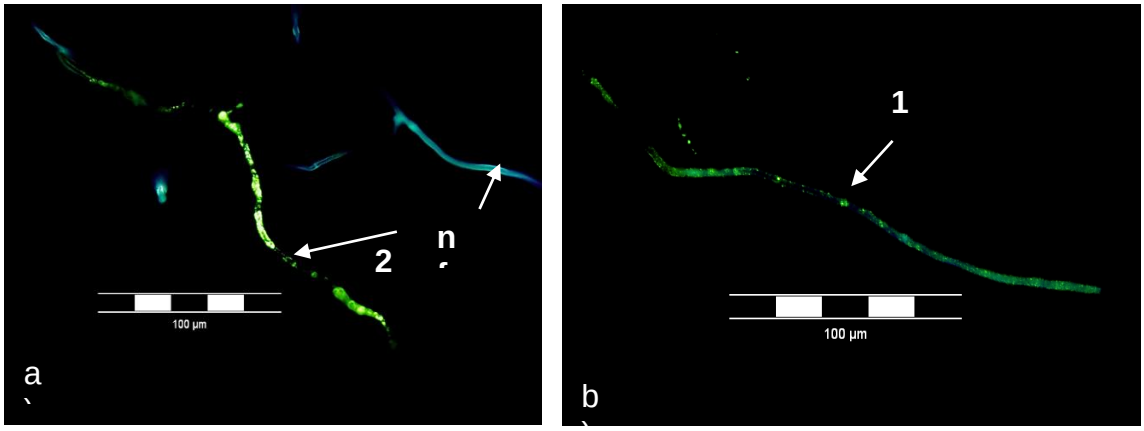

Figure 4.11 Phosphatase activity of hyphae in radiata pine rhizosphere from the field experiment as shown by ELF staining. (a) not showing (nf) and showing activity at acid pH with a brightness value of 2 in spiked sand, (b) showing activity with a brightness value of 1 at acid pH in non spiked sand. Examples are indicated by white arrows.

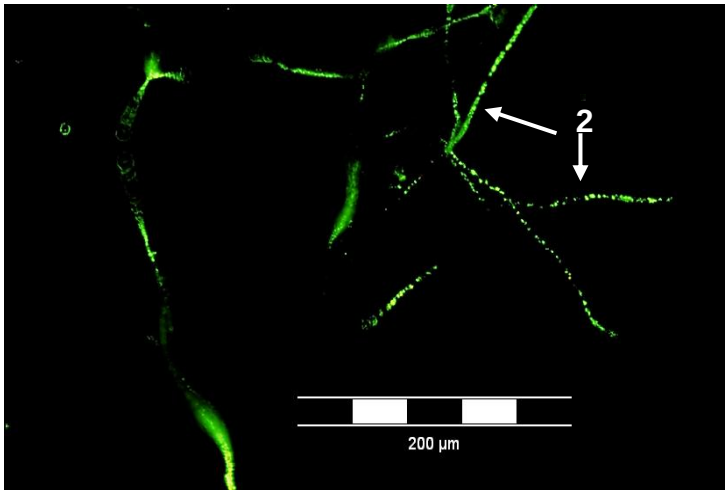

Figure 4.12 Phosphatase activity of hyphae in eucalypt rhizosphere from the field experiment as shown by ELF staining. Activity in P spiked sand with a brightness value of 2 at alkaline pH. Examples are indicated with white arrows.

4.4 Discussion

4.4.1 Inoculation experiment

The aim of this study was to determine the role of ectomycorrhizae and arbuscular mycorrhizae in the production of phosphatase enzymes and LMWOAs in the rhizosphere of radiata pine, eucalypt and macrocarpa trees. To do this, sterile trees were grown in sterile growth media with or without mycorrhizal inoculation. Ectomycorrhizal and arbuscular mycorrhizal treatments were used to compare the effects of both types of mycorrhizae on the processes under investigation. The use of sterile trees and sterile growth media eliminated any endogenous phosphatase levels that may mask the results and hinder detection of those produced during the experiment (Joner et al., 2000). Bacterial filtrate was given to all treatments to enable arbuscular mycorrhizal colonization and to reduce any variability between the treatments due to presence of bacteria and not the mycorrhizal treatments.

4.4.1.1 Treatment Success

All seedlings with Bac treatment showed no colonization with either type of mycorrhizae demonstrating that the non mycorrhizal controls were successful. The

ectomycorrhizal treatment was two isolates of *Rhizopogon rubescens* collected from sporocarps of five year old pine trees in Kaingaroa forest, Rotorua, New Zealand. This fungus is a common pine colonizer (Chilvers, 1973). It is well known to be a colonizer of radiata pine in New Zealand (Chu-Chou and Grace, 1984; 1988; 1990; Walbert et al., 2009) and did result in visible ectomycorrhizal colonization of radiata pine in this study. The yellow brown straight tips with dichotomous branching and smooth surface were similar to *R. rubescens* as described in Walbert (2008). This treatment was unsuccessful at colonizing eucalypt effectively. The short, dark tips found on eucalypt roots with this treatment (Figure 4.4a and b) were comparable to those found on *Eucalyptus camalgulensis* and *Eucalyptus marginata* when grown with other incompatible mycorrhizal fungi by Malajczuk et al. (1982). This darkening of epidermal and cortical cells was attributed to an accumulation of phenolic compounds where there was contact with the fungal mantle and is a common root trait when grown with incompatible fungi, likely because this is also a common plant reaction to pathogen invasion (Molina, 1981). Chilvers (1973) suggested that ectomycorrhizal fungi are host genus specific, not host species specific, a conclusion supported by this study and that of Malajczuk et al. (1982) where a pine specific ectomycorrhizae was incompatible with a non pine species. Malajczuk et al. (1984) found that incompatible mycorrhizal fungi initiated mantle formation but it was restricted to the thickness of a few hyphae and that cells were lysed after four months. Nyland and Unestam (1982) formed a hypothesis based on observed effects of eucalypt when inoculated with pine specific mycorrhizae. They stated that incompatible mycorrhizas form structures resembling compatible infections because eucalypts are 'phylogenetically adapted to the state of ectomycotrophy and built in controls regulate the fungal infection'.

The AM treatment was made from arbuscular mycorrhizal spores obtained from Orton Bradley Park soil in which macrocarpa was growing (plot 12). Macrocarpa roots were infected with this treatment. This was to be expected as the *Cupressus* family (of which macrocarpa is a member) is a highly dependent arbuscular mycorrhizal family (Ouahmane et al., 2007) and proves that this method was successful for initiating arbuscular mycorrhizal infection. *Eucalyptus* is a family that can form mycorrhizal associations with both types of mycorrhizal fungi (Chen et al., 2000b; Lodge, 2000) but no arbuscular infection was seen on eucalypt seedlings with AM treatment.

Ectomycorrhizal colonization was, however found on both radiata pine and eucalypt trees with AM treatment at a higher colonization rate than with the EM treatment. Most ectomycorrhizal species are basidiomycota with a few ascomycota (Smith and Read, 1997). All spores between 150 and 50 μm were collected for the arbuscular mycorrhizal inoculum. Ectomycorrhizal spores are predominantly basidiospores which have a usual size of 5-10 μm with some ascospores which can be thread like at 180 x 1.5 μm or more spherical at 60 μm (Ingold, 2001). This means that ascospores will likely have been caught on the 50 μm sieve and so will be present in this inoculum. Most basidiospores should have been washed out of the 50 μm sieve but it is possible some had been caught thus contributing to AM treatment. Ectomycorrhizal infection is established from spores or hyphae from resident mycorrhizae in the rhizosphere (Pritchett and Fisher, 1987) therefore the colonization resulting from this treatment will potentially be from a more diverse selection of ectomycorrhizae from the field soil. This would include fungal species compatible with eucalypt and Figure 4.4 c and d shows that there was indeed successful eucalypt ectomycorrhizal colonization with this treatment with less of the darkened short root tips, evident of incompatible fungal infection, present. The ectomycorrhizae in association with eucalypt roots appears to have a less obvious Hartig net than those formed with radiata pine (Fig. 4.4 c and d) and more superficial sheathing, typical of some eucalypt associations (Marschner and Dell, 1994). The ectomycorrhizal morphotype present on radiata pine root tips in AM treatment appeared to be the same as that found with the EM treatment. It is likely that *Rhizopogon rubescens* was present in this soil to infect the roots but without molecular identification, the fungal species colonizing the roots cannot be identified accurately and so the infecting species is/are not known with this treatment.

The fact that ectomycorrhizal colonization occurred in the AM treatment may explain why arbuscular mycorrhizal colonization was not seen in eucalypt roots with the AM treatment. The length of the experiment was long enough to see a succession from arbuscular to ectomycorrhizae colonization, as has occurred in other studies (Lapeyrie and Chilvers, 1985; Chilvers et al., 1987). For example, in an experiment where *Eucalyptus globulus* and *Eucalyptus urophylla* seedlings were inoculated with both ectomycorrhizae and arbuscular mycorrhizae, Chen et al. (2000b) found that arbuscular mycorrhizae initiated infections to begin with but after two months, the proportion of

roots showing infection decreased and those trees also inoculated with ectomycorrhizae then showed an increase in ectomycorrhizal colonization. In this experiment, seedlings were harvested after six months, long enough for a considerable ectomycorrhizal infection to build up a substantial fungal mantle thus forming an impenetrable barrier to arbuscular mycorrhizal infection (Chilvers et al., 1987; Lodge, 2000) and for the seedling to lose receptivity to arbuscular colonization (Chen et al., 2000b).

Treatments were successful for radiata pine, producing a non colonized treatment along with two rates of ectomycorrhizal colonization with *Rhizopogon rubescens* only and with field soil resident ectomycorrhizae (in the AM treatment). Macrocarpa treatments were also a success, producing two non colonized treatments for comparison with an AM colonized treatment. Eucalypt treatments were intended to be none mycorrhizal, arbuscular mycorrhizal and ectomycorrhizal. The non mycorrhizal treatment was a success but the other treatments resulted in incompatible and compatible ectomycorrhizal colonization.

4.4.1.2 Mycorrhizal role in rhizosphere processes

The most notable finding from the experiment is the correlation between mycorrhizal colonization and acid phosphomonoesterase activity (Table 4.6). Mycorrhizal colonization did not result in significantly different plant sizes, either above or below ground, therefore treatment differences must be a result of mycorrhizal colonization, not a confounding effect of plant size. Phosphatase enzyme activity measured in this experiment was much lower than that measured in other glasshouse studies using these species (see Chapter 3). The sterile conditions meant there were no endogenous phosphatases and therefore only those produced in the duration of the experiment were measured. The growth media of sand, potting mix and pumice also likely meant that less released phosphatases were stabilized in the environment as happens on soil surfaces such as clay or humic colloids (Burns, 1986; Sinsabaugh, 1994; George et al., 2005b) which contributes to the amount detected in other studies. Macrocarpa colonization with arbuscular mycorrhizae resulted in a significantly higher acid phosphatase activity than non mycorrhizal seedlings. This was the same with ectomycorrhizal colonization of eucalypt with a compatible fungus (in the AM treatment) compared to seedlings without colonization or with an incompatible species.

Radiata pine showed significantly different acid phosphomonoesterase activities in all treatments, correlating with the degree of ectomycorrhizal colonization. An increased phosphatase activity in the rhizosphere of plants with mycorrhizal colonization compared to uncolonized controls has been found in studies for both ectomycorrhizal, glasshouse grown radiata pine (Pasqualini et al., 1992; Liu et al., 2004) and arbuscular mycorrhizal, glasshouse grown wheat (Tarafdar and Marschner, 1994b) and rape and onion (Dodd et al., 1987).

Arbuscular mycorrhizal colonization resulted in a 2.8-3.8 μmol/g/hr increase in acid phosphatase activity in the macrocarpa rhizosphere compared to the rhizosphere without colonization. Ectomycorrhizal colonization with a compatible fungus resulted in a 6.5 μmol/g/hr activity increase in eucalypt seedling rhizosphere and ectomycorrhizal colonization of radiata pine resulted in a 1.8-5.5 μmol/g/hr activity increase, depending on the degree of colonization. Therefore it seems reasonable to conclude that ectomycorrhizae increase rhizosphere acid phosphomonoesterase activity to a greater extent than arbuscular mycorrhizae. Ectomycorrhizae are considered to be the more efficient mycorrhizal association. Ectomycorrhizal hyphae extend to much greater distances than arbuscular mycorrhizae (Finlay and Read, 1986; Marschner and Dell, 1994) and Häussling and Marschner (1989) found a positive correlation between hyphae length and phosphatase activity in spruce forest organic matter. The presence of phosphatases as ectoenzymes in ectomycorrhizal extraradical hyphae is well established (Ho, 1989; Hilger and Krause, 1989; Lapeyrie et al., 1990; Antibus et al., 1992; Nygren and Rosling, 2009) whereas evidence is conflicting for similar evidence in arbuscular mycorrhizae. Phosphatase production has been verified in and on the surface of arbuscular mycorrhizal hyphae (Joner and Johansen, 2000; Koide and Kabir, 2000; van Aarle et al., 2001; Feng et al., 2002) but with little evidence for release into the soil (Joner and Johansen, 2000) and many studies can only relate colonization to generally increasing rhizosphere enzyme activity, possibly by increasing root activity (Dodd et al., 1987; Tarafdar and Marschner, 1994a, b; Solaiman and Abbot, 2003). Alternatively, Joner et al. (1995) and Joner and Jakobsen (1995) in experiments using cucumber, did not find any increase in extracellular phosphatase in the presence of AM fungi. This was related to the non sterile conditions of these pot experiments. Endogenous phosphatases produced by the microorganisms were thought to mask any effect of the arbuscular

mycorrhizal hyphae, demonstrating their influence on rhizosphere processes is small (Joner et al., 2000).

It seems that it is not just mycorrhizal colonization that determines acid phosphomonoesterase activity differences in the rhizospheres of these three species. Without colonization, activity differences were smaller yet still significant with eucalypt having the highest activity, followed by radiata pine and then macrocarpa. The likely reason for higher acid phosphatase activity in eucalypt rhizosphere is the significantly larger root system as phosphatase activity increases with increasing root length density (Tarafdar and Jungk, 1987; Rubio et al., 1990).

Alkaline phosphomonoesterase and phosphodiesterase did not correlate with mycorrhizal colonization or plant P uptake. This is likely because levels were too low in the system to have an effect. Joner and Johansen (2000) showed that alkaline phosphatases in mycorrhizal hyphae are predominantly internal and therefore perform a different function to extracellular acid phosphatases and so would not be detected by the assay used in this study. It could also be that percentage of root colonized by mycorrhizae is not a good factor to correlate mycorrhizae effects to (Jones et al., 1998; McGonigle et al., 1990). The impact of percentage infection may be modified by the functioning of internal and external hyphae (Atkinson, 2006). Other researchers have suggested that the length of hyphae or the average distance of hyphae extension from the root are better factors to consider when assessing its ability to change factors in the rhizosphere (Jones, et al., 1990; Jakobsen et al., 1992) and do not necessarily relate to percentage root infection (Marschner and Dell, 1994).

Mycorrhizal colonization did not have an effect on growth or P content of any of the tree species. This is likely because hyphae length and density was not measured; only root colonization (see Table 4.2). However, acid phosphatase activity did correlate with the mycorrhizal colonization indicator used here so it seems likely that it is an effective means by which to assess mycorrhizae rhizosphere effects. On the other hand, phosphatase activity of any of the enzymes did not correlate with total plant P content. Eucalypt seedlings had a higher P content than the other two species in all treatments and treatment did not influence total plant P content. This was unexpected as

mycorrhizal inoculation usually increases plant P content (Bougher et al., 1990; Smith and Read, 1997). Although total plant P content was not affected by treatment, plant P concentration was. Acid phosphatase and mycorrhizal colonization increased plant P concentration. Under AM treatment, where all species had the highest level of mycorrhizal colonization, plant P concentrations (mg/g) were highest. It could possibly be that plants with mycorrhizal associations are more C limited due to the C transferred to the fungi. This means plants accumulate P compared to C and thus concentrations rise. If P was solely the limiting factor to plant growth, tissue concentrations would not rise (Smith and Read, 1997) so it appears that increased acid phosphatase activities, linked to mycorrhizal colonization, do indeed increase plant P concentration.

Treatment did not appear to greatly influence LMWOA concentration in the rhizosphere of any species. This is unusual in the case of ectomycorrhizae, especially with radiata pine as it has been found that seedlings of *P. pinaster* (Cassarin et al. 2003) and *P. radiata* (Liu et al. (2004a) infected with various ectomycorrhizal strains including *Rhizopogon roseolus* and *Rhizopogon rubescens* exuded more oxalate than those without. Liu et al. (2004a) determined *Rhizopogon rubescens* to produce the highest oxalate concentrations in soil solution compared to other fungal strains. Ectomycorrhizal hyphae have also been shown to produce oxalic acid when grown without their plant host (Lapeyrie et al., 1987, 1991; Lapeyrie, 1988) however, in these experiments it was *Paxillus involutus*, not *Rhizopogon rubescens* that was investigated. Also it should be noted that using HPLC, oxalate cannot be detected due to an unavoidable void peak overlapping with the oxalate peak in the chromatographs.

Arbuscular mycorrhizal exudation of LMWOAs has received relatively little attention but Antunes et al. (2007) found that hyphae did not release H^+ ions alone or in combination with organic anions. Citrate and oxalate are regarded as more effective at nutrient mobilization than other organic acids (Bar-Josef, 1991, cited in Richardson et al., 2009b). The macrocarpa rhizosphere contained the highest concentrations with most regularity of citrate and this species that has the highest concentration of P in plant biomass. It appears that organic acid concentration in rhizospheres of these species does not relate to mycorrhizal associations and does not influence plant P uptake greatly. This is possibly because these organic acids exuded in small amounts and are rapidly

adsorbed – limiting their influence, or rapidly metabolized by soil microorganisms resulting in a short lifespan in the soil (Hinsinger, 2001; Jones, 1998).

4.4.2 Hyphal phosphatase activity

The aim of this experiment was to assess phosphatase activity of mycorrhizal hyphae in the rhizosphere of eucalypt, radiata pine and macrocarpa using ELF staining and measure its response to an organic P source. Enzyme labeled fluorescence uses a fluorogenic substrate that forms a fluorescent crystalline precipitate at the site of phosphatase activity (van Aarle et al., 2001). The experiments were done in a glasshouse study, where a high concentration of hyphae would be present to enhance hyphosphere effects under investigation and ensure adequate number of hyphae for investigation, and also in a field situation using hyphae from tree rhizospheres under field conditions. Both experiments were set up to allow hyphae to grow in sand thus creating a hyphosphere that the hyphae could be easily extracted from. The response to an organic P source was assessed by spiking the sand of half the replicates with sparingly soluble calcium phytate and leaving the other half without.

