RHIZOSPHERE PROCESSES INFLUENCING SOIL AND FERTILISER PHOSPHORUS AVAILABILITY TO *PINUS RADIATA*

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ABSTRACT

Production of *Pinus radiata* is a major contributor to New Zealand's economy and new plantings are a valuable carbon sink. Phosphorus (P) deficiency and high P fixing capacity of some volcanic ash soils (e.g. Allophanic Soil) may constrain radiata productivity. This thesis investigates the role of ectomycorrhizal (ECM) root processes in the acquisition of P by *P. radiata* from native soil and soil fertilised with two reactive phosphate rock (RPR) fertilisers.

The application of finely-divided RPRs to a P deficient Allophanic Soil significantly increased *P. radiata* seedling growth and P uptake in 10 month pot trials. RPR dissolution was high in this soil, and it was further enhanced by the radiata rhizosphere processes. The development and formation of ECM in radiata seedlings was stimulated by low rates of RPR application but was hindered in unfertilised soils and high rates of RPR application.

The *P. radiata* ECM roots induced acidification and increased oxalate concentration and phosphatase activities in the rhizosphere soil. These changes in rhizosphere biochemical properties were associated with enhanced solubilisation of fertiliser and soil inorganic P and increased mineralisation of organic P, leading to increased P bioavailability in the rhizosphere.

ECM inoculation of *P. radiata* roots with *Rhizopogen rubescens* and *Suillus luteus* stimulated production of phosphatase enzymes and oxalate and induced acidification in the rhizosphere. The extent of root-induced changes in the rhizosphere soils was associated with ECM hyphae length density.

A technique using pulse labelling of radiata shoots with $^{14}CO_2$ showed promise in estimating the active ECM hyphae density. The ^{14}C activity was highly correlated with ECM hyphae density measured by an agar film technique.

Overall, observations made in this thesis indicate that sparingly soluble forms of organic and inorganic P in soils low in plant-available P are readily solubilised and utilised for *P. radiata* growth through ECM rhizosphere processes.

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General introduction

1.1 Background

Forestry is an important industry in New Zealand. For the year ended 30 June 2000, the forest sector earned NZ$3.1 billion in overseas trade (Ministry of Agricultural and Forestry, 2000). This was the third largest export earner after meat and dairy products. Newly planted commercial forests are also important to New Zealand for their value as major carbon sinks and their role in reducing global CO_2.

The soils supporting *Pinus radiata* plantations, which cover an area of 1.59 million ha in New Zealand and make up approximately 90% of New Zealand's total exotic forest estate (Ministry of Agriculture and Forestry, 2000), commonly contain lower plant-available phosphorus (P) compared to the pasture soils (New Zealand Soil Bureau, 1968; Gibson and Healy, 1982). The low plant-available soil P concentration in *P. radiata* plantations is mainly due to the absence of, or low rates of, fertiliser P use. The inherently low P status and high P sorption capacity of those soils formed on volcanic materials in most forest plantations of the North Island of New Zealand, due to the presence of large amounts of P-sorbing Fe and Al minerals and allophane in these soils, are other reasons for the low concentration of plant-available soil P. Although P deficiency has been recognised in many soils beneath *P. radiata* plantations (New Zealand Soil Bureau, 1968; Gibson and Healy, 1982; Will, 1985), unlike the growth of agricultural crops which is largely dependent on fertiliser-P utilisation, *P. radiata* generally grows well in these low P status soils (Hunter et al., 1991; Sparling et al., 1994; Liu et al., 2004). The reasons for the low fertiliser P requirement of *P. radiata* are not well understood.