The glasshouse experiment set up meant the hyphae containing sand was surrounded by an impenetrable pot. Therefore hyphae were confined to this small sand volume once they had penetrated. Hyphae from the entire root system could have penetrated into the sand from the root bag, all of which was used for hyphae collection thus accounting for the large hyphal density in this experiment compared to the field experiment. In the field, hyphae could penetrate into the sand bag but could also grow out of this bag into surrounding soil. As the volume of the sand bag is small in relation to the entire root system, the chances of hyphae actually growing directly into the bag were low, probably accounting for the lower number of hyphae recovered from the field experiment compare to the glasshouse experiment. A larger number of hyphae were found in P spiked bags perhaps indicating a proliferation of hyphae in nutrient rich zones (St John et al., 1983; Warner, 1984; Bending and Read, 1995; Smith and Read, 1997). The phosphatase activity differences between species and treatments were much lower in the field experiment. This is likely due to the fact that rhizosphere effects are confined and enhanced in pot experiments (Li et al., 1991) and thus not likely representing processes under field conditions. In the field it is more likely that hyphae of saprotrophic fungi as

well as mycorrhizal fungi penetrated the sand bags. Functional and species differences between saprotrophic fungi will not be strictly related to tree species and so be more similar between plots. The saprotrophic fungi *Aspergillus niger* has been shown to possess an acid phosphatase gene (McRae et al., 1988) and *Aspergillus fumigatus* (van Aarle et al., 2000) has been demonstrated to produce phosphatases at acid and alkaline pH using the same technique as this experiment. Thus it seems saprotrophic fungi phosphatases would be detected in all hyphal analysis.

4.4.2.1 Species differences in hyphal phosphatase activity

Hyphae in the rhizospheres of all three species showed phosphatase activity at both acid and alkaline pH. The activity shown was internal or wall bound. Hyphae in macrocarpa rhizosphere showed the highest activity levels at both pHs in the glasshouse and field experiment. Some of these hyphae from the glasshouse experiment were seen to have produced spores (Figure 4.8) demonstrating they were definitely arbuscular mycorrhizal hyphae. These spores were young and intact and shown to produce phosphatase enzymes at acid and alkaline pH as found by van Aarle et al. (2001) in a previous ELF study of saprotrophic and arbuscular mycorrhizal fungal hyphae. The determination of phosphatase activity in arbuscular mycorrhizal hyphae is in accordance with other studies (Joner and Johansen, 2000; Koide and Kabir, 2000; van Aarle et al., 2001; Feng et al 2002). Noticeably less hyphae were extracted overall from macrocarpa, compared to eucalypt and radiata pine rhizosphere sand from the glasshouse experiment. This probably reflects the contrasting mycorrhizal partners with macrocarpa being an arbuscular mycorrhizal host and the biomass of arbuscular mycorrhizal hyphae in soils is generally lower than that of ectomycorrhizae (Smith and Read, 1997; Jones et al., 1998) and low arbuscular mycorrhizal hyphae biomass in soil was given as a reason for the contradictory results found in studies on arbuscular mycorrhizal role in rhizosphere effects (Joner and Johansen, 2000; Joner et al., 2000).

Hyphae from the eucalypt hyphosphere were the most prolific with many hyphae forming large clumps in the glasshouse experiment. It is likely that the eucalypts in this experiment were predominately ectomycorrhizal as ectomycorrhizal root tips could be seen with the naked eye and as stated earlier, once ectomycorrhizal associations are formed (after 2-3 months [Chen et al., 2000b]), subsequent arbuscular mycorrhizal

colonization is hindered by the impenetrable fungal sheath barrier (Lodge, 2000). Ectomycorrhizal hyphae have been demonstrated to produce phosphatases (Dighton, 1983; Häussling and Marschner, 1989; Ho, 1989; Antibus et al., 1992). The large amount of hyphae in eucalypt rhizosphere was particularly evident in the glasshouse experiment with P spiked sand. Much of the P present in the sand was likely utilized by the tree in the 14 months of growth (see below) but initially the sand would have been a nutrient rich patch and therefore hyphae would proliferate in this area (Bending and Read, 1995). Hyphae that formed large clumps in eucalypt sand displayed much lower levels of phosphatase activity. Less than half of the hyphae in these clumps displayed any fluorescence and those that did displayed few crystals. Van Aarle et al. (2001) concluded that fluorescence shown by ELF staining was a good indicator of live, active hyphae so therefore it is likely that the non fluorescing hyphae were no longer active due to the utilization of the P source.

Ectomycorrhizal structures were seen on the root tips of radiata pine in this experiment thus hyphae in the radiata pine hyphosphere would have been predominantly ectomycorrhizal (Chu-Chou and Grace, 1984). Hyphal phosphatase activity of this species was higher than that of eucalypt hyphae and lower than that of macrocarpa hyphae in the glasshouse experiment. This appears to be related to hyphal density as the intermediate activity levels correspond with the intermediate hyphal density (observed not measured) in this experiment. This is also in agreement with data from the field experiment where differences in hyphal density were not so pronounced and phosphatase activities between species were much closer. In the glasshouse study, activity was higher at alkaline pH, whereas in the field, activity was higher at acid pH. This is possibly because there is more P in the field to be accessed so more phosphatases are exuded to access it in the field experiment. Acid phosphatases have been demonstrated to be the enzymes exuded to obtain P from soil and are extracellular or wall bound (Joner and Johansen, 2000; Joner et al., 2000). The prolific hyphae of the glasshouse experiment have likely already utilized most of the P and so now concentrate on transferring it to the plant. Alkaline phosphatase has been found be involved in the internal transfer of P from the hyphae to the plant (Joner and Johansen; 2000; Joner et al., 2000; van Aarle et al., et al., 2002).

4.4.2.2 Phosphatase response to organic P source

The provision of a sparingly soluble organic P source only appeared to influence hyphal phosphatase activity in the field experiment. The enhancing effect was slight and mainly at acid pH with the biggest difference between spiked and non-spiked in the radiata pine rhizosphere. It has been demonstrated previously that the addition of an organic P substrate enhances fungal phosphatase activity (Tarafdar and Marschner, 1994a and b). Extracellular phosphatases will be the enzymes produced in response to an external P source and it has been demonstrated that acid phosphatases in hyphae are mainly external or wall bound (Joner and Johansen, 2000; Joner et al., 2000) thus explaining why the difference was evident mainly at acid pH. Alkaline phosphatases are mainly internal and are more involved in regulating P transfer to the plant and regulated by internal plant P requirements (van Aarle et al., 2002).

A lack of response to the organic P source in the glasshouse experiment is unusual because addition of substrates, especially to a non fixing media such as sand results in unrealistically high availabilities and solubilities and therefore enzyme activities to utilize them (Joner et al., 2000). However, addition of Na-phytate has been shown to have negative effects on the phosphatase activity of mycorrhizal roots (Azcon et al., 1982) and active hyphal lengths (Baláz and Vosatka, 1997). Calcium phytate is less soluble than Na-phytate and is only soluble at pH <6 (Anderson, 1967). The pH of the sand was not measured in these experiments but it was unlikely to be less than 6 therefore rendering the Ca-phytate insoluble. This would mean that it is not solely phosphatases that control the mineralization of this P source, solubilizing agents such as LMWOAs would be necessary for plant utilization (Condron et al., 2005; Richardson et al., 2006; Turner, 2008). However the demonstration of a P spiking effect in the field experiment indicates that insolubility is probably not the limiting factor. It is possible that the plants were P deficient and thus more phosphatase enzymes were produced by a negative feedback mechanism (Quiquampoix and Mousain, 2005). The trees in this experiment were grown in a small mesh bag for 14 months. They had gained a considerable amount of biomass and had an extensive root system. It is likely that much of the P present in the soil/potting mix and also the Ca-phytate added to the sand had been utilized already by the trees and so phosphatase was induced across all species in these P deficient conditions. Phosphatase activity of mycorrhizae has been shown to be

greatest at low concentrations of P (Antibus et al., 1992). However, van Aarle et al. (2002) found that phosphatase activity of extraradical arbuscular mycorrhizal hyphae did not correlate with external P availability.

4.5 Conclusions

Both ectomycorrhizae and arbuscular mycorrhizae increased rhizosphere acid phosphomonoesterase activity under radiata pine, eucalypt and macrocarpa with ectomycorrhizal colonization resulting in the highest phosphatase activity and plant P concentration. Mycorrhizal association did not significantly affect rhizosphere alkaline phosphomonoesterase, phosphodiesterase activity or LMWOA type and concentration under radiata pine, eucalypt or macrocarpa. Both ectomycorrhizal and arbuscular mycorrhizal hyphae associated with different tree species were demonstrated to produce phosphatases. The activity was related to the number of hyphae present with greater activity in single macrocarpa hyphae and lower activity in clumped eucalypt hyphae. Therefore the larger rhizosphere effect from ectomycorrhizae was possibly related to the greater biomass of these hyphae in soil compared to that of arbuscular mycorrhizae and not the capabilities of the fungi to produce phosphatases. In field situations mycorrhizal hyphae increase phosphatase activity in response to an organic P source, especially at acid pH, with some evidence for proliferation in nutrient rich patches.

Chapter 5

Soil Organic Phosphorus Utilization by Different Tree Species

5.1 Introduction

Organic P has long been recognized as a source for replenishment of soil solution P and therefore plant nutrition via the process of mineralization (Dalal, 1979; Firsching and Claasen, 1996). It can comprise up to 90% of total soil P although it is usually between 30 and 65% thus only a small fraction of this organic P need be hydrolyzed to meet the P requirement of plants (Harrison, 1987). There are many chemically different types of organic and inorganic P in soil and plants have a range of adaptations to acquire both organic and inorganic P types from the soil so a resource partitioning hypothesis, i.e. plants specializing in the utilization of different types of P to avoid competition, seems very likely (Turner, 2008). In brief, the resource partitioning hypothesis speculates that different plant species are able to utilize different forms of P. Some species can utilize only dissolved phosphate that can be taken up directly by roots. Other species can also utilize weakly sorbed forms of P such as simple monoesters like glucose 6 phosphate, that require hydrolysis by phosphomonoesterases whereas other species can also utilize phosphate diesters, such as RNA, efficiently that require hydrolysis by both phosphodiesterases and phosphomonoesterases. Some plants can also utilize inositol phosphates, such as phytic acid, efficiently - something that requires both solubilization and hydrolysis by specialized enzymes called phytases to release phosphate.

In previous chapters it was established that the mycorrhizal associations of ectomycorrhizal eucalypt and radiata pine play a large role in P dynamics by increasing acid phosphomonoesterase activity and the microbial community play a large role in P dynamics of eucalypt and macrocarpa because of the greater exudation of low molecular weight organic anions from these species. However, how this influences the actual utilization and uptake of organic P from the soil by eucalypt and macrocarpa and

whether growth of these species is limited by its ability to utilize soil organic P remains to be investigated. Accordingly, in this chapter, P uptake of radiata pine, eucalypt and macrocarpa was investigated in two different P limiting situations:

i. Trees were grown in soil taken from the Orton Bradley Park field site as used in previous chapters but in a confined space for a long period of time to assess the exhaustive effects of these trees on a single soil (exhaustive pot trial).

ii. Trees were grown in soils from a chronosequence with contrasting levels of total P and proportions of organic P to assess the ability of these species to utilize organic P to meet their P requirements in a range of related soils (organic P gradient pot trial).

5.2 Materials and Methods

5.2.1 Exhaustive Pot Trial

A pot trial was established in June 2007 to determine the exhaustive effects of radiata pine, eucalypt and macrocarpa trees on soil P. Four 30 cm high replicates of each species (supplied by Southern Woods Nurseries Ltd, New Zealand) were planted into liver pails packed with 1.5 kg of 4 mm sieved soil. The soil was from the Orton Bradley Park field site and taken from the top 10 cm and bulked from plots 9, 8 and 7 (see Chapter 2 for full site description). Five 10 mm holes were drilled into the bottom of each liver pail to allow for drainage. This system was maintained in a randomized design in a glasshouse between 15 and 19^{o}C and watered daily. The experiment was monitored weekly for weeds and any were removed from the pail by hand. The experiment was terminated after 22 months growth (Figure 5.1).

5.2.2 Organic P Gradient pot trial

In November 2008, three mineral soils from a chronosequence in Haast, New Zealand were chosen for differences in total P and percentage of organic P. This relatively young chronosequence is a series of dunes formed when earthquake debris from the Southern

Alps washes into the Haast River and is deposited along the shoreline. Some of these dunes have been dated and the chronosequence spans from 4000 BC (6000 years old) to 1826 AD (200 years old). Temperate rainforest is at various stages of development on these dunes and between each dune is wetland which can be clearly seen in Figure 5.2. Table 5.1 presents the total and organic P contents of each soil from the chronosequence used in this experiment.

These soils were sieved to <2 mm and 450 g was packed into plastic pots (RX Plastics Ltd, New Zealand). Twenty, approximately 20 cm high, seedlings of each species (supplied by Southern Woods Nursery Ltd, New Zealand) were washed free of potting mix. Five plants of each species were placed into each soil type, and five of each species were dried and kept for later analysis. The experiment was terminated after six months of growth. Due to the sandy nature of these soils, a large amount of soil was attached to the root system of the trees upon removal which was then easily removed by shaking. This meant that using only soil attached to the roots upon plant removal resulted in sufficient soil for analysis and this was deemed as rhizosphere soil.

Figure 5.1 The trees of the pot trial at experiment termination.

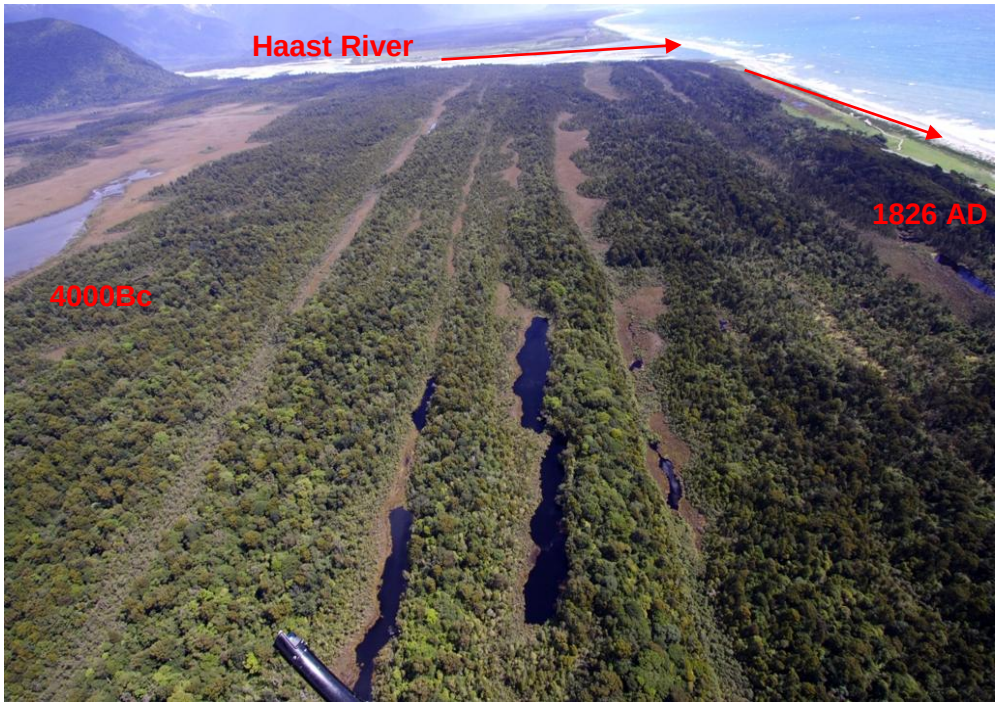

Figure 5.2 The location and formation of the Haast soil chronosequence from which the organic P gradient soils were taken. Red arrows indicate direction of the dune forming debris flow.

Table 5.1 Age and P contents of soils from the Haast chronosequence used in the organic P gradient pot trial.

Date formed	Total P (mg/kg)	Organic P (mg/kg)	% organic P
1826	312	46	15
1490	211	147	70
4000bc	102	69	67

5.2.3 Laboratory Analysis

Upon experiment termination, the trees of both experiments were removed from the soil, shaken to remove soil particles and then roots were washed free of any remaining soil particles.

The soils were sieved to <4mm, air dried at 20^{o}C and finely ground prior to analysis. The trees of both experiments were dried at 70^{o}C for 72 hours, weighed to obtain dry weight biomass and then finely ground. Percentage P was then determined in the ground trees using acid digestion and ICP.

Soils of the exhaustive pot trial experiment were analyzed for NaOH-EDTA extractable P and resin P using methods described in Chapter 2. The resin P extraction was carried out on air dried soil in this experiment. Soils from the organic P gradient experiment were analyzed for total P using the ignition and acid extraction method of Saunders and Williams, (1955) as described in Chapter 2. Another sample of soil also underwent this method without the ignition step to determine total inorganic P. Organic P was determined by subtracting inorganic P values from total P values for each sample.

5.2.4 Statistical Analysis

All statistical analysis was performed using GenStat 10 (Lawes Agricultural Trust, Rothamstead Experimental Station, UK). For the exhaustive pot trial, effects of species on soil P concentrations, plant P content and plant P concentrations were analyzed using a general ANOVA of unbalanced design to account for varying sample sizes. Complete ANOVA results are presented in Appendix 5.1. For the organic P gradient pot trial, significant (P<0.05) interactions between species and soil type in plant biomass, plant P content, plant P concentration and soil P content were analyzed using a 2-way ANOVA and compared by 5% least significant differences (LSD). Complete ANOVA results are presented in Appendix 5.2.

5.3 Results

5.3.1 Exhaustive Pot Trial

Plant growth and P uptake

Table 5.2 gives dry weights and P content of the original trees and of the trees after growth in the exhaustive pot trial experiment. All species gained considerable biomass.

Eucalypt gained an average of 63 g, radiata pine gained 176 g and macrocarpa 105 g. Originally, macrocarpa and radiata pine both had a significantly larger biomass than eucalypt. However, after the experiment duration, radiata pine had a significantly larger biomass than macrocarpa, which had a significantly higher biomass than eucalypt.

As expected, all species acquired a significant amount of P in the duration of the experiment. Radiata pine gained 232 mg, eucalypt gained 25 mg and macrocarpa gained 47 mg. Originally, macrocarpa contained significantly less P than eucalypt and radiata pine. At experiment termination, radiata pine contained significantly more P than eucalypt and macrocarpa which did not differ significantly in P content.

Soil P concentrations

Results presented in Table 5.3 show concentrations of all P forms measured in the original soil and the soil after 22 months growth of all three species. All species slightly decreased resin P compared to the original soil but did not differ significantly between each other. All species decreased NaOH-EDTA extractable P compared to the original soil with the largest decrease under radiata pine. The organic portion of this P pool was significantly lower under radiata pine than the original soil and of that under macrocarpa and eucalypt, whereas the inorganic proportion was significantly higher than that of eucalypt and macrocarpa and markedly higher than the original soil.

Table 5.2 Mean weight and P content of radiata pine, eucalypt and macrocarpa roots and shoots determined before planting and after 22 months growth in soil in the exhaustive pot trial. Values in parenthesis are standard errors of means, n = 4 for radiata pine and macrocarpa, n = 3 for eucalypt. Except in original trees where n = 3 for macrocarpa and n = 4 for the other two.

Soil	Radiata pine		Eucalypt		Macrocarpa		Significance	
Original	7.5 a (0.50)	21.9 a (1.92)	5.1 b (0.22)	21.1 a (1.47)	6.6 a (0.56)	14.6 b (1.21)	*P* = 0.009	*P* = 0.036
							M + E/R LSD	
							1.32	5.71
							E+R LSD	
							1.41	6.11
Roots	69.9 a (2.44)	102.3 a (13.67)	26.0 c (8.49)	20.6 b (4.81)	48.6 b (5.74)	32.4 b (2.95)	*P* = 0.002	*P*<0.001
							E+M/R LSD	
							18.67	31.04
							M+R LSD	
							17.28	28.74
Shoots	113.7 a (5.70)	151.4 a (16.76)	42.1 b (5.83)	25.2 b (3.50)	63.1 b (7.39)	29.0 b (6.99)	*P*<0.001	*P*<0.001
							E+M/R LSD	
							22.01	39.54
							M+R LSD	
							20.38	36.63
Total	183.7 a (7.33)	253.7 a (28.60)	68.1 c (13.39)	45.8 b (7.86)	111.8 b (9.57)	61.4 b (4.53)	*P*<0.001	*P*<0.001
							E+M/R LSD	
							33.06	63.6
							M+R LSD	
							30.60	58.9

□ = Plant dry weight (g) ▒ = Plant P content (mg)

Different letters represent significantly different means between species using a 5% LSD. A different LSD is used to compare a 3 replicate species with a 4 replicate species than a 4 replicates with another 4 replicate species.

Table 5.3 Mean P concentrations determined in soils of the exhaustive pot trial before planting and after 2 years growth of radiata pine, eucalypt or macrocarpa. Values in parenthesis are standard errors of means, n = 4 for radiata pine and macrocarpa, n = 3 for eucalypt. Except in original trees where n = 3 for macrocarpa and n = 4 for the other two.

	P concentration (mg/kg)			
	Resin P	**NaOH-EDTA tP**	**NaOH-EDTA oP**	**NaOH-EDTA iP**
Original	14.0	534	488	46
Radiata pine	11.9	456 a	384 a	72 a
	(0.89)	(10.1)	(11.9)	(17.9)
Eucalypt	11.5	512 b	471 b	41 b
	(0.41)	(3.5)	(3.7)	(13.7)
Macrocarpa	9.9	520 b	477 b	43 b
	(0.32)	(8.9)	(8.8)	(10.9)
Significance		*P* = 0.00l	*P*<0.001	*P*<0.001
E+R or M	NSD	LSD = 29.5	LSD = 32.5	LSD = 7.7
R+M		LSD = 27.3	LSD = 30.1	LSD = 7.1

tP = total phosphorus, oP = organic phosphorus, iP = inorganic phosphorus
Different letters represent significantly different means between species using a 5% LSD. A different LSD is used to compare a 3 replicate species with a 4 replicate species than a 4 replicates with another 4 replicate species.
NSD = no significant difference

5.3.2 Organic P Gradient Pot Trial

Plant Growth and P uptake

Results presented in Table 5.4 show the original plant biomass and P content and the biomass and P content determined after six months growth in soils from the Haast chronosequence. Originally no species differed significantly from another in dry weight however after growth in all soils, macrocarpa always had a significantly higher biomass than radiata pine, which always had a significantly higher biomass than eucalypt. Only radiata pine and macrocarpa gained significant biomass during the six months of the experiment but this did not differ significantly between soils.

There was no interaction between soil type and species on plant P content. Macrocarpa had a significantly higher P content than radiata pine and both these species contained significantly more P than eucalypt. Growth in the 1826 soil resulted in significantly higher P contents than the trees originally and after growth in 4000BC soils. Growth in 1490 soils resulted in plant P contents not significantly different to that after growth in another soil or the original trees.

Table 5.4 Mean dry weight and P content of radiata pine, eucalypt and macrocarpa determined before planting and after 6 months growth in soils from the Haast chronosequence. Values in parenthesis are standard errors of means, n = 3.

	Radiata pine		Eucalypt		Macrocarpa	
Original	5.5	10.2	2.8	5.2	7.8	14.4
	(0.14)	(1.49)	(0.26)	(0.61)	(0.27)	(2.06)
1826	20.6	16.9	4.1	5.5	27.2	20.7
	(1.80)	(1.22)	(0.75)	(1.25)	(5.57)	(4.18)
1490	17.9	13.4	7.6	5.3	26.5	18.1
	(0.11)	(0.67)	(0.13)	(0.21)	(2.93)	(1.92)
4000BC	18.4	13.1	8.5	5.2	25.9	16.0
	(1.0)	(1.39)	(0.27)	(0.32)	(1.67)	(1.60)

□ = Plant dry weight (g) ▒ = Plant P content (mg)

Soil P Depletion

Total, organic and inorganic P concentrations of all soils before and after growth of all species are presented in Table 5.5 and the percentage change of each P form after growth of all species is presented in Table 5.6. Planting of eucalypt decreased total P by 1-3%, thus the total P concentration after growth in any soil was not significantly different from the original soil. Radiata pine and macrocarpa however, decreased total P concentration significantly in all soils compared to the original. Growth of macrocarpa in the 1826 soil decreased total P by 17% which meant total P concentration was significantly lower than after radiata pine growth which decreased concentration by 13%. On the other hand, in the 4000BC soil, growth of radiata pine decreased total P by

16% which meant total P concentration was significantly lower than after macrocarpa growth which decreased total P by 8%. There was no significant difference in total P concentration between these two species in the 1490 soil, both decreasing total P by 7-8%.

In the 1826 soil, organic P concentrations were significantly lower after both radiata pine and macrocarpa growth (39 and 38% decrease, respectively) compared to that of eucalypt (9% decrease). It was radiata pine and eucalypt growth that significantly decreased organic P concentration (26% decrease for both species) compared to that of macrocarpa (14% decrease) in the 1490 soils. However in the 4000BC soil, only growth of radiata pine significantly decreased soil organic P (by 32%) compared to eucalypt (20%) and macrocarpa (13%).

Growth of both radiata pine and macrocarpa decreased the inorganic P concentration in 1826 soil compared to before planting (by 8% and 14%, respectively) therefore macrocarpa soil had a significantly lower inorganic P concentration than radiata pine soil, which, in turn, had a significantly lower concentration than eucalypt. In 1490 soils, both radiata pine and eucalypt growth significantly increased soil inorganic P concentrations by 37% and 55%, respectively, but macrocarpa did not have a significant effect. Therefore macrocarpa soil had a significantly lower inorganic P concentration than radiata pine soil which had a significantly lower concentration than eucalypt soil. In the 4000BC soil, only growth of eucalypt significantly increased inorganic P concentration (by 35%) compared to before planting therefore eucalypt soil had a significantly higher inorganic P concentration than radiata pine and macrocarpa soil which increased inorganic P by only 16% and 2%, respectively.

Table 5.5 Mean total, organic and inorganic P concentrations determined in soils from the Haast chronosequence before planting and after 6 months growth of eucalypt, macrocarpa and radiata pine. Values in parenthesis are standard errors of means, n = 3.

	Total P (mg/kg)			Inorganic P (mg/kg)			Organic P (mg/kg)		
	1826	**1490**	**4000BC**	**1826**	**1490**	**4000BC**	**1826**	**1490**	**4000BC**
Original	312	211	102	266	64	33	46	147	69
Radiata pine	273 b	196 b	85 b	244 b	88 b	38 ab	28 b	108 b	47 b
	(5.9)	(2.0)	(3.3)	(6.2)	(4.0)	(1.1)	(0.4)	(2.4)	(2.8)
Eucalypt	304 a	208 a	100 a	262 a	99 a	44 a	42 a	109 b	55 a
	(1.3)	(1.3)	(1.3)	(0.7)	(2.3)	(0.5)	(1.5)	(3.5)	(1.1)
Macrocarpa	258 c	195 b	94 a	230 c	68 c	34 b	29 b	127 a	60 c
	(2.7)	(2.6)	(1.6)	(3.0)	(1.1)	(1.0)	(0.7)	(1.9)	(2.5)

= 1826 soil = 1490 soil = 4000BC soil

Species*year interaction *P* <0.001 for all P pools.
Different letters represent significantly different means between species within a soil year using a 5% LSD of 8.3 for total P, 8.5 for inorganic P and 6.2 for organic P.

Table 5.6 Mean percent change in total, organic and inorganic P concentrations determined in soils from the Haast chronosequence after 6 months growth of eucalypt, macrocarpa and radiata pine.

Soil	Total P (mg/kg)			Inorganic P (mg/kg)			Organic P (mg/kg)		
	1826	**1490**	**4000BC**	**1826**	**1490**	**4000BC**	**1826**	**1490**	**4000BC**
Radiata pine	-13 %	-7 %	-16 %	-8 %	+37 %	+16 %	-39 %	-26 %	-32 %
Eucalypt	-3 %	-1 %	-2 %	-2 %	+55 %	+35 %	-9 %	-26 %	-20 %
Macrocarpa	-17 %	-8 %	-8 %	-14 %	+6 %	+2 %	-38 %	-14 %	-13 %

= 1826 soil = 1490 soil = 4000BC soil

5.4 Discussion

5.4.1 Exhaustive Pot trial

The aim of this experiment was to assess the effects of radiata pine, eucalypt and macrocarpa in a single soil P source over an extended period in a controlled environment. To do this these species were grown in soil in a confined space for 22 months. The most prominent finding of this experiment was that radiata pine grew best, utilized the largest amount of P which resulted in the largest decrease in soil P. Eucalypt did not grow as well in this experiment. This species has much finer roots than the other two species therefore it is possible that the transplanting shock was too much for this species to overcome and maintain adequate growth.

Radiata pine biomass increased by 71 g more than macrocarpa and 113 g more than eucalypt. This meant radiata pine took up 185 mg more P than macrocarpa and 207 mg more P than eucalypt. The greater plant P concentration in radiata pine than the other two species indicates that the higher P uptake of radiata pine was not only due to higher growth. The P concentration of the radiata pine trees (1.4 mg/g) was approximately double that of eucalypt and macrocarpa (0.7 and 0.6 mg/g, respectively) at experiment termination, indicating that radiata pine was less P limited in this experiment and therefore better adapted to utilize P in an exhaustive situation.

The fact that radiata pine depleted total soil NaOH-EDTA extractable P to a much larger extent than eucalypt and macrocarpa is linked to its higher growth rate. It is the re distribution of P within this pool by radiata pine that is important. Radiata pine decreased a significant amount of organic NaOH-EDTA extractable P which resulted in an increase of inorganic P compared to the original soil and a significantly higher amount than found under the other two species, indicating excess mineralization by radiata pine. This is not an unusual result as radiata pine, along with other *Pinus* spp. has been found to increase inorganic P and decrease organic P fractions in many other afforestation studies. For example, Chen et al. (2000a) found all forms of organic P were lower and all forms of inorganic P were higher under a mixed forest of *Pinus ponderosa* and *Pinus nigra* compared to those under adjacent grassland. Furthermore, in

a glass house experiment, Davis (1995) grew radiata pine and cocksfoot in soils for one year and found a similar result. The chemical nature of this P change was not measured but conifers have been shown to decrease both orthophosphate monoesters and diesters by Condron et al. (1996) and McDowell and Stewart (2006). Also, using ^{31}P NMR spectroscopy, Chen et al. (2004b) and Turner et al. (2005b) confirmed that mineralization of *myo*-inositol hexakisphosphate accounted for 18-100% of the orthophosphate monoester mineralization under radiata pine.

One of the mechanisms thought to be responsible for the increased mineralization under pine forest compared to pasture is the difference in mycorrhizal association between them (Chen et al., 2008). In the present study, ectomycorrhizal radiata pine mineralized a greater amount of P than arbuscular mycorrhizal macrocarpa therefore these results are in agreement with this hypothesis. Ectomycorrhizae are considered to be more efficient than AM at utilizing soil P for plant uptake (Lodge, 2000) through larger hyphae biomass in soils (Jones et al., 1998), greater hyphal production of extracellular phosphatase enzymes (Nygren and Rosling, 2009) and release of P solubilizing agents such as LMWOAs (Lapeyrie et al., 1987; Lapeyrie, 1988; Lapeyrie et al., 1991).

The reason for excess P mineralization, beyond what is required by the plant, is unclear. In a short term glasshouse experiment, Scott and Condron (2004) found that compartments containing solely ectomycorrhizal hyphae from fungi associated with radiata pine had a higher mineralization rate than those containing plants with an arbuscular mycorrhizal association. It is possible that the ectomycorrhizal hyphae are responding to the organic P in the environment and thus mineralizing it, however the fungi and therefore the plant are not utilizing the inorganic P. It is well known that ectomycorrhizal fungi produce phosphatases which mineralize organic P (Dighton, 1983; Häussling and Marschner, 1989; Ho, 1989; Antibus et al., 1992). It has been demonstrated previously that organic P presence enhances fungal phosphatase activity (Tarafdar and Marschner, 1994a and b) and that it is acid phosphatases that are mainly external or wall bound in mycorrhizal hyphae (Joner and Johansen, 2000; Joner et al., 2000) and thus responsible for the mineralization of soil P. Ectomycorrhizal hyphae are known to store P as inorganic polyphosphates which act to increase P flow into the host plant (Bücking and Heyser, 2000) and ectomycorrhizae are known to have a much

greater hyphal biomass in soil compared to arbuscular mycorrhizae (Jones et al., 1998) therefore there is potential for a large amount of inorganic P to be stored within ectomycorrhizal hyphae. Hyphae would have been severed in the sieving and grinding during sample preparation thus releasing whatever P was stored within them and this P is possibly contributing to that which is detected in the extract, resulting in seemingly higher soil inorganic P levels under radiata pine. However, it unlikely that hyphal P is the sole contributor to the high inorganic P levels detected as high intracellular P concentrations in ectomycorrhizal hyphae limit absorption of phosphate from the external environment by the hyphae (Cairney and Smith, 1992) and so limit excess storage in hyphae without plant uptake.

Eucalypt utilized a similar amount of total P and mineralized a similar amount of organic P as macrocarpa despite its significantly lower growth. In all previous experiments in this thesis (Chapters 2, 3 and 4), eucalypt has been predominantly ectomycorrhizal, thus it seems reasonable to assume that it is also ectomycorrhizal in the present study, especially due to the length of time being more than sufficient to allow the succession from arbuscular mycorrhizae to ectomycorrhizae that commonly occurs in tripartite eucalypt roots (Chen et al., 2000b). The P mineralization and utilization by eucalypt indicates that this ectomycorrhizal species can utilize a similar amount of soil P to the arbuscular mycorrhizal macrocarpa despite a much poorer condition resulting in lower growth which indicates that perhaps eucalypt is better adapted to utilize P in an exhaustive situation but growth was hampered in this experiment due its poor condition. In a previous pot trial (Chapter 3) eucalypt soil P concentrations were lowest, indicating that eucalypt can, in some situation deplete soil P and therefore it is likely that the poor condition of eucalypt in this experiment prevented much P uptake by this species.

5.4.2 Soil Organic P Gradient Pot Trial

This experiment was designed to assess how radiata pine, eucalypt and macrocarpa were able to utilize P from related soils containing different amounts of organic P. To do this the trees were grown in soils from a chronosequence where the different soil ages result in different concentrations of P and proportions of organic P. The trees used

in this experiment were taken from a nursery environment where the trees were in a high nutrient potting mix for optimum health. Therefore transplanting the trees to a more nutrient limiting soil would have put the trees under stress. It is not surprising that removing the trees from the potting mix and replanting them caused a decrease in plant P concentration. Therefore it is not useful to compare P content and concentration between the original trees and trees at experiment termination, the important comparison is between the trees grown in each soil.

The 1826 soil was the youngest soil used and had the highest P concentration with the lowest organic P proportion (Table 5.1). This soil was the least P limiting environment for the trees. The young age of these soils means that P had not been lost through leaching (Walker and Syers, 1976) and there had not been sufficient time for the primary mineral phosphate to be converted by biological and chemical processes to organic and occluded forms (Turner et al., 2007). The lack of soil development resulted in a higher bioavailability of P in the younger soils and means that vegetation is likely N, not P, limited (Richardson et al., 2004). This explains the higher amount and concentration of P in the trees after growth in the 1826 soil compared to growth in the older 1490 and 4000BC soils with less bioavailable P. The 1826 soil was the only soil that resulted in significantly higher plant P contents than that which the trees had originally.

All of the species reduced total P in the 1826 soil. As related to the growth and P content of the species, macrocarpa drew down the largest amount of P, followed by radiata pine and then eucalypt by a considerably smaller amount. The shock of transplanting from the nutrient rich potting mix to the relatively nutrient poor soil had a greater influence on eucalypt than the other two species and so prevented adequate growth to result in significant P draw down. When comparing radiata pine and macrocarpa, both species reduced organic P by 17-18 mg/kg or 38-39% indicating both species are adapted to utilize young, relatively labile forms of organic P to the same extent. Results from a ^{31}P NMR study on another chronosequence in New Zealand by Turner et al. (2007) showed that in soils approximately 200 years old (as is the 1826 soil), soil organic P consisted largely of monoesters. Inositol phosphates, considered to be relatively recalcitrant in soils (Turner et al., 2005b) were at a low proportion in soils

of this age on this chronosequence thus the remaining monoesters are likely to be more labile simple monoesters requiring only hydrolysis by phosphomonoesterases for plant uptake (Turner, 2008). The greater draw down of soil P by macrocarpa than radiata pine is due to a greater utilization of inorganic P by this species.

The 1490 soils have undergone approximately 500 years of soil development. Total P has declined by approximately a third of that in the 1826 soil and organic forms of P now make up 70% of the 1490 soil P. In order to grow and uptake P in the 1490 soil the trees must be efficiently adapted to utilize organic forms of P. Results from the Turner et al. (2007) study on another chronosequence reveal that the composition of organic P is unlikely to have changed greatly compared to the 1826 soil, except a slightly higher proportion of phosphonates and DNA, however, the relative importance of organic P is increased due to the lower proportion of inorganic P in the 1490 soil. This decrease in P availability explains the overall lower tree P contents and concentrations after growth in 1490 soil compared to the 1826 soils. Again, radiata pine and macrocarpa drew down significantly more P than eucalypt and were almost the same as each other in total P utilization. The major difference between the species is in the utilization of organic P in these soils. Radiata pine appeared to be more efficient at utilizing organic P than macrocarpa in 1490 soils. It decreased 20% of organic P compared to macrocarpa which decreased only 14%. However, this organic P decrease under radiata pine lead to a 37% increase in inorganic P indicating that radiata pine was mineralizing excess P to its requirement and its growth and P uptake was not limited by its ability to mineralize organic P. Macrocarpa organic P decreases only resulted in a 6% inorganic P increase and so it would appear that the macrocarpa was only mineralizing the P it requires and is therefore more efficient. On the other hand it could be mineralizing all it can and growth was therefore limited by its mineralization capacity which could explain its lower plant P concentration compared with radiata pine.

Eucalypt growth and therefore total P draw down was low in the 1490 soil. However, this species depleted very similar amounts of organic P to radiata pine. Labile organic P forms have been shown to be closely correlated with productivity on other eucalypt species (Adams et al., 1989) and due to extremely high phosphatase enzyme activity levels recorded under *Eucalyptus regnans*, Polglase et al. (1992a) showed that soil

organic P represented significant stores of potentially available P. However, in this experiment, due to the lack of growth and P uptake, mineralization of organic P resulted in a 55% increase in inorganic P in the soils. Why would this species mineralize such a large amount of soil P but not utilize it? Something in this soil may be preventing growth of this species independent of P concentration or forms.

The behavior of radiata pine in this study is in accordance with many studies investigating afforestation with conifer species and its effects on soil P in New Zealand. These afforestation studies found a decrease in organic P and an increase in inorganic P under conifer trees (Chen et al., 2000a; 2002; 2004b; Davis, 1995; Scott and Condron, 2004). This change in soil P has been attributed to the ectomycorrhizal association of the trees compared to the arbuscular mycorrhizal association of the pastures species, increased mineralization in early stages of forest development and increased P flux through the microbial biomass (Chen et al., 2008). Ectomycorrhizae are considered to be more efficient than AM at utilizing soil P for plant uptake (Marschner and Dell, 1994; Jones et al., 1998; Lodge, 2000). The mycorrhizal associations could explain the mineralization performance of eucalypt and radiata pine compared to macrocarpa. Radiata pine is known to be ectomycorrhizal (Chu-Chou and Grace, 1984; 1988; 1990; Walbert et al., 2009) and in previous chapters in this thesis, eucalypt has been shown (Chapter 2 and 4) or assumed (Chapter 3) to be ectomycorrhizal whereas macrocarpa is known to be arbuscular mycorrhizal. Therefore it appears that ectomycorrhizal tree species are better adapted to mineralize organic P than arbuscular mycorrhizal tree species.