Experimental evidence suggests that conifers including *P. radiata* are able to utilise soil and fertiliser P forms that are not considered readily available to most other plant

species, such as soil organic P (Davis and Lang, 1991; Davis, 1995; Condron et al., 1996) and insoluble inorganic P compounds (Wallander et al., 1997; Wallander, 2000). Because *P. radiata* is able to utilise P from insoluble inorganic P compounds in soil, reactive phosphate rock (RPR) is commonly recommended as the preferred P fertiliser in New Zealand *P. radiata* plantations (Payn et al., 1998). Studies (e.g. Chen et al., 2002) suggest that radiata rhizosphere processes influence the bioavailability of soil and fertiliser P. Nevertheless, several complex mechanisms involved in the uptake of P by radiata pine roots are still not clearly understood mainly due to the association of ectomycorrhizal fungi with radiata roots.

Evolution in plants has resulted in a range of morphological, biochemical and symbiotic adaptive strategies in plants whereby the acquisition of inorganic phosphate (P_i) and/or the efficiency of external utilisation of P_i are enhanced (Bolan, 1991; Smith et al., 2003; Trolove et al., 2003). *P. radiata* developed these adaptations in a remarkable way by the formation of P-efficient ectomycorrhizal roots. The mycorrhizal fungi associated with *P. radiata* are known to improve P uptake. They increase the absorptive surface of roots and solubilise insoluble-P and assist P transport from soil to tree roots when available P in the soil becomes limited (Bowen, 1968; Skinner and Bowen, 1974a; 1974b; Chu-Chou and Grace, 1985; Smith and Read, 1997). However, limited studies have been conducted on the influence of mycorrhizal association on changes in the chemical and biochemical properties of the rhizosphere and their effects on changes in soil P fractions and the availability to *P. radiata*, particularly under field conditions (Chen et al., 2002). This is an important area requiring further studies so that the nutritional requirements of large tracts of managed *P. radiata* in New Zealand can be better understood.

1.2 Objectives of the thesis

The research reported in this thesis attempts to increase the knowledge of rhizosphere processes influencing the soil P chemistry under *P. radiata*. The specific objectives of the research are to test the hypotheses listed below:

- The ectomycorrhizal roots can induce changes in soil chemical properties, particularly the P chemistry, and selected biochemical characteristics in the rhizosphere of *P. radiata*;
- The root processes of *P. radiata* are largely associated with ectomycorrhizal hyphae;
- The type of fungal species involved in ECM association with *P. radiata* influences the nature of biochemical change in the rhizosphere and seedling growth;
- Reactive phosphate rocks are agronomically effective for *P. radiata* in soils with high P fixing capacity and marginally acidic pH;
- $^{14}CO_2$ pulse-labelling technique can be used to quantify the active mycorrhizal hyphae distribution in soil.

CHAPTER 2

Rhizosphere processes influencing soil and fertiliser phosphorus availability to *Pinus radiata* – A literature review

2.1 Introduction

In the year 2000 it was estimated that more than 1.59 million hectares of *Pinus radiata* plantations (Ministry of Agriculture and Forestry, 2000) have been established in New Zealand on soils with a wide range of phosphorus (P) fertility levels (Gibson and Healy, 1982). Many of the plantations have grown 2 – 4 generations of trees, resulting in soil P deficiency. In these P deficient soils satisfactory growth of *P. radiata* relies partly on P fertiliser application (Will, 1985; Payn et al., 1998). The capacity of ectomycorrhizal roots of radiata pines to increase soil and fertiliser P bioavailability also plays an important role in maintaining sustainable growth of radiata forests (Bowen, 1984; Davis and Lang, 1991; Parfitt et al., 1994; Condron et al., 1996; Perrott et al., 1999; Chen et al., 2000; 2002). Amongst the factors influencing the availability of soil and fertiliser P, *P. radiata* rhizosphere processes are thought to be the most important.

The rhizosphere processes, occurring in agricultural crops, have been comprehensively reviewed (Hinsinger, 2001; Jones et al., 2003; Trolove et al., 2003). These processes are mediated mainly by root hairs, or endomycorrhizal associations. Several complex mechanisms involved in the uptake of nutrients, particularly P, by association of ectomycorrhizal fungi with coniferous roots are still not fully understood. This is an important area requiring further studies so that the nutritional requirements of large tracts of managed *P. radiata* in New Zealand can be better understood. This chapter

reviews the rhizosphere processes with particular attention paid to P acquisition by *P. radiata* with ectomycorrhizal association.