Inorganic P stored within the ectomycorrhizal hyphae is likely to contribute to the inorganic P detected in the extract as discussed in Section 5.4.1 however this is unlikely to account for the total increase in inorganic P detected. Microbial activity has been shown to be high in eucalypt soils due to high levels of root exudation of LMWOAs (Chapter 3) providing the microbial biomass with a readily metabolizable C source (Jones et al., 2003; Richardson et al., 2009a). Ectomycorrhizal hyphae have also been repeatedly demonstrated to stimulate microbial activity through exudation of organic substances (Timonen and Marschner, 2006; Calvaruso et al., 2007) and microbial activity has been shown to be high in ectomycorrhizal hyphae compartments under

radiata pine in Chapter 3. Microorganisms are primarily responsible for the mineralization of organic C and associated P (Stewart and Tiessen, 1987; Jakobsen et al., 2005; Oberson and Joner, 2005; Bünemann et al., 2008) and microbial P has been shown to be correlated with productivity of *Eucalyptus* species in Tasmania (Adams et al., 1989). Water soluble organic carbon (WSOC) is mainly derived from root exudates (McGill et al., 1986). Chen et al. (2002) and Scott and Condron (2004) found mineralization rate correlated closely with WSOC levels in short term glasshouse experiments. Therefore it is possible that the microbial biomass, stimulated by root and mycorrhizal hyphae exudation of C compounds, is contributing to the high levels of mineralization found under radiata pine and eucalypt in this study. The flux of P through the biomass is likely to be high, especially in eucalypt soils, as the struggling tree is not competing effectively with the microbial biomass for mineralized P in the rhizosphere.

Recently, more work has been conducted on the mutualism-parasitism continuum between host plant and their mycorrhizal associates (Johnson et al., 1997; Klironomos, 2003; Landis and Fraser, 2007). Plant growth responses to mycorrhizal infection can range from very positive (mutualism) to highly negative (parasitism) depending on plant and fungal species involved and the nutrient status of the environment (Klironomos, 2003). Large response differences were observed with the same arbuscular mycorrhizal fungus but different host, same host but different fungus and the same partners but a different soil by Klironomos (2003). The same could occur for ectomycorrhizal associates. Therefore the very low growth but unusually high mineralization under eucalypt could be a result of a parasitic association with its mycorrhizal association. Further work would need to be conducted involving different soils and different mycorrhizal associates to assess if this was the case and further determine the role of mycorrhizae in P utilization by eucalypt.

The 4000BC soils have undergone 6000 years of soil development. There is now approximately half the amount of P in this soil compared to 1490 soil and a third the amount of that in 1826 soil. The 4000BC soil has a similar proportion of organic P to that in the 1490 soils but the remaining inorganic P is also likely to be of a less available nature thus the relative importance of the organic proportion will increase. The behavior

of the three species is very similar to that in the 1490 soils. Growth, P content and P concentration of the trees is slightly less than 1490 soils because the plants are working harder to obtain P from the soils. It is clear that radiata pine is the best adapted tree to utilize the forms of organic P in these soils as this species has decreased organic P by 32%. Again, this has resulted in a 16% increase in inorganic P. Macrocarpa utilized organic P in these soils, decreasing it by 13% but this resulted in only a 2% increase in inorganic P. The opposite occurs again with eucalypt trees. This species only decreases 2% of total soil P, due to low growth but there was a 20% organic P decrease in these soils but a 35% inorganic P increase. Again, this species is mineralizing organic P but is not utilizing it. As discussed above, in a previous pot trial (Chapter 3) soil P concentrations were lowest under eucalypt so it seems likely that the difference between these experiments is plant condition.

5.5 Conclusions

From the experiments described in this chapter, it appears that radiata pine and eucalypt are well adapted to mineralize organic P, most likely through their ectomycorrhizal associate. Even in an exhaustive situation, radiata pine still caused excessive mineralization, increasing concentrations of inorganic P and decreasing concentration of organic P compared to original soil. In soils with high proportions of organic P (70%) radiata pine again caused excess mineralization even in an older soil that likely increased the importance of organic P to plant nutrition due to increasing insolubility of the inorganic P.

In the exhaustive situation, soil organic P depletion by eucalypt was not significantly different to macrocarpa despite a lower growth and biomass indicating that it was the poor condition of the tree that affected its performance. In the organic P gradient pot trial, eucalypt behaved very similarly to radiata pine in its effect on soil P, however it did not take this P up into the biomass. The shock of transplanting on the finer roots of eucalypt likely prevented the tree from utilizing the P that was mineralized beneath it. In both ectomycorrhizal species, tree growth was not limited by organic P mineralization capacity.

Macrocarpa can utilize organic P in an exhaustive situation but to a smaller extent than radiata pine. This also occurred in related soils of varying organic P proportions. Organic P utilization by macrocarpa did not cause excess mineralization perhaps indicating a greater efficiency of this species – only mineralizing what was required by the plant or indicating that macrocarpa plant growth is limited by its capacity to mineralize organic P.

Chapter 6

Summary, Conclusions and Future Research

6.1 Summary

Enhanced soil organic P mineralization under forest that results in a decrease in organic P and an increase in inorganic P compared with pasture soils has been largely attributed to the contrasting mycorrhizal associations of *Pinus* and pasture species. To further investigate this phenomenon, three tree species (*Pinus radiata*, *Eucalyptus nitens* and *Cupressus macrocarpa*) with contrasting mycorrhizal associations were chosen for further detailed investigation of rhizosphere P dynamics.

A replicated field trial located at Orton Bradley Park on Banks Peninsula was chosen to assess short term, *in situ* rhizosphere P dynamics of ectomycorrhizal radiata pine, arbuscular mycorrhizal macrocarpa and tripartite (associated with both ectomycorrhizae and arbuscular mycorrhizae) eucalypt (Chapter 2). Results revealed large differences between the ectomycorrhizal and arbuscular mycorrhizal species in acid phosphomonoesterase activity and therefore in potential organic P mineralization. Acid phosphomonoesterase activity was significantly higher in the ectomycorrhizal species and correlated significantly with mycorrhizal colonization. A field experiment was also carried out which involved sampling rhizosphere and non rhizosphere soil to investigate the cumulative effects of nine years tree growth on P dynamics. Results revealed differences in the nature of P cycling under the three species, which was related to a combination of factors including differences in understory composition related to growth rate and canopy closure. All species utilized soil organic P, especially in the top 10 cm. Radiata pine had a significant litter layer (2-11 cm) which had considerably higher concentrations of resin P and microbial biomass than the under lying soil. Microbial activity was 6-9 fold higher and phosphatase enzyme activity was also higher in the litter layer than the under lying soil. It was speculated that much of the organic P

mineralization processes were occurring in the litter layer under this species through the microbial biomass and possibly ectomycorrhizal hyphae. Eucalypt and macrocarpa had comparable levels of microbial activity and phosphatase enzyme activities despite continued inputs of organic matter under macrocarpa from the pasture understory. This indicated that eucalypt was a more efficient phosphatase producer, related to its tripartite mycorrhizal association.

Field trial results supported the conclusion drawn from afforestation studies that increased mineralization under forest is due to the ectomycorrhizal association of the trees. However, many questions remained regarding the direct or indirect mycorrhizal contribution to P dynamics. To investigate this, a short-term glasshouse experiment was carried out on all three species, using mesh of different pore sizes to separate roots, mycorrhizal hyphae and microorganisms to confine and enhance rhizosphere processes in distinct measurable compartments. This split pot design was used to determine the relative contribution of mycorrhizae to P dynamics within the rhizosphere of radiata pine, eucalypt and macrocarpa and compare the contribution between the trees. The ectomycorrhizal association of radiata pine depleted organic P directly through phosphatase enzyme exudation and indirectly though stimulation of microbial activity, without necessitating the plant to invest excess energy into root exudation other than the C supply for its symbiont. Under eucalypt, root exudation of LMWOAs stimulated microbial activity and its mycorrhizal association increased phosphatase enzyme activity and possibly further selected for an efficient P solubilizing microbial community. For macrocarpa, it appeared that the very active bacterial microbial community, possibly stimulated by a large efflux of root exudates including LMWOAs, was a greater driver of organic P dynamics through the production of phosphatase enzymes. This large and active microbial community may have compensated for the less efficient arbuscular mycorrhizal association with macrocarpa. Alternatively, macrocarpa may not need a more efficient mycorrhizal symbiont as it would have little benefit because soil microbes appeared to drive P dynamics.

The results presented in Chapters 2 and 3 provided a clear story about the influence of various rhizosphere components on P dynamics under the three species, but what still remained to be answered was what proportion of phosphatase activity and LMWOA

concentrations could be directly attributed to mycorrhizal hyphae. Accordingly, a series of experiments were carried out to determine the direct contribution of mycorrhizal extraradical hyphae to P dynamics of ectomycorrhizal radiata pine, tripartite eucalypt and arbuscular mycorrhizal macrocarpa (Chapter 4). Phosphatase enzyme activity and LMWOA concentrations were measured in inoculated and non inoculated sterile trees and then the hyphae of mycorrhizal fungi associated with these tree species both in the field and in a glasshouse study were extracted from sand that was also spiked with a sparingly soluble organic P source. The hyphal capacity for producing phosphatase enzymes in response to organic P was assessed using enzyme labeled fluorescence. The inoculation experiment results showed that both ectomycorrhizae and arbuscular mycorrhizae increased acid phosphomonoesterase activity with ectomycorrhizal colonization resulting in the highest level of phosphatase activity which was linked to plant P uptake. However, mycorrhizal association did not appear to significantly affect rhizosphere alkaline phosphomonoesterase, phosphodiesterase activity or LMWOA type and concentration under all three species. When assessing the phosphatase activity of mycorrhizal hyphae directly, both ectomycorrhizae and arbuscular mycorrhizal hyphae were found to produce phosphatases. The activity was related to the number of hyphae present, with greater activity in single macrocarpa hyphae and lower activity in the more abundant eucalypt hyphae. It was concluded that the greater rhizosphere effect observed for ectomycorrhizae was related to the greater biomass of these hyphae compared to arbuscular mycorrhizae and not the capacities of the fungal types to produce phosphatases. There was also some evidence that hyphae increased phosphatase activity in response to the presence of the sparingly soluble organic P source, especially at low pH.

The utilization of soil organic P by the three species was determined in two glasshouse experiments. The first experiment involved growing the trees in Orton Bradley Park soil (as used in previous experiments) in a confined space for 22 months to create an exhaustive situation with a single soil. The second experiment involved growing the three species in selected soils from a chronosequence with different concentrations of total and organic P to assess the ability of each species to utilize organic P from a range of related soils. In an exhaustive situation, radiata pine utilized the largest amount of P but also caused excess organic P mineralization, increasing the concentration of

inorganic P and decreasing the concentration of organic P compared to the original soil. Eucalypt and macrocarpa utilized organic P but the concentration was decreased by far less than under radiata pine all P depleted from the soil was incorporated into the biomass with no excess mineralization. Soil organic P depletion by eucalypt in the exhaustive situation was not significantly different to macrocarpa despite a much lower plant biomass and P utilization. When grown in the related soils with contrasting levels of organic P, radiata pine caused excess mineralization again. In this experiment, macrocarpa utilized organic P but mineralization was at a much lower rate than under eucalypt and radiata pine as this did not result in an increase in soil inorganic P concentration - P was utilized and incorporated into the plant biomass. Eucalypt also caused excess mineralization, increasing inorganic P concentrations by as much as 55% however the released inorganic P was not utilized by the plant.

6.2 Conclusions

The major conclusions derived from this study were as follows:

i. Rhizosphere processes and organic P utilization varied significantly with tree species.

ii. All three tree species utilized organic P but ectomycorrhizal trees caused excess mineralization, increasing inorganic P concentrations in the soil.

iii. In the field, the dynamics and bioavailability of P cycles maybe significantly influenced by the presence of an understory or litter.

iv. The ectomycorrhizal association of radiata pine played a significant role in rhizosphere processes by increasing phosphatase activity and microbial activity. This increased activity was possibly the reason for the lower exudation of LMWOAs by roots of radiata pine compared to the others. Radiata pine growth and P uptake was not limited by its ability to mineralize organic P.

v. Eucalypt was mainly ectomycorrhizal in this study. This ectomycorrhizal association increased rhizosphere phosphatase activity but roots of eucalypt also exuded large amounts of LMWOAs which acted to stimulate microbial activity and further increase phosphatase activity in the rhizosphere. Therefore it was the microbial community as well the ectomycorrhizal association that contributed to P dynamics in the eucalypt rhizosphere. Growth and P uptake of eucalypt was not limited by organic P mineralization.

vi. The microbial community under macrocarpa was found to be very active and likely stimulated by a large efflux of LMWOAs by the roots. This meant it was the microbial community that had the largest influence on P dynamics in this species rhizosphere, perhaps a compensation for its less effective arbuscular mycorrhizal association. Growth and P uptake of macrocarpa may have been limited by organic P mineralization ability.

vii. The capabilities of ectomycorrhizal and arbuscular mycorrhizal hyphae to produce phosphatase enzymes at acid and alkaline pH were similar. Therefore the difference in rhizosphere phosphatase enzyme activity between the two types was likely due to the much greater biomass of ectomycorrhizal hyphae.

viii. Mycorrhizal infection did not appear to influence rhizosphere LMWOA concentration, alkaline phosphatase activity or phosphodiesterase activity but did have a significant influence on acid phosphomonoesterase activity in the rhizospheres.

6.3 Future Research

The findings of this study contributed to improved understanding of the rhizosphere processes influencing P dynamics in forest ecosystems and the role of mycorrhizae in particular. Further research could investigate the following:

i. Results presented in Chapter 5 revealed obvious differences in organic P utilization and redistribution of P forms between species. Further differences would become apparent if ^{31}P NMR was conducted on samples from experiments like these to assess changes in more specific P pools in the soil.

ii. The root sampling study presented in Chapter 2 and work by Chen et al. (2002a) revealed the potential importance of the radiata pine litter layer in P dynamics. Further work investigating processes at the soil-litter interface such as ELF staining for phosphatase activity in the fungal hyphae present in this zone would clarify the speculations made about rhizosphere processes essentially 'by-passing' the soil under radiata pine.

iii. Where a mycorrhizal association falls in the mutualism-parasitism continuum is very system specific (Johnson et al., 1997; Klironomos, 2003). Therefore to fully understand the mycorrhizal role in tree rhizosphere processes, more extensive studies need to be conducted using different tree species, different mycorrhizal hosts and different soil environments. This would involve identification at the molecular level of mycorrhizal species associated with the trees.

iv. Results from Chapter 3 demonstrated that a stimulation of microbial activity occurred in hyphal zones. Mycorrhizal fungi have previously been demonstrated to select for a more efficient P solubilizing microbial community (Frey-Klett et al., 2005; Calvaruso et al., 2007). It would be useful to understand the change in microbial community driven by the mycorrhizal association of these species to further understand the indirect role of mycorrhizal fungi in rhizosphere dynamics.

References

Adams, J. F., Wood, C. W., and Mitchell, R. I. (1999). Loblolly pine plant community structure and soil solution aluminium, organic acids, calcium, magnesium and pH. *Communications in Soil Science and Plant Analysis, 30,* 1939-1950.

Adams, M. A. (1992). Phosphatase activity and phosphorus fractions in Karri (*Eucalyptus diversicolor* F. Muell.) forest soils. *Biology and Fertility of Soils, 14*, 200-204.

Adams, M. A., Attiwill, P. M., and Polglase, P. J. (1989). Availability of nitrogen and phosphorus in forest soils in north-eastern Tasmania. *Biology and Fertility of Soils, 8*, 212-218.

Adams, M. A., and Pate, J. S. (1992). Availability of organic and inorganic forms of phosphorus to lupins. *Plant and Soil, 145*, 107-113.

Alef, K. (1995). Dehydrogenase activity. In K. Alef and P. Nannipieri (Eds.), *Methods in Applied Soil Microbiology and Biochemistry* (pp. 228-231). London: Academic Press.

Aleksieva, P., and Micheva-Viteva, S. (2000). Regulations of extra-cellular acid phosphatase biosynthesis by phosphates in proteinase producing fungus *Humicola lutea* 120-5. *Enzyme and Microbial Technology, 27*, 570-575.

Anderson, G. (1967). Nucleic acids, derivatives and organic phosphates. In A. D. McLaren and G. H. Peterson (Eds.), *Soil Biochemistry* (Vol. 1, pp. 67-90). New York: Marcell Dekker Inc.

Anderson, J. P., and Domsch, K. H. (1975). Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. *Canadian Journal of Microbiology, 21*, 314-322.

Anderson, J. P., and Domsch, K. H. (1980). Quantities of plant nutrients in the microbial biomass of selected soils. *Soil Science, 130*, 211-215.

Andrade, G., Mihara, K. L., Linderman, R. G., and Bethlenfalvay, G. J. (1997). Bacteria from rhizosphere and hyphosphere soils of different arbuscular mycorrhizal fungi. *Plant and Soil, 192,* 71-79.

Antelmann, H., Scharf, C., and Hecker, M. (2000). Phosphate starvation-inducible proteins of *Bacillus subtilis*: proteomics and transcriptional analysis. *Journal of Bacteriology, 182*, 4478-4490.

Antibus, R. K., Bower, D., and Dighton, J. (1997). Root surface phosphatase activities and uptake of 32P-labelled inositol phosphate in field-collected gray birch and red maple roots. *Mycorrhiza, 7*, 39-46.

Antibus, R. K., Sinsabaugh, R. L., and Linkins, A. E. (1992). Phosphatase activities and phosphorus uptake from inositol phosphate by ectomycorrhizal fungi. *Canadian Journal of Botany, 70*, 794-801.

Antunes, P. M., Schneider, K., Hillis, D., and Klironomos, J. N. (2007). Can the arbuscular mycorrhizal fungus *Glomus intraradices* actively mobilize P from rock phosphates? *Pedobiologia, 51*, 281-286.

Arshad, M., and Franken-berger, W. T. (1998). Plant growth regulating substances in the rhizosphere: Microbial production and functions. *Advances in Agronomy, 62*, 45-151.

Asmar, F., Gahoonia, T. S., and Nielsen, J. S. (1995). Barley genotypes differ in activity of soluble extracellular phosphatase and depletion of organic phosphorus in the rhizosphere soil. *Plant and Soil, 172*, 117-122.

Atkinson, D. (2006). Arbuscular mycorrhizal fungi and the form and functioning of the root system. In K. G. Mukerji, C. Manoharachary and J. Singh (Eds.), *Microbial Activity in the Rhizosphere* (Vol. 7, pp. 199-222). Berlin: Springer-Verlag.

Attiwill, P. M., and Adams, M. A. (1993). Nutrient cycling in forests. *New Phytologist, 124*, 561-582.

Azcon, R., Borie, F., and Barea, J.-M. (1982). Exocellular phosphatase activity of lavender and wheat roots as affected by phytate and mycorrhizal inoculation. In S. Gianinazzi, V. Gianinazzi-Pearson and A. Trouvelot (Eds.), *Les Mycorrhizes: Biologie et Utilisation* (Vol. 13, pp. 83-85). Dijon: INRA.

Badalucco, L., and Kuikman, P. J. (2001). Mineralization and immobilization in the rhizosphere. In R. Pinton, Z. Varanini and P. Nannipieri (Eds.), *The Rhizosphere*. (pp. 159-196) New York: Mercel Dekker.

Baláz, M., and Vosatka, M. (1997). Vesicular-arbuscular mycorrhiza of *Calamagrostis villosa* supplied with organic and inorganic phosphorus. *Biologia Plant Arum, 39*, 281-288.

Barea, J.-M., Pozo, M. J., Azcon, R., and Azcon, C. (2005). Microbial co-operation in the rhizosphere. *Journal of Experimental Botany, 56*, 1761-1778.

Barea, J.-M., Toro, M., Orozco, M. O., Campos, E., and Azcon, R. (2002). The application of isotopic ^{32}P and ^{15}N-dilution techniques to evaluate the interactive effect of phosphate-solubilizing rhizobacteria, mycorrhizal fungi and *Rhizobium* to improve the agronomic efficiency of rock phosphate for legume crops. *Agroecosystems, 63*, 35-42.

Bending, G. D., and Read, D. J. (1995). The structure and function of the vegetative mycelium of ectomycorrhizal plants. V. Foraging behaviour and translocation of nutrients from exploited litter. *New Phytologist, 130*, 401-409.

Berg, M. P., Kniese, J. P., and Verhoef, H. A. (1998). Dynamics and stratification of bacteria and fungi in the organic layers of a Scots pine forest soil. *Biology and Fertility of Soils, 26*, 313-322.

Blakemore, L. C., Searle, P. L., and Daly, B. K. (1987). Methods for chemical analysis of soils. *NZ Soil Bureau Scientific Report, 80*, 103p.

Bolan, N. S. (1991). A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil, 134*, 189-207.

Bougher, N. L., Grove, T. S., and Malajczuk, N. (1990). Growth and phosphorus acquisition of karri (*Eucalyptus diversicolor* F. Muell.) seedlings inoculated with ectomycorrhizal fungi in relation to phosphorus supply. *New Phytologist, 114*, 77-85.

Bouillet, J.-P., Laclau, J.-P., Arnaud, M., M'Bou, A. T., Saint-Andre, L., and Jourdan, C. (2002). Changes with age in the spatial distribution of roots of *Eucalyptus* clone in Congo. Impact on water and nutrient uptake. *Forest Ecology and Management, 171*, 43-57.

Bowman, R. A., and Cole, C. V. (1978). Transformations of organic phosphorus substrates in soils as evaluated by $NaHCO_3$ extraction. *Soil Science, 125*, 49-54.

Bowman, R. A., and Moir, J. O. (1993). Basic EDTA as an extractant for soil organic phosphorus. *Soil Science Society of America Journal, 57*, 1516-1518.

Browman, M. G., and Tabatabai, M. A. (1978). Phosphodiesterase activity of soils. *Soil Science Society of America Journal, 42*, 284-290.

Brundrett, M. C., Dell, B., Grove, T. L., and Malajczuk, N. (1996). *Working with mycorrhizas in forestry and agriculture* (ACIAR monograph no. 32). Canberra: Australian Centre for International Agricultural Research.

Bücking, H., and Heyser, W. (2000). Subcellular compartmentation of elements in non-mycorrhizal and mycorrhizal roots of *Pinus sylvestris*: an X-ray microanalytical study. I. The distribution of phosphate. *New Phytologist, 145*, 311-320.

Bünemann, E. K., and Condron, L. M. (2006). Phosphorus and sulphur cycling in Terrestrial Ecosystems. In P. Marschner and R. Z (Eds.), *Nutrient Cycling in Terrestrial Ecosystems*. New York: Springer-Verlag.

Bünemann, E. K., Smernik, R. J., Marschner, P., and McNeill, A. M. (2008). Microbial Synthesis of organic and condensed forms of phosphorus in acid and calcareous soils. *Soil Biology and Biochemistry, 40*, 932-946.

Burns, R. G. (1982). Enzyme activity in soil: Location and a possible role in microbial ecology. *Soil Biology and Biochemistry, 14*, 423-427.

Burns, R. G. (1986). Interaction of enzymes with soil mineral and organic colloids. In P. M. Huang and M. Schnitzer (Eds.), *Interactions of Soil Minerals With Natural Organics and Microbes* (pp. 429-451). Madison: Soil Science Society of America.

Cade-Menun, B. J. (2005). Using phosphorus 31-P nuclear magnetic resonance spectroscopy to characterize organic phosphorus in environment samples. In B. L. Turner, E. Frossard and D. Baldwin (Eds.), *Organic Phosphorus in the Environment* (pp. 21-44). Wallingford, UK: CAB international.

Cairney, J. W., and Smith, S. E. (1992). Influence if intracellular phosphorus concentration on phosphate absorption by the ectomycorrhizal basidiomycete *Pisolithus tinctorius*. *Mycological Research, 96*, 673-676.

Calvaruso, C., Turpault, M. P., Leclerc, E., and Frey-Klett, P. (2007). Impact of Ectomycorrhizosphere on the functional diversity of soil bacterial and fungal communities from a forest stand in relation to nutrient mobilization processes. *Microbial Ecology, 54*, 567-577.

Cassarin, V., Plassard, C., Souche, G., and Arvieu, J. C. (2003). Quantification of oxalate ions and protons released by ectomycorrhizal fungi in rhizosphere soil. *Agronomie, 23*, 461-469.

Cavagnaro, T. R., Smith, S. E., and Dickson, S. (2001). Backseat driving? Accessing phosphate beyond the rhizosphere- depletion zone. *Trends in Plant Science, 6*, 194-195.

Celi, L., and Barberis, E. (2005). Abiotic reactions of inositol phosphates in soil. In B. L. Turner, A. E. Richardson and E. J. Mullaney (Eds.), *Inositol Phosphates - Linking Agriculture and the Environment* (pp. 207-221). Wallingford: CAB international.

Chen, C. R., Condron, L. M., Davis, M. R., and Sherlock, R. R. (2000a). Effects of afforestation on phosphorus dynamics and biological properties in a New Zealand grassland soil. *Plant and Soil, 220*, 151-163.

Chen, C. R., Condron, L. M., Davis, M. R., and Sherlock, R. R. (2002). Phosphorus dynamics in the rhizosphere of perennial ryegrass (*Lolium perenne* L.) and radiata pine (*Pinus radiata* D.Don.). *Soil Biology and Biochemistry, 34*, 487-499.

Chen, C. R., Condron, L. M., Davis, M. R., and Sherlock, R. R. (2003a). Seasonal changes in soil phosphorus and associated microbial properties under adjacent grassland and forest in New Zealand. *Forest Ecology and Management, 177*, 539-557.

Chen, C. R., Condron, L. M., Davis, M. R., and Sherlock, R. R. (2004a). Effects of plant species on microbial biomass phosphorus and phosphatase activity in a range of grassland soils. *Biology and Fertility of Soils, 40*, 313-322.

Chen, C. R., Condron, L. M., Sinaj, S., Davis, M. R., Sherlock, R. R., and Frossard, E. (2003b). Effects of plant species on phosphorus availability in a range of grassland soils. *Plant and Soil, 256*, 115-130.

Chen, C. R., Condron, L. M., Turner, B. L., Mahieu, N., Davis, M. R., and Xu, Z. H., (2004b). Mineralization of soil orthophosphate monoesters under pine seedlings and ryegrass. *Australian Journal of Soil Research, 42*, 189-196.

Chen, C. R., Condron, L. M., and Xu, Z. H. (2008). Impacts of grassland afforestation with coniferous trees on soil phosphorus dynamics and associated microbial processes: A review. *Forest Ecology and Management, 255*, 396-409.

Chen, Y. L., Brundrett, M. C., and Dell, B. (2000b). Effects of Ectomycorrhizas and Vesicular-Arbuscular Mycorrhizas, Alone or in Competition, on Root Colonization and Growth of *Eucalyptus globulus* and *E. urophylla*. *New Phytologist, 146*, 545-556.

Chilvers, G. A. (1973). Host range of some eucalypt mycorrhizal fungi. *Australian Journal of Botany, 21*, 103-111.

Chilvers, G. A., Lapeyrie, F., and Horan, D. P. (1987). Ectomycorrhizal vs endomycorrhizal fungi within the same root system. *New Phytologist, 107*, 441-448.

Chu-Chou, M., and Grace, L. J. (1982). Mycorrhizal fungi of *Eucalyptus* in the north island of New Zealand. *Soil Biology and Biochemistry, 14*, 133-137.

Chu-Chou, M., and Grace, L. J. (1984). Cultural characteristics of *Rhizopogon* Spp. associated with *Pinus radiata* seedlings. *New Zealand Journal of Botany, 22*, 35-41.

Chu-Chou, M., and Grace, L. J. (1988). Mycorrhizal fungi of *Pinus radiata* in the different forests of the North and South islands in New Zealand. *Soil biology and Biochemistry, 20*, 883-886.

Chu-Chou, M., and Grace, L. J. (1990). Mycorrhizal fungi of radiata pine seedlings in nurseries and trees in forests. *Soil Biology and Biochemistry, 22*, 959-966.

Cole, C. V., Elliot, E. T., Hunt, H. W., and Coleman, D. C. (1978). Trophic interactions in soils as they affect energy and nutrient dynamics. V. Phosphorus transformation. *Microbial Ecology, 4*, 381-387.

Comerford, N. B., and De Barros, N. F. (2005). Phosphorus nutrition of forest trees. In J. T. Sims and A. N. Sharpley (Eds.), *Phosphorus: Agriculture and the Environment*. (pp. 541-558) Madison, Wisconsin: ASA/ CSSA/ SSSA.

Condron, L. M., Davis, M. R., Newman, R. H., and Cornforth, I. S. (1996). Influence of conifers on the forms of phosphorus in selected New Zealand grassland soils. *Biology and Fertility of Soils, 21*, 37-42.

Condron, L. M., and Newman, R. H. (1998). Chemical nature of soil organic matter under grassland and recently established forest. *European Journal of Soil Science, 49*, 597-603.

Condron, L. M., and Newman, S. (2009). Revisiting the fundamentals of phosphorus fractionation of sediments and soils. *Journal of Environmental Quality, in prep*

Condron, L. M., and Teissen, H. (2005). Interactions of organic phosphorus in terrestrial ecosystems. In B. L. Turner, E. Frossard and D. Baldwin (Eds.), *Organic Phosphorus in the Environment* (pp. 295-307). Wallingford, UK: CAB international.

Condron, L. M., Turner, B. L., and Cade-Menun, B. J. (2005). Chemistry and dynamics of soil organic phosphorus. In J. T. Sims and A. N. Sharpley (Eds.), *Phosphorus: Agriculture and the Environment* (pp. 87-121). Madison, Wisconsin: ASA/ CSSA/ SSSA.

Conn, C., and Dighton, J. (2000). Litter quality influences on decomposition, ectomycorrhizal community structure and mycorrhizal root surface acid phosphatase activity. *Soil Biology and Biochemistry, 32*, 489-496.

Cortina, J., Romanya, J., and Vallejo, V. R. (1995). Nitrogen and phosphorus leaching from the forest floor of a mature *Pinus radiata* stand. *Geoderma, 66*, 321-330.

Courtey, P.E., Pouysegur, R., Buee, M., and Garbaye, J. 2006. Laccase and phosphatase acitivities of the dominant ectomycorrhizal types in a lowland oak forest. *Soil Biology and Biochemistry 38*, 1219-1222.

Dahlberg, A. (2001). Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. *New Phytologist, 150*, 555-562.

Dalal, R. C. (1979). Mineralization of carbon and phosphorus from carbon-14 and phosphorus-32 labelled plant material added to soil. *Soil Science Society of America Journal, 43*, 913-916.

Darrah, P. R. (1991). Models of the Rhizosphere: I. Microbial population dynamics around a root releasing soluble and insoluble carbon. *Plant and Soil, 133*, 187.

Davis, M. R. (1994). Topsoil properties under tussock grassland and adjoining pine forest in Otago, New Zealand. *New Zealand Journal of Agricultural Research, 37*, 465-469.

Davis, M. R. (1995). Influence of radiata pine seedlings on chemical properties of some New Zealand montane grassland soils. *Plant and Soil, 176*, 255-262.

Davis, M. R., and Condron, L. M. (2002). Impact of grassland afforestation on soil carbon in New Zealand: a review of paired-site studies. *Australian Journal of Soil Research, 40*, 675-690.

Davis, M. R., and Lang, M. H. (1991). Increased nutrient availability in topsoils under conifers in the south island high country. *New Zealand Journal of Forestry Sciences, 21*, 165-179.

Diaz-Raviña, M., Acea, M. J., and Carballas, T. (1995). Seasonal changes in microbial biomass and nutrient flush in forest soil. *Biology and Fertility of Soils, 19*, 220-226.

Dighton, J. (1983). Phosphatase production by mycorrhizal fungi. *Plant and Soil, 71*, 455-462.

Dodd, J. C., Boddington, C. L., Rodriguez, A., Gonzalez-Chavez, C., and Mansur, I. (2000). Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: form function and detection. *Plant and Soil, 226*, 131-151.

Dodd, J. C., Burton, C. C., Burns, R. G., and Jeffries, P. (1987). Phosphatase activity associated with the roots and the rhizosphere of plants infected with vesicular-arbuscular mycorrhizal fungi. *New Phytologist, 107*, 163-172.

Don, A., and Kalbitz, K. (2005). Amounts and degradability of dissolved organic carbon from foliar litter at different decomposition stages. *Soil Biology and Biochemistry, 37*, 2171-2179.

Eivazi, F., and Tabatabai, M. A. (1977). Phosphatases in Soils. *Soil Biology and Biochemistry, 9*, 167-172.

Eleanor, M. B., and Lewis, D. H. (1978). Surface phosphatase activity of mycorrhizal roots of beech. *Soil Biology and Biochemistry, 5*, 249-257.

Facelli, E., Smith, S. E., and Smith, F. A. (2009). Mycorrhizal symbiosis - overview and new insights into roles of arbuscular mycorrhizas in agro- and natural ecosystems. *Australasian Plant Pathology, 38*, 338-344.

Farr, E., Vaidyanathan, L. V., and Nye, P. H. (1969). Measurement of ionic concentrations gradients in soil near roots. *Soil Science, 102*, 385-391.

Feng, G., Su, Y., Xiaolin, L., Wang, H., Zhang, F., and Tang, C. (2002). Histochemical visualization of phosphatase released by arbuscular mycorrhizal fungi in soil. *Journal of Plant Nutrition, 25*, 969-980.

Fillion, M., St-Arnaud, M., and Fortin, J. A. (1999). Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere micro-organisms. *New Phytologist, 141*, 525-533.

Finlay, R., and Read, D. (1986). The structure and function of the vegetative mycelium of ectomycorrhizal plants. II. The uptake and distribution of phosphorus by mycelial strands in the connecting host plants. *New Phytologist, 103*, 157-165.

Firsching, B.-M., and Claassen, N. (1996). Root phosphatase activity and soil organic phosphorus utilization by Norway spruce [*Picea abies* (L.) Karst.]. *Soil Biology and Biochemistry, 28*, 1417-1424.

Fox, T. R., and Comerford, N. B. (1990). Water soluble organic acids in selected forest soils of the south eastern United States. *Soil Science Society of America Journal, 54*, 1139-1144.

Fox, T. R., and Comerford, N. B. (1992). Rhizosphere phosphatase activity and phosphatase hydrolyzable organic phosphorus in two forested spodosols. *Soil Biology and Biochemistry, 24*, 579-583.

Fransson, A. M., and Jones, D. L. (2007). Phosphatase activity does not limit the microbial use of low molecular weight organic -P substrates in soil. *Soil Biology and Biochemistry, 39*, 1213-1217.

Frey-Klett, P., Chavatte, M., Clausse, M. L., Courrier, S., Le Roux, C., and Raaijmakers, J. (2005). Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. *New Phytologist, 165*, 317-328.

Frossard, E., Brossard, M., Hedley, M. J., and Metherell, A. (1995). Reactions controlling the cycling of P in soils. In H. Teissen (Ed.), *Phosphorus in the Global Environment* (pp. 107-141): John Wiley and Sons Ltd.

Frossard, E., Condron, L. M., Oberson, A., Sinaj, S., and Fardeau, J. C. (2000). Processes governing Phosphorus Availability in temperate soils. *Journal of Environmental Quality, 29*, 15-23.

Gahoonia, T. S., and Nielsen, J. S. (1997). Variation in root hairs of barley cultivars doubles soil phosphorus uptake. *Euphytica, 98*, 177-182.

Gahoonia, T. S., and Nielsen, J. S. (2004). Root traits as tools for creating phosphorus efficient crop varieties. *Plant and Soil, 260*, 47-57.

Gahoonia, T. S., and Nielsen, N. E. (1991). A Method to study rhizosphere processes in thin soil layers of different proximity to roots. *Plant and Soil, 143*, 143-146.

Gautam, M. K. (1998). *Rooting characteristics of Pinus radiata D. Don as influenced by understorey competition in an agroforestry system.* Unpublished doctoral thesis, Lincoln University, New Zealand.

Gautam, M. K., Mad, D. J., Chang, S. X., and Clinton, P. W. (2002). Spatial variation and understorey competition effect of *Pinus radiata* fine roots in a silvopastoral system in New Zealand. *Agroforestry Systems, 55*, 89-98.

George, T. S., Gregory, P. J., Hocking, P. J., and Richardson, A. E. (2008). Variation in root-associated phosphatase activities in wheat contributes to the utilization of organic P substrates *in-vitro* , but does not explain differences in the P nutrition of plants when grown in soils. *Environmental Experimental Botany, 64*, 239-249.

George, T. S., Gregory, P. J., Robinson, J. S., Buresh, R. J., and Jama, B. (2002a). Utilisation of soil organic P by agroforestry and crop species in the field, western Kenya. *Plant and Soil, 246*

George, T. S., Gregory, P. J., Wood, M., Read, D., and Buresh, R. J. (2002b). Phosphatase activity and organic acids in the rhizosphere of potential agriforestry species and maize. *Soil Biology and Biochemistry, 34*, 1487-1494.

George, T. S., Richardson, A. E., Barry Smith, J., Hadobas, P. A., and Simpson, R. J. (2005a). Limitations to the potential of transgenic *Trifolium subterraneum* L. plants that exude phytase when grown in soils with a range of organic P content. *Plant and Soil, 258*, 263-274.

George, T. S., Richardson, A. E., and Simpson, R. J. (2005b). Behaviour of plant derived extracelluar phytase upon addition to soil. *Soil Biology and Biochemistry, 37*, 977-988.

George, T. S., Turner, B. L., Gregory, P. J., Cade-Menun, B. J., and Richardson, A. E. (2006). Depletion of organic phosphorus from Oxisols in relation to phosphatase activities in the rhizosphere. *European Journal of Soil Science, 57*, 47-57.

Girisha, G. K., Condron, L. M., Clinton, P. W., and Davis, M. R. (2003). Decomposition and nutrient dynamics of green and freshly fallen radiata pine (*Pinus radiata*) needles. *Forest Ecology and Management, 179*, 169-181.

Goldstein, A. H., Baertlein, D. A., and McDaniel, R.G. (1988). Phosphate starvation inducible metabolism in *Lycopersicon esculentum.* I. Excretion of acid phosphatase by tomato plants and suspension cultured cells. *Plant Physiology, 87*, 711-715.

Grierson, P. F., and Adams, M. A. (2000). Plant species affect acid phosphatase, ergosterol and microbial P in a Jarrah (*Eucalyptus marginata* Donn ex Sm.) forest in south-western Australia. *Soil Biology and Biochemistry, 32*, 1817-1827.

Griffiths, R. P., Baham, J. E., and Caldwell, B. A. (1994). Soil solution chemistry of ectomycorrhizal mats in forest soil. *Soil Biology and Biochemistry, 26*, 331-337.

Guggenberger, G., Christensen, B. T., and Rubaek, G. H. (2000). Isolation and characterization of labile phosphorus pools in soils from the Askov long-term field experiments. *Journal of Plant Nutrition and Soil Science, 163*, 151-155.

Hariprasad, P., and Niranjana, S. R. (2009). Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. *Plant and Soil, 316,* 13-24.

Harley, J. L. (1969). *The Biology of Mycorrhiza*. London: Leonard Hill.

Harrison, A. F. (1987). *Soil Organic Phosphorus - A Review of World Literature*. Wallingford: CAB international.

Häussling, M., and Marschner, H. (1989). Organic and inorganic soil phosphates and acid phosphatase activity in the rhizosphere of 80-year-old Norway spruce [Picea abies (L.) Karst.] trees. *Biology and Fertility of Soils, 8*, 128-133.

Hayes, J. E., Richardson, A. E., and Simpson, R. J. (1999). Phytase and acid phosphatase activities in roots of temperate pasture grasses and legumes. *Australian Journal of Plant Physiology, 26*, 801-809.

Hendriks, L., and Jungk, A. (1981). Erfassung der Mineralstoffverteilung in Wurzelnana durch getrennte analyse von Rhizo- und Restboden. *Z. Pflanzenernahr Bodenkd, 144*, 276-282.

Hilger, A. B., and Krause, H. H. (1989). Growth characteristics of *Laccaria laccata* and *Paxillus involutus* in liquid culture media with inorganic and organic phosphorus sources. *Canadian Journal of Botany, 67*, 1782-1789.

Hinsinger, P. (2001). Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil, 237*, 173-195.

Hinsinger, P., Bengough, A. G., Vetterlein, D., and Young, I. M. (2009). Rhizosphere: biophysics, biogeochemistry and ecological relevance. *Plant and Soil, 321*, 117-152.

Ho, I. (1989). Acid phosphatase, alkaline phosphatase and nitrate reductase activity of selected ectomycorrhizal fungi. *Canadian Journal of Botany, 67*, 750-753.

Ho, M. D., Rosas, J. C., Brown, K. M., and Lynch, J. P. (2005). Root architectural trade offs for water and phosphorus acquisition. *Functional Plant Biology, 32*, 737-748.

Holford, I. C. (1997). Soil phosphorus: its measurement, and its uptake by plants. *Australian Journal of Soil Research, 35*, 227-240.

Ingold, C. T. (2001). Range in size and form of basidiospores and ascospores. *Mycologist, 15*, 165-166.

Jackson, R. M., and Mason, P. A. (1984). *Mycorrhiza*. London: Edward Arnold Ltd.

Jakobsen, I., Abbott, L. K., and Robson, A. D. (1992). External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. *New Phytologist, 120*, 371-380.

Jakobsen, I., Leggett, M. E., and Richardson, A. E. (2005). Rizosphere microorganisms and plant phosphorus uptake. In J. T. Sims and A. N. Sharpley (Eds.), *Phosphorus: Agriculture and the Environment* (pp. 437-494). Madison: ASA/CSSA/SSSA.

Jakobsen, I., and Rodsendahl, L. (1990). Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytologist, 115*, 77-83.

Jayachandran, K., Schwab, A. P., and Hetrick, B. A. D. (1989). Mycorrhizal mediation of phosphorus availability: synthetic iron chelate effects on phosphorus solubilization. *Soil Science Society of America Journal, 53*, 1701-1706.

Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K., and Barea, J.-M. (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils, 37*, 1-16.

Jentschke, G., Bonkowski, M., Godbold, D. L., and Scheu, S. (1995). Soil protazoa and forest tree growth: non nutritional effects and interaction with mycorrhizae. *Biology and Fertility of Soils, 19*, 215-219.

Johnson, N. C., Graham, J. H., and Smith, F. A. (1997). Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist, 135*, 575-586.

Joner, E. J., and Jakobsen, I. (1994). Contribution by two arbuscular mycorrhizal fungi to P uptake by cucumber (*Cucumis sativus* L.) from32P-labelled organic matter during mineralization in soil. *Plant and Soil, 163*, 203-209.

Joner, E. J., and Jakobsen, I. (1995). Growth and extracellular phosphatase activity of arbuscular mycorrhizal hyphae as influenced by soil organic matter. *Soil Biology and Biochemistry, 27*, 1153-1159.

Joner, E. J., and Johansen, A. (2000). Phosphatase activity of external hyphae of two arbuscular mycorrhizal fungi. *Mycological Research, 104*, 81-86.

Joner, E. J., Magid, J., Gahoonia, T. S., and Jakobsen, I. (1995). P depletion and activity of phosphatases in the rhizosphere of mycorrhizal and non-mycorrhizal cucumber (*cucumis sativus* L.). *Soil Biology and Biochemistry, 27*, 1145-1151.

Joner, E. J., van Aarle, I. M., and Vosatka, M. (2000). Phosphatase activity of extra-radical arbuscular mycorrhizal hyphae: A review. *Plant and Soil, 226*, 199-210.

Jones, D. L. (1998). Organic acids in the rhizosphere – a critical review. *Plant and Soil, 205*, 25-44.

Jones, D. L., and Darrah, P. R. (1994). Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant and Soil, 166*, 247-257.

Jones, D. L., Dennis, P. G., Owen, A. G., and Hees, P. A. (2003). Organic acid behaviour in soils – misconceptions and knowledge gaps. *Plant and Soil, 248*, 31-41.

Jones, M. D., Durall, D. M., and Tinker, P. B. (1998). A comparison of arbuscular and ectomycorrhizal Eucalyptus coccifera: growth response, phosphorus uptake efficiency and external hyphal production. *New Phytologist, 140*, 125-134.

Jones, M. D., Durrall, D. M., and Tinker, P. B. (1990). Phosphorus relationships and production of extramatrical hyphae by two types of willow ectomycorrhizas at different soil phosphorus levels. *New Phytologist, 115*, 259-267.

Jungk, A. (1996). Dynamics of nutrient movement and the soil-root interface. In Y. Waisel, A. Eshel and U. Kafkafi (Eds.), *Plants Roots: The Hidden Half* (pp. 581-605). New York: Marcel Dekker.

Kanerva, S., Kitunen, V., Loponen, J., and Smolander, A. (2008). Phenolic compounds and terpenes in soil organic horizon layers under silver birch, Norway spruce and Scots pine. *Biology and Fertility of Soils, 44*, 547-556.

Kanerva, S., and Smolander, A. (2007). Microbial activities in forest floor layers under silver birch, Norway Spruce and Scots pine. *Soil Biology and Biochemistry, 39*, 1459-1467.

Kavvadais, V.A., Alifragis, D., Tsiontsis, A., Brofas, G., and Stamatelos, G. 2001. Litterfall, litter accumulation and litter decomposition rates in four forest ecosystems in northern Greece. *Forest Ecology and Management 144*,113-127.

Klironomos, J. N. (2003). Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology, 84*, 2292-2301.

Koide, R. T., and Kabir, Z. (2000). Extraradical hyphae of the mycorrhizal fungus Glomus intraradices can hydrolyse organic phosphate. *New Phytologist, 148*, 511-517.

Kouno, K., Wu, J., and Brookes, P. C. (2002). Turnover of microbial biomass C and P in soil following the incorporation of glucose or ryegrass. *Soil Biology and Biochemistry, 34*, 617-622.

Kovar, J. L., and Claassen, N. (2005). Soil-root interactions and phosphorus nutrition of plants. In J. T. Sims and A. N. Sharpley (Eds.), *Phosphorus: Agriculture and the Environment* (pp. 379-415). Madison, Wisconsin: ASA/ CSSA/ SSSA.

Kuchenbuch, R., and Jungk, A. (1982). A method for determining concentration profiles at the soil-root interface by thin slicing rhizosphere soil. *Plant and Soil, 68*, 391-394.

Landis, F. C., and Fraser, L. H. (2007). A new model of carbon and phosphorus transfers in arbuscular mycorrhizas. *New Phytologist, 177*, 466-479.

Lajtha, K., and Harrison, A. F. (1995). Strategies of phosphorus acquisition and conservation by plant species and communities. In H. Teissen (Ed.), *Phosphorus in the Global Environment* (pp. 139-147) New York: John Wiley and Sons Ltd.

Lapeyrie, F. (1988). Oxalate synthesis from soil bicarbonate by the mycorrhizal fungus *Paxillus involutus*. *Plant and Soil, 110*, 3-8.

Lapeyrie, F., and Chilvers, G. A. (1985). An endo mycorrhiza-ectomycorrhiza succession associated with enhanced growth of *Eucalyptus dumosa* seedlings planted in a calcareous soil. *New Phytologist, 100*, 93-104.

Lapeyrie, F., Chilvers, G. A., and Bhem, C. A. (1987). Oxalic acid synthesis by the mycorrhizal fungus *Paxillus involutus* (Batsch. Ex. Rf.) Fr. *New Phytologist, 106*, 139-146.

Lapeyrie, F., Ranger, J., and Vairelles, D. (1991). Phosphatase-solubilizing activity of ectomycorrhizal fungi *in vitro*. *Canadian Journal of Botany, 69*, 342-346.

Lathwell, D. J., and Grove, T. L. (1986). Soil-plant relationships in the tropics. *Annual Review of Ecology and Systematics, 17*, 1-16.

Li, H., Smith, S. E., Holloway, R. E., Zhu, Y. G., and Smith, A. (2006). Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus fixing soil even in the absence of positive growth responses. *New Phytologist, 172*, 536-543.

Li, X., Ecckhard, G., and Marschner, H. (1991). Phosphorus depletion and pH decrease at the root-soil and hyphae-soil interfaces of VA mycorrhizal white clover fertilized with ammonium. *New Phytologist, 119*, 397-404.

Liu, Q., Loganathan, P., and Hedley, M. J. (2005). Influence of ectomycorrhizal hyphae on phosphate fractions and dissolution of phosphate rock in rhizosphere soils of *Pinus radiata*. *Journal of Plant Nutrition, 28*, 1525-1540.

Liu, Q., Loganathan, P., Hedley, M. J., and Grace, L. J. (2004a). *Effect of mycorrhizal inoculation on rhizosphere properties of Pinus radiata seedlings*. Paper presented at the Super Soil 2004.

Liu, Q., Loganathan, P., Hedley, M. J., and Skinner, M. F. (2004b). The mobilisation and fate of soil and rock phosphate in the rhizosphere of ectomycorrhizal *Pinus radiata* seedlings in Allophanic soil. *Plant and Soil, 264*, 219-229.

Lodge, D. J. (2000). Ecto- or arbuscular mycorrhizas - which are best? *New Phytologist, 146*, 353-354.

Lopez-Hernandez, D., Siehert, G., and Rodriguez, J V. 1986. Competitive adsorbtion of phosphate with malate and oxalate by tropical soils. *Soil Science Society of America Journal 50,* 1460-1462.

Luster, J., Gottlein, A., Nowack, B., and Sarret, G. (2009). Sampling, defining, characterising and modeling the rhizosphere - the soil science tool box. *Plant and Soil, 321*, 457-482.

Lynch, J. M. (1982). The rhizosphere. In R. G. Burns and S. J. H (Eds.), *Experimental Microbial Ecology* (pp. 395-411). Oxford: Blackwell Scientific Publications.

Macklon, A. E., Grayston, S. J., Shand, C. A., Sim, A., Sellars, S., and Ord, B. G. (1997). Uptake and transport of phosphorus by *Agrostis capillaris* seedlings from rapidly hydrolyzed organic sources extracted from ^{32}P labelled bacterial cultures. *Plant and Soil, 190*, 163-167.

Magid, J. (1993). Vegetation effects on phosphorus fractions in set-aside soils. *Plant and Soil, 149*, 111-119.

Magid, J., Teissen, H., and Condron, L. M. (1996). Dynamics of organic phosphorus in soils under natural and agricultural ecosystems. In A. Piccolo (Ed.), *Humic Substances in Terrestrial Ecosystems* (pp. 429-466) New York: Elsevier Science B.V.

Malajczuk, N., Molina, R., and Trappe, J. M. (1982). Ectomycorrhiza Formation in *Eucalyptus* I. Pure culture synthesis, host specificity and mycorrhiza compatibility with *Pinus radiata. New Phytologist, 91*, 467-482.

Malajczuk, N., Molina, R., and Trappe, J. M. (1984). Ectomycorrhiza formation in *Eucalyptus* II. The ultra structure of compatible and incompatible mycorrhizal fungi and associated roots. *New Phytologist, 96*, 43-53.

Manoharachary, C., and Mukerji, K. G. (2006). Rhizosphere biology - an overview. In K. G. Mukerji, C. Manoharachary and J. Singh (Eds.), *Microbial Activity in the Rhizosphere* (pp. 349). Berlin: Springer-Verlag.

Manske, G.G., Ortiz-Monasterio, J. I., Van Grinkel, M., Rajaram, S., Molina, E., and Vick, P L. 2000. Traits associated with improved P uptake efficiency in CIMMYT's semi dwarf spring bread wheat grown on an acid Andisol in Mexico. *Plant and Soil 221*,189-204.

Marschner, H. (1998). Role of root growth, arbuscular mycorrhiza and root exudates for the efficiency in nutrient acquisition. *Field Crops Research, 56*, 203-207.

Marschner, H., and Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil, 159*, 89-102.

Martin, F. M., Perotto, S., and Bonfante, P. (2001). Mycorrhizal fungi: A fungal community at the interface between soils and roots. In R. Pinton, Z. Varanini and P. Nannipieri (Eds.), *The Rhizosphere* (pp. 263-296). New York: Marcel Dekker.

McDowell, R. W., and Stewart, I. (2006). The phosphorus composition of contrasting soil in pastoral, native and forest management soils in Otago, New Zealand: sequential extraction and ^{31}P NMR. *Geoderma, 130*, 176-189.

McGill, W. B., Cannon, K. R., Robertson, J. A., and Cook, F. D. (1986). Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. *Canadian Journal of Soil Science, 66*, 1-19.

McGill, W. B., and Cole, C. V. (1981). Comparative aspects of cycling of organic C, N, S and P through soil organic matter. *Geoderma, 26*, 267-286.

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., and Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist, 115*, 495-501.

McKelvie, I. D. (2005). Separation, preconcentration and speciation of organic phosphorus in environmental samples. In B. L. Turner, E. Frossard and D. Baldwin (Eds.), *Organic Phosphorus in the Environment* (pp. 1-21). Wallingford: CAB international.

McRae, W. D., Buxton, F. P., Sibley, S., Garven, S., Gwynne, D. I., and Davies, R. W. (1988). A phosphate repressible acid phosphatase gene from *Aspergillus niger*: its cloning, sequencing and transcriptional analysis. *Gene, 71*, 339-348.

Merharg, A. A., and Killham, K. (1995). Loss of exudates from the roots of perennial ryegrass inoculated with a range of microorganisms. *Plant and Soil, 170*, 345-349.

Miller, S. S., Liu, J., Allan, D. L., Menzhuber, C. J., Fedorova, M., and Vance, C. P. (2001). Molecular control of acid phosphatase secretion into the rhizosphere of proteoid roots from phosphorus stressed white lupin. *Plant Physiology, 127*, 594-606.

Mimmo, T., Ghizzi, M., Marzadori, C., and Gessa, C. E. (2008). Organic extraction from rhizosphere soil: effect of field moist, dried and frozen samples. *Plant and Soil, 312*, 175-184.

Molina, R. 1981. Ectomycorrhizal specificity in the genus *Alnus. Canadian Journal of Botany 59*, 325-334.

Morgan, J. A. W., Bending, G. D., and White, P. J. (2005). Biological costs and benefits to plant-microbe interactions in the rhizosphere. *Journal of Experimental Botany, 56*, 1729-1739.

Murphy, J., and Riley, J. P. (1962). A modified single solution method for the determination of phosphatase in natural waters. *Analytica Chimica Acta, 27*, 31-36.

Myers, R. G., Thein, S. J., and Pierzynski, G. M. (1999). Using an ion sink to extract microbial phosphorus from soil. *Soil Science Society of America Journal, 63*, 1229-1237.

Nambier, S. E. (1990). Interplay between nutrients, water, root growth and productivity in young plantations. *Forest Ecology and Management, 30*, 213-232.

Nardi, S., Concheri, G., Pizzeghello, D., Sturaro, A., Rella, R., and Parvoli, G. (2000). Soil organic matter mobilization by root exudates. *Chemosphere, 41*, 653-658.

Newman, E. I. (1988). Mycorrhizal links between plants: their functioning and ecological relevance. *Advances in Ecological Research, 18*, 243-270.

Nygren, C. M., and Rosling, A. (2009). Localisation of phosphomonoesterase activity in ectomycorrhizal fungi grown on different phosphorus sources. *Mycorrhiza, 19*, 197-204.

Nyland, J. E., and Unestam, T. (1982). Structure and physiology of ectomycorrhizae I. The process of mycorrhiza formation in Norway Spruce in vitro. *New Phytologist, 91*, 65-79.

Oberson, A., and Joner, E. J. (2005). Microbial turnover of phosphorus in soil. In B. L. Turner, E. Frossard and D. Baldwin (Eds.), *Organic Phosphorus in the Environment* (pp. 133-164). Wallingford, Oxfordshire: CABI.

O' Grady, A. P., Worledge, D., and Battaglia, M. (2005). Temporal and spatial changes in fine root distributions in a young *Eucalyptus globulus* stand in southern Tasmania. *Forest Ecology and Management, 214*, 373-383.

Ouahmane, L., Hafidi, M., Thioulouse, J., Ducousso, M., Kisa, M., and Prin, Y. (2007). Improvement of *Cupressus atlantica* Gaussen growth by inoculation with native arbuscular mycorrhizal fungi. *Journal of Applied Microbiology, 103*, 683-690.

Pasqualini, S., Panara, F., and Antonielli, M. (1992). Acid phosphatase activity in *Pinus pinea* - *Tuber albidum* ectomycorrhizal association. *Canadian Journal of Botany, 70*, 1377-1383.

Perez-Moreno, J., and Read, D. J. (2000). Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. *New Phytologist, 145*, 301-309.

Perry, D. A., Molina, R., and Amaranthus, M. P. (1986). Mycorrhizae, mycorrhizospheres and reforestation: current knowledge and research needs. *Canadian Journal of Forest Research, 17*, 929-940.

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

Polglase, P., Attiwill, P., and Adams, M. (1992a). Nitrogen and phosphorus cycling in relation to stand age of *Eucalyptus regnans* F. Muell. *Plant and Soil, 142,* 177-185.

Polglase, P. J., Jokela, E. J., and Comerford, N. B. (1992b). Nitrogen and phosphorus release from decomposing needles of southern pine plantations. *Soil Science Society of America Journal, 56,* 914-920.

Pritchett, W. L., and Fisher, R. F. (1987). *Properties and management of forest soils* (second ed.) New York: John Wiley & Sons, Inc.

Pulleman, M., and Tietema, A. (1999). Microbial C and N transformations during drying and rewetting of coniferous forest floor material. *Soil Biology and Biochemistry, 31,* 275-285.

Quiquampoix, H., and Mousain, D. (2005). Enzymatic Hydrolysis of Organic Phosphorus. In B. L. Turner, E. Frossard and D. Baldwin (Eds.), *Organic Phosphorus in the Environment* (pp. 89-112). Wallingford, Oxfordshire: CABI.

Radersma, S., and Grierson, P. F. (2004). Phosphorus mobilization in agroforestry: Organic anions, phosphatase activity and phosphorus fractions in the rhizosphere. *Plant and Soil, 259,* 209-219.

Raghothama, K. G. (2005). Phosphorus and plant nutrition: an overview. In J. T. Sims and A. N. Sharpley (Eds.), *Phosphorus: Agriculture and the Environment*. (pp. 355-378) Madison, Wisconsin: ASA/CSSA/SSSA.

Raghothama, K. G., and Karthikeyan, A. S. (2005). Phosphate acquisition. *Plant and Soil, 274,* 37-49.

Renella, G., Landi, L., Valori, F., and Nannipieri, P. (2007). Microbial and hydrolase activity after release of low molecular weight organic compounds by a model root surface in a clayey and a sandy soil. *Applied Soil Ecology, 36,* 124-129.

Reyes, I., Valery, A., and Valduz, Z. (2006). Phosphate solubilizing microorganisms isolated from rhizospheric and bulk soils of colonizer plants at an abandoned rock phosphate mine. *Plant and Soil, 287,* 69-75.

Richardson, A. E. (2001). Prospects for utilizing soil microorganisms to improve the acquisition of phosphorus by plants. *Australian Journal of Plant Physiology, 28,* 897-906.

Richardson, A. E., George, T. S., Hens, M., and Simpson, R. J. (2005). Utilization of soil organic phosphorus by higher plants. In B. L. Turner, E. Frossard and D. Baldwin (Eds.), *Organic Phosphorus in the Environment* (pp. 165-184). Wallingford, Oxfordshire: CABI.

Richardson, A. E., George, T. S., Jakobsen, I., and Simpson, R. J. (2006). Plant utilization of inositol phosphates. In B. L. Turner, A. E. Richardson and E. J. Mullaney (Eds.), *Inositol Phosphates: Linking Agriculture and Environment* (pp. 242-260). Wallingford, UK: CAB International.

Richardson, A. E., Hadobas, P. A., Hayes, J. E., O'Hara, C. P., and Simpson, R. J. (2001). Utilization of phosphorus by pasture plants supplied with *myo*-inositol hexaphosphate is enhanced by the presence of soil micro-organisms. *Plant and Soil, 229*, 47-56.

Richardson, A. E., Hocking, P. J., Simpson, R. J., and George, T. S. (2009a). Plant mechanisms to optimise access to soil phosphorus. *Crop and Pasture Science, 60*, 124-143.

Richardson, A. E., José-Miguel, B., McNeill, A. M., and Prigent-Combaret, C. (2009b). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant and Soil, 321*, 305-339.

Richardson, S., Peltzer, D. A., Allen, R. B., McGlone, M. S., and Parfitt, R. L. (2004). Rapid Development of phosphorus limitation in temperate rainforest along the Franz Josef chronosequence. *Ecosystem Ecology, 139*, 267-276.

Rillig, M. C. (2004). Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology Letters, 7*, 740-754.

Rubaek, G. H., Guggenberger, G., Zech, W., and Christensen, B. T. (1999). Organic phosphorus in soil size separates characterized by phosphorus-31 nuclear magnetic resonance and resin extraction. *Soil Science Society of America Journal, 63*, 1123-1132.

Rubio, R., Moraga, E., and Borie, F. (1990). Acid phosphatase activity and vesicular-arbuscular mycorrhizal infection associated with roots of four wheat cultivars. *Journal of Plant Nutrition, 13*, 585-598.

Saggar, S., Parfitt, R. L., and Skinner, M. F. (1998). Carbon and phosphorus transformations during decomposition of pine forest floor with different phosphorus status. *Biology and Fertility of Soils, 27*, 197-204.

Saunders, W. M., and Williams, E. G. (1955). Observations on the determination of total phosphorus in soils. *Journal of Soil Science, 6*, 254-267.

Schachman, D. P., Reid, R. J., and Ayling, S. M. (1998). Phosphorus uptake by plants: from soil to cell. *Plant Physiology*, 116, 447-453.

Schefe, C. R., Watt, M., Slattery, W. J., and Mele, P. M. (2008). Organic anions in the rhizosphere of Al-tolerant and Al-sensitive wheat lines grown in an acid soil in controlled and field environments. *Australian Journal of Soil Research, 46*, 257-264.

Scott, J. T. (2002). *Soil phosphorus dynamics in a temperate silvopastoral system.* Unpublished doctoral thesis, Lincoln University, New Zealand.

Scott, J. T., and Condron, L. M. (2003). Dynamics and availability of phosphorus in the rhizosphere of a temperate silvopastoral system. *Biology and Fertility of Soils, 39*, 65-73.

Scott, J. T., and Condron, L. M. (2004). Short term effects of radiata pine and selected pasture species on soil organic phosphorus mineralisation. *Plant and Soil, 266*, 153-163.

Seeling, B., and Zasoski, R. J. (1993). Microbial effects in maintaining organic and inorganic solution phosphorus concentrations in a grassland topsoil. *Plant and Soil, 148*, 277-284.

Singh, B. K., and Walker, A. (2006). Microbial degradation of organophosphorus compounds. *FEMS Microbiology Reviews, 30*, 428-471.

Sinsabaugh, R. L. (1994). Enzymatic analysis of microbial pattern and process. *Biology and Fertility of Soils, 17*, 69-74.

Smith, S. E., and Read, D. (1997). *Mycorrhizal Symbiosis*. San Diego, California: Elsevier Science Ltd.

Smith, W. H. (1990). The atmosphere and the rhizosphere: linkages with potential significance for forest tree health. In A. A. Lucier and S. G. Haines (Eds.), *Mechanisms of Forest Response to Acidic Deposition* (pp. 188-242). New York: Springer-Verlag.

Solaiman, M. Z., and Abbott, L. K. (2003). Phosphorus uptake by a community of arbuscular mycorrhizal fungi in jarrah forest. *Plant and Soil, 248*, 313-320.

Sparling, G. P., Hart, P. B., August, J. A., and Leslie, D. M. (1994). A comparison of soil and microbial carbon, notrogen and phosphorus contents and macro-aggregate stability of a soil under native forest and after clearance for pastures and plantation forest. *Biology and Fertility of Soils, 17*, 91-100.

Speir, T. W., and Ross, D. J. (1978). Soil phosphatase and sulphatase. In R. G. Burns (Ed.), *Soil Enzymes* (pp. 197-250). London: Academic Press.

St John, T. V., Coleman, D. C., and Reid, C. P. (1983). Growth and Spatial distribution of nutrient absorbing organs: selective exploitation of soil heterogeneity. *Plant and Soil, 71*, 487-493.

Stevenson, B. A., Sparling, G. P., Schipper, L. A., Degens, B. P., and Duncan, L. C. (2004). Pasture and forest soil microbial communities show distinct patterns in their catabolic respiration responses at a landscape scale. *Soil Biology and Biochemistry, 36*, 49-55.

Stewart, J. W., and Tiessen, H. (1987). Dynamics of soil organic phosphorus. *Biogeochemistry, 4*, 41-60.

Ström, L., Godbold, D. L., Owen, A. G., and Jones, D. L. (2001). Organic acid behaviour in a calcareous soil: sorption reactions and biodegradation rates. *Soil Biology and Biochemistry, 33*, 2125-2133.

Ström, L., Godbold, D. L., Owen, A. G., and Jones, D. L. (2002). Organic acid mediated P mobilization in the rhizosphere and uptake by maize roots. *Soil Biology and Biochemistry, 34*, 703-710.

Sylvia, D. M. (1994). Vesicular-Arbuscular mycorrhizal fungi. In R. W. Weaver, S. Angle, P. Bottomley, D. Bezdicek, S. Smith, M. A. Tabatabai and A. Wollum (Eds.), *Methods of soil Analysis, part 2. Microbiological and Biochemical Properties* (Vol. 5, pp. 351-378). Wisconsin: Soil Science Society of America.

Tabatabai, M. A. (1994). Soil Enzymes. In *Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties* (pp. 775-833). Madison: soil science Society of America.

Tabatabai, M. A., and Bremner, J. M. (1969). Use of *P*-nitrophenyl phosphate for assay of phosphatase activity. *Soil Biology and Biochemistry, 1*, 307-309.

Tadano, T., and Sakai, H. (1991). Secretion of acid phosphatase by the roots of several crop species under phosphorus deficient conditions. *Soil Science and Plant Nutrition, 37*, 129-140.

Tang, J., Leung, A., Leung, C., and Lim, B. L. (2006). Hydrolysis of precipitated phytate by three distinct families of phytases. *Soil Biology and Biochemistry, 38*, 1316-1324.

Tarafdar, J. C., and Claassen, N. (1988). Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. *Biology and Fertility of Soils, 5*, 308-312.

Tarafdar, J. C., and Jungk, A. (1987). Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. *Biology and Fertility of Soils, 3*, 199-204.

Tarafdar, J. C., and Marschner, H. (1994a). Efficiency of VAM hyphae in utilization of organic phosphorus by wheat plants. *Soil Science and Plant Nutrition, 40*, 593-600.

Tarafdar, J. C., and Marschner, H. (1994b). Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biology and Biochemistry, 26*, 387-395.

Tiessen, H. (2005). Phosphorus dynamics in tropical trees. In J. T. Sims and A. N. Sharpley (Eds.), *Phosphorus: Agriculture and the Environment*. (pp. 253-262) Madison, Wisconsin: ASA/ CSSA/ SSSA.

Tiessen, H., and Moir, J. O. (1993). Characterization of available P by sequential extraction. In M. R. Carter (Ed.), *Soil Sampling and Methods of Analysis* (pp. 75-87). Boca Raton, Florida: Lewis Publishers.

Tiessen, H., Weisbach, C., and Menezes, R. S. C. (1999). Estimating the contribution of trees to available phosphorus. *Agroforestry Forum, 9*, 57-60.

Timonen, S., and Marschner, P. (2006). Mycorrhizosphere concept. In K. G. Mukerji, C. Manoharachary and J. Singh (Eds.), *Microbial Activity in the Rhizosphere* (Vol. 7, pp. 155-173). New York: Springer-Verlag.

Tinker, P. B. (1980). The role of rhizosphere microorganisms in mediating phosphorus uptake by plants . In F. E. Kwasenah (Ed.), *The Role of Phosphorus in Agriculture* (pp. 617-654). Madison, Wisconsin: ASA. CSSA and SSSA.

Thein, S. J., and Myers, R. G. (1992). Determination of bioavailable phosphorus in soil. *Soil Science Society of America Journal, 56*, 814-818.

Toro, M., Azcon, R., and Barea, J.-M. (1997). Improvement of arbuscular mycorrhizal development by inoculation with phosphate solubilizing rhizobacteria to improve rock phosphate bioavailability (^{32}P) and nutrient cycling. *Applied Environmental Microbiology, 63*, 4408-4412.

Treseder, K. K., and Cross, A. (2006). Global Distributions of Arbuscular Mycorrhizal Fungi. *Ecosystems, 9*, 305-316.

Trolove, S. N., Hedley, M. J., Kirk, G. J., Bolan, N. S., and Loganathan, P. (2003). Progress in selected areas of rhizosphere research on P aquisition. *Australian Journal of Soil Research, 41*, 471-499.

Turner, B. L. (2008). Resource partitioning for soil phosphorus: a hypothesis. *Journal of Ecology, 96*, 698-702.

Turner, B. L., Cade-Menun, B. J., Condron, L. M., and Newman, S. (2005a). Extraction of soil organic phosphorus. *Talanta, 66*, 294-306.

Turner, B. L., Condron, L. M., Richardson, S. J., Peltzer, D. A., and Allison, V. J. (2007). Soil Organic phosphorus transformations during pedogenesis. *Ecosystems, 10*, 1166-1181.

Turner, B. L., and Haygarth, P. M. (2005). Phosphatase activity in temperate pasture soils: Potential regulation of labile organic phosphorus turnover by phosphodiesterase activity. *Science of the Total Environment, 344*, 27-36.

Turner, B. L., Mahieu, N., and Condron, L. M. (2003a). Phosphorus-31 Nuclear Magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. *Soil Science Society of America Journal, 67*, 497-510.

Turner, B. L., Mahieu, N., and Condron, L. M. (2003b). The phosphorus composition of temperate pasture soils determined by NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy. *Organic Geochemistry, 34*, 1199-1210.

Turner, B. L., Mahieu, N., Condron, L. M., and Chen, C. R. (2005b). Quantification and bioavailability of *scyllo*-inositol hexa*kis*phosphate in pasture soils. *Soil Biology and Biochemistry, 37*, 2155-2158.

Turner, B. L., Paphazy, M. J., Haygarth, P. M., and McKelvie, I. D. (2002). Inositol phosphates in the environment. *Philosophical Transactions of the Royal Society of London, series B, 357*, 449-469.

Turner, J., and Lambart, M. J. (1985). Soil phosphorus forms and related tree growth in a long term *Pinus radiata* phosphate fertilizer trial. *Communications in Soil Science and Plant Analysis, 16*, 275-288.

van Aarle, I. M., Olsson, P. A., and Soderstrom, B. (2001). Microscopic Detection of phosphatase activity of saprophytic and arbuscular mycorrhizal fungi using a flourogenic subtrate. *Mycologia, 93*, 17-24.

Vance, C. P., Uhde-stone, C., and Allan, D. L. (2003). Phosphorus acquisition and use: critical adaptations for securing a non renewable resource. *New Phytologist, 157*, 423-447.

Vasquez, M. M., Cesar, S., Azcon, R., and Barea, J.-M. (2000). Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum, Psreudomonas*, Trichoderma) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. *Applied Soil Ecology, 15*, 261-272.

Vitousek, P. M. (1984). Litterfall, nutrient cycling and nutrient limitation in tropical forests. *Ecology, 65*, 285-298.

Vogt, K. A., Edmonds, R. L., and Grier, G. C. (1981). Seasonal changes in biomass and vertical distribution of mycorrhizal and fibrous-textured conifer fine roots in 23 and 180 year old subalpine *Abies amabilis* stands. *Canadian Journal of Forest Research, 11*, 223-229.

Walbert, K. (2008). *Ectomycorrhizal communities associated with a* Pinus radiata *plantation in the North Island, New Zealand.* Unpublished doctoral thesis. Lincoln University, New Zealand.

Walbert, K., Ramsfield, T. D., Ridgway, H. J., and Jones, E. E. (2010). Ectomycorrhizal species associated with *Pinus radiata* in New Zealand including novel associations determined by molecular analysis. *Mycorrhiza, 20,* 209-215.

Walker, T. S., Pal Bais, H., Grotewold, E., and Vivanco, J. M. (2003). Root exudation and rhizosphere biology. *Plant Physiology, 132*, 44-51.

Walker, W., and Syers, J. K. (1976). The fate of phosphorus during pedogenesis. *Geoderma, 15*, 1-19.

Warner, A. (1984). Colonization of organic matter by vesicular-arbuscular mycorrhizal fungi. *Transactions of the British Mycological Society, 80*, 352-354.

Watt, M., Silk, W. K., and Passioura, J. B. (2006). Rates of Root and Organism Growth, Soil Conditions, and Temporal and Spatial Development of the Rhizosphere. *Annals of Botany, 97*, 839-855.

Wulfsohn, D., Nyengaard, J. R., Gunderson, H. J., Cutler, A. J., and Squires, T. M. (1999). Non-destructive, steriological estimation of plant root lengths, branching pattern and diameter distribution. *Plant and Soil*, *214*, 15-16.

Yanai, R. D., Majdi, H., and Park, B. B. (2003). Measured and modelled differences in nutrient concentrations between rhizosphere and bulk soil in a Norway spruce stand. *Plant and Soil, 257*, 133-142.

Yeates, G. W., and Saggar, S. (1998). Comparison of Soil microbial properties and fauna under tussock-grassland and pine plantation. *Journal of the Royal Society of New Zealand, 28*, 523-535.

Yeates, G. W., Saggar, S., and Daly, B. K. (1997). Soil microbial C, N, and P, and microfaunal populations under *Pinus radiata* and grazed pasture land-use systems. *Pedobiologia, 41*, 549-565.

Zoysa, A. K., Loganathan, P., and Hedley, M. J. (1997). A technique for studying rhizosphere processes in tree crops: soil phosphorus depletion around camellia (*Camellia japonica* L.) roots. *Plant and Soil, 190*, 253-265.

Zoysa, A. K., Loganathan, P., and Hedley, M. J. (1998). Effects of forms of nitrogen supply on mobilization of phosphorus from a phosphatase rock and acidification in the rhizosphere of tea. *Australian Journal of Soil Research, 39*, 373-387.

Appendix

Appendix 2.1 Statistical Analysis Tables of Root Sleeve Data

Significantly influenced parameters are in bold.

pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**3**	**0.5200594**	**0.1733531**	**704.74**	**<.001**
Residual	28	0.0068875	0.0002460		
Total	31	0.5269469			

Total P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	3	4854.5	1618.2	2.79	0.059
Residual	28	16252.6	580.4		
Total	31	21107.1			

Organic P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	3	2313.3	771.1	1.36	0.276
Residual	28	15896.7	567.7		
Total	31	18210.0			

Acid PME

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**3**	**128980.0**	**42993.3**	**123.25**	**<.001**
Residual	28	9767.5	348.8		
Total	31	138747.6			

Alkaline PME

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**3**	**889.02**	**296.34**	**18.72**	**<.001**
Residual	28	443.26	15.83		
Total	31	1332.29			

Appendix 2.2 Statistical Analysis Tables of Root Sampling Experiment Data

A2.2.1 Effects of, and interactions between, species, distance and depth on fine root biomass measured in the root sampling experiment. Most important effects are in bold.

Fine root Biomass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	2	31.88347	15.94174	215.80	<.001
Depth	1	106.95007	106.95007	1447.77	<.001
Distance	**1**	**0.36602**	**0.36602**	**4.95**	**0.036**
Species.Depth	**2**	**27.93514**	**13.96757**	**189.08**	**<.001**
Species.Distance	2	0.18472	0.09236	1.25	0.304
Depth, Distance	1	0.00514	0.00514	0.07	0.794
Species.Depth.Distance	2	0.19861	0.09930	1.34	0.280
Residual	24	1.77293	0.07387		
Total	35	169.29610			

A2.2.2 Effects of, and interactions between, species, soil type and soil depth on all parameters measured in the root sampling experiment. Most important interactions are in bold.

Total P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Depth	1	209141.	209141.	75.95	<.001
Soil type	1	5448.	5448.	1.98	0.165
Species	2	237612.	118806.	43.14	<.001
Depth.Soil type	1	10558.	10558.	3.83	0.055
Depth.Species	**2**	**59029.**	**29515.**	**10.72**	**<.001**
Soil Type.Species	2	1847.	924.	0.34	0.716
Depth.Soil type.Species	2	3925.	1962.	0.71	0.494
Residual	60	165225.	2754.		
Total	71	692785.			

Organic P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Depth	**1**	**19691.**	**19691.**	**8.60**	**0.005**
Soil type	1	5461.	5461.	2.39	0.128
Species	2	11939.	5969.	2.61	0.082
Depth.Soil type	1	4688.	4688.	2.05	0.158
Depth.Species	2	314.	157.	0.07	0.934
Soil type.Species	2	5254.	2627.	1.15	0.324
Depth.Soil type.Species	2	1132.	566.	0.25	0.782
Residual	60	137317.	2289.		
Total	71	185795.			

Resin P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Depth	1	55.864	55.864	39.85	<.001
Soil type	1	131.035	131.035	93.46	<.001
Species	**2**	**95.779**	**47.890**	**34.16**	**<.001**
Depth.Soil type	**1**	**25.787**	**25.787**	**18.39**	**<.001**
Depth.Species	2	3.577	1.789	1.28	0.287
Soil type.Species	2	5.305	2.653	1.89	0.160
Depth.Soil type.Species	2	0.590	0.295	0.21	0.811
Residual	60	84.120	1.402		
Total	71	402.058			

Microbial Activity

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Depth	1		4.5895	4.5895	39.31	<.001
Soil type	1		19.5500	19.5500	167.44	<.001
Species	2		15.7462	7.8731	67.43	<.001
Depth.Soil type	**1**		**3.8984**	**3.8984**	**33.39**	**<.001**
Depth.Species	**2**		**1.1204**	**0.5602**	**4.80**	**0.028**
Soil type.Species	2		0.7694	0.3847	3.29	0.070
Depth.Soil type.Species	2		0.4826	0.2413	2.07	0.166
Residual	13	(47)	1.5179	0.1168		
Total	24	(47)	16.9209			

Acid PME

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Depth	1	44662.2	44662.2	55.97	<.001
Soil type	1	192450.5	192450.5	241.17	<.001
Species	2	5328.8	2664.4	3.34	0.042
Depth.Soil type	**1**	**34461.8**	**34461.8**	**43.19**	**<.001**
Depth.Species	**2**	**7710.0**	**3855.0**	**4.83**	**0.011**
Soil type.Species	2	4015.7	2007.9	2.52	0.089
Depth.Soil type.Species	2	878.0	439.0	0.55	0.580
Residual	60	47878.4	798.0		
Total	71	337385.6			

PDE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Depth	**1**	**23.5786**	**23.5786**	**56.05**	**<.001**
Soil type	**1**	**63.5855**	**63.5855**	**151.15**	**<.001**
Species	**2**	**3.8559**	**1.9280**	**4.58**	**0.014**
Depth.Soil type	1	0.1855	0.1855	0.44	0.509
Depth.Species	2	0.4458	0.2229	0.53	0.591
Soil type.Species	2	0.4305	0.2152	0.51	0.602
Depth.Soil type.Species	2	0.5403	0.2701	0.64	0.530
Residual	60	25.2415	0.4207		
Total	71	117.8635			

Mean values of all variables determined in each compartment of radiata pine, eucalypt and macrocarpa rhizosphere in the root study container preliminary experiment. Values in parenthesis are standard errors of mean, n = 6.

Soil Property	Pine			Eucalyptus			Macrocarpa		
	RHM	*HM*	*M*	*RHM*	*HM*	*M*	*RHM*	*HM*	*M*
pH	5.7 (0.03)	5.9 (0.08)	6.0 (0.06)	5.1 (0.02)	5.6 (0.05)	5.8 (0.06)	5.2 (0.01)	5.5 (0.06)	5.8 (0.09)
Total P (mg/kg)	1147 (11.5)	1134 (5.5)	1210 (20.3)	1043 (7.7)	1064 (19.9)	1179 (21.7)	1076 (0.7)	1092 (7.1)	1361 (55.5)
Organic P (mg/kg)	446 (4.5)	455 (1.4)	463 (4.0)	476 (5.8)	491 (1.8)	465 (2.9)	458 (5.0)	460 (2.5)	436 (5.8)
Resin P (mg/kg)	13.9 (1.2)	19.8 (1.4)	36.3 (3.3)	3.1 (0.5)	20.6 (1.5)	18.4 (3.0)	8.2 (0.5)	21.7 (2.6)	29.4 (1.5)
Microbial biomass P (mg/kg)	20.8 (2.9)	35.1 (4.3)	26.1 (4.4)	21.6 (0.7)	50.7 (5.6)	36.0 (4.2)	15.9 (0.7)	28.0 (3.3)	22.6 (1.8)
Microbial : Resin P	2.6 (0.3)	2.9 (0.4)	1.8 (0.3)	9.2 (1.5)	4.0 (0.7)	3.1 (0.4)	3.0 (0.2)	2.5 (0.3)	1.8 (0.1)
Microbial Activity (gDHE/g/hr)	5.5 (0.3)	4.3 (0.3)	4.1 (0.1)	6.1 (0.2)	4.3 (0.1)	5.0 (0.1)	5.3 (0.1)	4.1 (0.2)	3.9 (0.1)
Acid PME (µmol/g/hr)	402 (8.5)	334 (12.3)	344 (17.4)	412 (14.2)	391 (16.9)	395 (25.6)	376 (22.3)	332 (10.6)	346 (6.0)
Alkaline PME (µmol/g/hr)	111 (4.8)	108 (5.1)	107 (7.2)	167 (6.2)	123 (7.6)	139 (7.4)	139 (1.8)	130 (7.8)	130 (7.2)

PME = phosphomonoesterase; DHE = dehydrogenase activity

Appendix 3.2 Statistical Analysis Tables for Root Compartment Study Data

A3.2.1 Effects of, and interactions between, species and compartment on all parameters of the root compartment study. Most important interactions are in bold.

pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	2	0.832626	0.416313	97.73	<.001
Species	2	0.160959	0.080480	18.89	<.001
Compartment.Species	**4**	**0.054041**	**0.013510**	**3.17**	**0.022**
Residual	45	0.191700	0.004260		
Total	53	1.239326			

Total P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	2	2036.1	1018.1	8.81	<.001
Species	2	7120.0	3560.0	30.81	<.001
Compartment.Species	**4**	**4860.5**	**1215.1**	**10.52**	**<.001**
Residual	45	5200.3	115.6		
Total	53	19216.9			

Organic P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	2	4302.28	2151.14	36.58	<.001
Species	2	5383.71	2691.86	45.78	<.001
Compartment.Species	**4**	**4303.62**	**1075.90**	**18.30**	**<.001**
Residual	45	2646.04	58.80		
Total	53	16635.65			

Resin P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	2	142.1963	71.0982	93.02	<.001
Species	2	69.0811	34.5406	45.19	<.001
Compartment.Species	**4**	**16.8350**	**4.2087**	**5.51**	**0.001**
Residual	45	34.3934	0.7643		
Total	53	262.5059			

Microbial biomass P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**97.628**	**48.814**	**6.06**	**0.005**
Species	**2**	**406.164**	**203.082**	**25.21**	**<.001**
Compartment.Species	4	63.575	15.894	1.97	0.115
Residual	45	362.459	8.055		
Total	53	929.825			

Microbial activity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	2	20.6020	10.3010	85.24	<.001
Species	2	2.8840	1.4420	11.93	<.001
Compartment.Species	**4**	**6.7359**	**1.6840**	**13.94**	**<.001**
Residual	45	5.4379	0.1208		
Total	53	35.6598			

Acid PME

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	2	7441.7	3720.8	25.47	<.001
Species	2	67975.4	33987.7	232.66	<.001
Compartment.Species	**4**	**4359.2**	**1089.8**	**7.46**	**<.001**
Residual	45	6573.8	146.1		
Total	53	86350.1			

Alkaline PME

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	2	2282.6	1141.3	5.95	0.005
Species	2	23000.5	11500.2	59.91	<.001
Compartment.Species	**4**	**7893.8**	**1973.4**	**10.28**	**<.001**
Residual	45	8638.8	192.0		
Total	53	41815.7			

PDE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	2	19.1146	9.5573	10.08	<.001
Species	2	44.4550	22.2275	23.45	<.001
Compartment.Species	**4**	**37.9789**	**9.4947**	**10.02**	**<.001**
Residual	45	42.6538	0.9479		
Total	53	144.2023			

A3.2.2 Effects of compartment on all parameters under radiata pine measured in the root compartment study. Significant effects are in bold.

pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**0.129478**	**0.064739**	**24.03**	**<.001**
Residual	15	0.040417	0.002694		
Total	17	0.169894			

Total P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**6033.2**	**3016.6**	**28.52**	**<.001**
Residual	15	1586.4	105.8		
Total	17	7619.6			

Organic P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**7845.67**	**3922.84**	**77.55**	**<.001**
Residual	15	758.74	50.58		
Total	17	8604.42			

Resin P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**59.5273**	**29.7637**	**59.98**	**<.001**
Residual	15	7.4428	0.4962		
Total	17	66.9701			

Microbial Biomass P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	2	9.877	4.939	0.51	0.612
Residual	15	145.744	9.716		
Total	17	155.621			

Microbial Activity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**2.87804**	**1.43902**	**22.34**	**<.001**
Residual	15	0.96630	0.06442		
Total	17	3.84434			

Acid PME

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**8808.0**	**4404.0**	**40.40**	**<.001**
Residual	15	1635.3	109.0		
Total	17	10443.4			

Alkaline PME

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**1809.5**	**904.8**	**6.49**	**0.009**
Residual	15	2089.8	139.3		
Total	17	3899.3			

PDE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**9.1035**	**4.5518**	**5.20**	**0.019**
Residual	15	13.1350	0.8757		
Total	17	22.2385			

A3.2.3 Effects of compartment on all parameters under eucalypt measured in the root compartment study. Significant effects are in bold.

pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**0.414978**	**0.207489**	**36.34**	**<.001**
Residual	15	0.085650	0.005710		
Total	17	0.500628			

Total P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**347.70**	**173.85**	**4.08**	**0.039**
Residual	15	639.44	42.63		
Total	17	987.14			

Organic P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**636.93**	**318.46**	**6.06**	**0.012**
Residual	15	788.03	52.54		
Total	17	1424.96			

Resin P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**83.906**	**41.953**	**32.41**	**<.001**
Residual	15	19.417	1.294		
Total	17	103.322			

Microbial biomass P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**94.237**	**47.118**	**9.25**	**0.002**
Residual	15	76.416	5.094		
Total	17	170.653			

Microbial Activity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**2.5076**	**1.2538**	**7.57**	**0.005**
Residual	15	2.4841	0.1656		
Total	17	4.9917			

Acid PME

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	2	36.9	18.4	0.11	0.898
Residual	15	2565.6	171.0		
Total	17	2602.5			

Alkaline PME

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**6034.4**	**3017.2**	**20.84**	**<.001**
Residual	15	2171.6	144.8		
Total	17	8206.0			

PDE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**4.40256**	**2.20128**	**26.34**	**<.001**
Residual	15	1.25341	0.08356		
Total	17	5.65597			

A3.2.4 Effects of compartment on all parameters under macrocarpa measured in the root compartment study. Significant effects are in bold.

pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**0.342211**	**0.171106**	**39.10**	**<.001**
Residual	15	0.065633	0.004376		
Total	17	0.407844			

Total P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	2	515.7	257.9	1.30	0.301
Residual	15	2974.4	198.3		
Total	17	3490.2			

Organic P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	2	123.29	61.65	0.84	0.451
Residual	15	1099.27	73.28		
Total	17	1222.56			

Resin P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**15.5985**	**7.7992**	**15.53**	**<.001**
Residual	15	7.5337	0.5022		
Total	17	23.1322			

Microbial biomass P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	2	57.088	28.544	3.05	0.077
Residual	15	140.298	9.353		
Total	17	197.387			

Microbial Activity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**21.9522**	**10.9761**	**82.84**	**<.001**
Residual	15	1.9875	0.1325		
Total	17	23.9398			

Acid PME

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**2956.0**	**1478.0**	**9.34**	**0.002**
Residual	15	2372.9	158.2		
Total	17	5328.9			

Alkaline PME

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**2332.5**	**1166.2**	**4.00**	**0.041**
Residual	15	4377.4	291.8		
Total	17	6709.9			

PDE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Compartment	**2**	**43.587**	**21.794**	**11.57**	**<.001**
Residual	15	28.265	1.884		
Total	17	71.853			

Appendix 4.1 Preliminary Spore Count at Orton Bradley Park

Average number of spores/g of soil determined in macrocarpa plot 12 at Orton Bradley Park

Overall	50cm from tree	80cm from tree	0-10 cm depth	10-15 cm depth
0.22	0.25	0.19	0.27	0.18

Appendix 4.2 Statistical Analysis Tables for Mycorrhizal Inoculation Experiment

A4.2.1 Effects of and interactions between, species and treatment on plant growth and P content parameters measured in the mycorrhizal inoculation study. Important effects are in bold.

Root fresh weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**9.4226**	**4.7113**	**14.28**	**<.001**
Treatment	2	0.2995	0.1498	0.45	0.639
Species.Treatment	4	1.8210	0.4553	1.38	0.260
Residual	36	11.8753	0.3299		
Total	44	23.4185			

Root length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**18892805.**	**9446402.**	**214.17**	**<.001**
Treatment	2	25686.	12843.	0.29	0.749
Species.Treatment	4	367661.	91915.	2.08	0.103
Residual	36	1587863.	44107.		
Total	44	20874015.			

Seedling fresh weight (above ground)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**15.3934**	**7.6967**	**11.45**	**<.001**
Treatment	2	0.1262	0.0631	0.09	0.911
Species.Treatment	4	2.6170	0.6543	0.97	0.434
Residual	36	24.1957	0.6721		
Total	44	42.3323			

Seedling height (above ground)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**246.08**	**123.04**	**7.72**	**0.002**
Treatment	2	3.61	1.81	0.11	0.893
Species.Treatment	4	40.29	10.07	0.63	0.643
Residual	36	573.50	15.93		
Total	44	863.48			

Plant P content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**18.6667**	**9.3334**	**30.41**	**<.001**
Treatment	2	0.4562	0.2281	0.74	0.483
Species.Treatment	4	0.3945	0.0986	0.32	0.862
Residual	36	11.0507	0.3070		
Total	44	30.5682			

A4.2.2 Effects of and interactions between, species and treatment on phosphatase enzymes measured in the mycorrhizal inoculation study. Important effects are in bold.

Acid PME

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	2	224.867	112.434	66.66	<.001
Treatment	2	215.912	107.956	64.00	<.001
Species.Treatment	**4**	**17.984**	**4.496**	**2.67**	**0.048**
Residual	36	60.724	1.687		
Total	44	519.488			

Alkaline PME

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**0.15296**	**0.07648**	**7.42**	**0.002**
Treatment	2	0.02470	0.01235	1.20	0.314
Species.Treatment	4	0.03555	0.00889	0.86	0.496
Residual	36	0.37115	0.01031		
Total	44	0.58435			

PDE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**0.0097472**	**0.0048736**	**12.05**	**<.001**
Treatment	**2**	**0.0101355**	**0.0050677**	**12.53**	**<.001**
Species.Treatment	4	0.0029866	0.0007466	1.85	0.141
Residual	36	0.0145556	0.0004043		
Total	44	0.0374249			

Appendix 5.1 Statistical Analysis Tables for Exhaustive Pot Trial data

A5.1.1 Effects of, and interactions between, species and soil type on plant biomass measured in the exhaustive pot trial. Important effects are in bold.

Original tree weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**10.7648**	**5.3824**	**10.15**	**0.009**
Residual	7	3.7116	0.5302		
Total	9	14.4764	1.6085		

Root weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**3321.7**	**1660.8**	**14.78**	**0.002**
Residual	8	898.8	112.4		
Total	10	4220.5	422.0		

Shoot weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**9811.5**	**4905.8**	**31.41**	**<.001**
Residual	8	1249.3	156.2		
Total	10	11060.8	1106.1		

A5.1.2 Effects of, and interactions between, species and soil type on plant P content and plant P concentrations measured in the exhaustive pot trial. Important effects are in bold.

Original plant P content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**111.173**	**55.586**	**5.56**	**0.036**
Residual	7	69.986	9.998		
Total	9	181.158	20.129		

Root P content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**14563.7**	**7281.9**	**23.44**	**<.001**
Residual	8	2484.8	310.6		
Total	10	17048.5	1704.8		

Shoot P content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**39175.5**	**19587.8**	**38.87**	**<.001**
Residual	8	4031.7	504.0		
Total	10	43207.3	4320.7		

Original tree P concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**7.3943**	**3.6972**	**22.57**	**<.001**
Residual	7	1.1467	0.1638		
Total	9	8.5410	0.9490		

Root P concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**1.40742**	**0.70371**	**8.26**	**0.011**
Residual	8	0.68167	0.08521		
Total	10	2.08909	0.20891		

Shoot P concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**1.71159**	**0.85580**	**34.67**	**<.001**
Residual	8	0.19750	0.02469		
Total	10	1.90909	0.19091		

Total plant P concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**1.55555**	**0.77777**	**17.12**	**0.001**
Residual	8	0.36354	0.04544		
Total	10	1.91909	0.19191		

A5.1.3 Effects of, and interactions between, species and soil type on soil P concentrations measured in the exhaustive pot trial. Important effects are in bold.

Resin P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	2	9.159	4.580	3.10	0.101
Residual	8	11.811	1.476		
Total	10	20.970	2.097		

Total NaOH-EDTA extractable P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**9574.7**	**4787.3**	**17.08**	**0.001**
Residual	8	2242.2	280.3		
Total	10	11816.9	1181.7		

NaOH-EDTA extractable organic P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**20731.6**	**10365.8**	**30.52**	**<.001**
Residual	8	2717.1	339.6		
Total	10	23448.7	2344.9		

NaOH-EDTA extractable inorganic P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**2170.99**	**1085.49**	**56.84**	**<.001**
Residual	8	152.77	19.10		
Total	10	2323.76	232.38		

Appendix 5.2: Statistical Analysis Tables for Chronosequence Experiment Data

A5.2.1 Effects of, and interactions between, species and soil type on plant dry weight, total plant P content and plant P concentration measured in the organic P gradient pot trial. Important effects are in bold.

Plant dry Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	2	1586.46	793.23	66.88	<.001
Year	3	979.15	326.38	27.52	<.001
Species.Year	**6**	**300.04**	**50.01**	**4.22**	**0.005**
Residual	24	284.64	11.86		
Total	35	3150.28			

Plant P content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	**2**	**900.855**	**450.427**	**49.89**	**<.001**
Year	**3**	**93.408**	**31.136**	**3.45**	**0.032**
Species.Year	6	42.616	7.103	0.79	0.589
Residual	24	216.670	9.028		
Total	35	1253.549			

Plant P concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Species	2	0.15242	0.07621	2.10	0.145
Year	**3**	**8.41836**	**2.80612**	**77.14**	**<.001**
Species.Year	6	0.46698	0.07783	2.14	0.086
Residual	24	0.87300	0.03637		
Total	35	9.91076			

A5.2.2 Effects of, and interactions between, species and soil type on soil P concentrations measured in the organic P gradient pot trial. Important effects are in bold.

Total soil P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Soil type	2	155955.92	77977.96	3296.97	<.001
Species	2	2502.82	1251.41	52.91	<.001
Soil type.Species	**4**	**1349.14**	**337.29**	**14.26**	**<.001**
Residual	18	425.73	23.65		
Total	26	160233.60			

Organic P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Soil type	2	53447.15	26723.58	2039.36	<.001
Species	2	509.73	254.86	19.45	<.001
Soil type.Species	**4**	**399.43**	**99.86**	**7.62**	**<.001**
Residual	18	235.87	13.10		
Total	26	54592.17			

Inorganic P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Soil type	2	275412.33	137706.17	5673.92	<.001
Species	2	3854.54	1927.27	79.41	<.001
Soil type.Species	**4**	**1100.75**	**275.19**	**11.34**	**<.001**
Residual	18	436.86	24.27		
Total	26	280804.48			