2.2 Phosphorus deficiency in soils supporting *P. radiata*

Phosphorus deficiency is acknowledged as a common nutrient disorder in New Zealand's forests, particularly in some *P. radiata* plantations located in Auckland, Northland, Coromandel, Nelson, and Westland areas (Will, 1985; Hunter et al., 1991; Payn et al., 1998). Owing to the genesis of most of these soils from volcanic ash, the soils are inherently low in P, and have high P sorption capacity due to the presence of large amounts of P-sorbing, non-crystalline Fe and Al minerals weathered from the parent materials (New Zealand Soil Bureau, 1968). In contrast to the low P status, these soils must supply 2 - 18 kg P ha^{-1} yr^{-1} to meet the P requirement for growth of *P. radiata* with varying ages and at different locations (Table 2.1). Webber and Madgwick (1983) reported that 29-year-old radiata pine takes up more than 66 kg P ha^{-1} during its growth (Figure 2.1) and at harvest more than 29 kg P ha^{-1} may be removed in tree stems (Payn et al., 1998). Therefore, in radiata plantations more than two rotations old, particularly those in the 3rd or 4th generation, the considerable amount of P removed by previous tree harvesting and the large P requirement of the trees places the current generation under some P stress. As a result, without fertiliser P application, P deficiency in these soils is expected to limit radiata productivity, especially in moderately weathered soils formed from volcanic ashes that have high allophane contents.

2.3 Phosphorus fertiliser requirement

Many studies have reported that *P. radiata* responds to the application of soluble fertiliser P (mainly as superphosphate) (Will, 1964; Ballard, 1972; Mead, 1974). Unlike soluble P fertilisers, however, few experimental data are currently available to demonstrate the effectiveness of phosphate rock on *P. radiata* productivity (Mead, 1974; Hunter and Graham, 1983). Payn et al. (1998) predicted that phosphate rock will be the form of P fertiliser that will be mostly used in the future (Table 2.2), because this source of P is considered to be able to provide the same growth response as superphosphate but

Table 2.1 Estimated annual P uptake by and return from *Pinus radiata* in New Zealand

Annual P uptake by trees (kg $ha^{-1}y^{-1}$)		
22 years-old (net uptake)[1]	1.5	Madgwick et al. (1977)
2 – 4 years-old (net uptake)[1]	7.9	Madgwick et al. (1977)
8 – 10 years-old (net uptake)[1]	7.4	Madgwick et al. (1977)
4 – 8 years-old (gross uptake)[1]	13.8	Madgwick et al. (1977)
7 – 8 years-old[2]	3 - 5	Parfitt et al. (1994)
11 years-old[3]	6 - 8	Parfitt et al. (1994)
12 years-old (overall uptake)[4]	54	Sparling et al. (1994)
P return by trees		
Returned at thinning at age 8 (% of total P returned to the forest floor)[1]	60	Madgwick et al. (1977)
Returned at thinning in relation to amounts returned by harvesting at age 22 (%)[1]	118	Madgwick et al. (1977)
Returned in previous harvest residues (kg P ha^{-1})	30	Madgwick (1994)
Annual plant turnover (kg P ha^{-1})[4]	5	Sparling et al. (1994)

[1]Stands located in Kaingaroa Forest; Pekepeke sandy or Matahina soil. [2]Stand located in Shenstone Forest. [3]Stand located in Te Kao Forest. [4]Stands located in Taita Experimental Station, Lower Hutt; Taita hill soil (Typic Dystrochrept).

Table 2.2 Past P fertiliser use in forestry and predictions of use in 2025 under three scenarios (Payn et al., 1998)

Year	1986	1997	2025 Deficiency correction	2025 Maintenance dressings	2025 Maximum production
P usage (tonnes P y^{-1})	1350	350	972	1598	3658

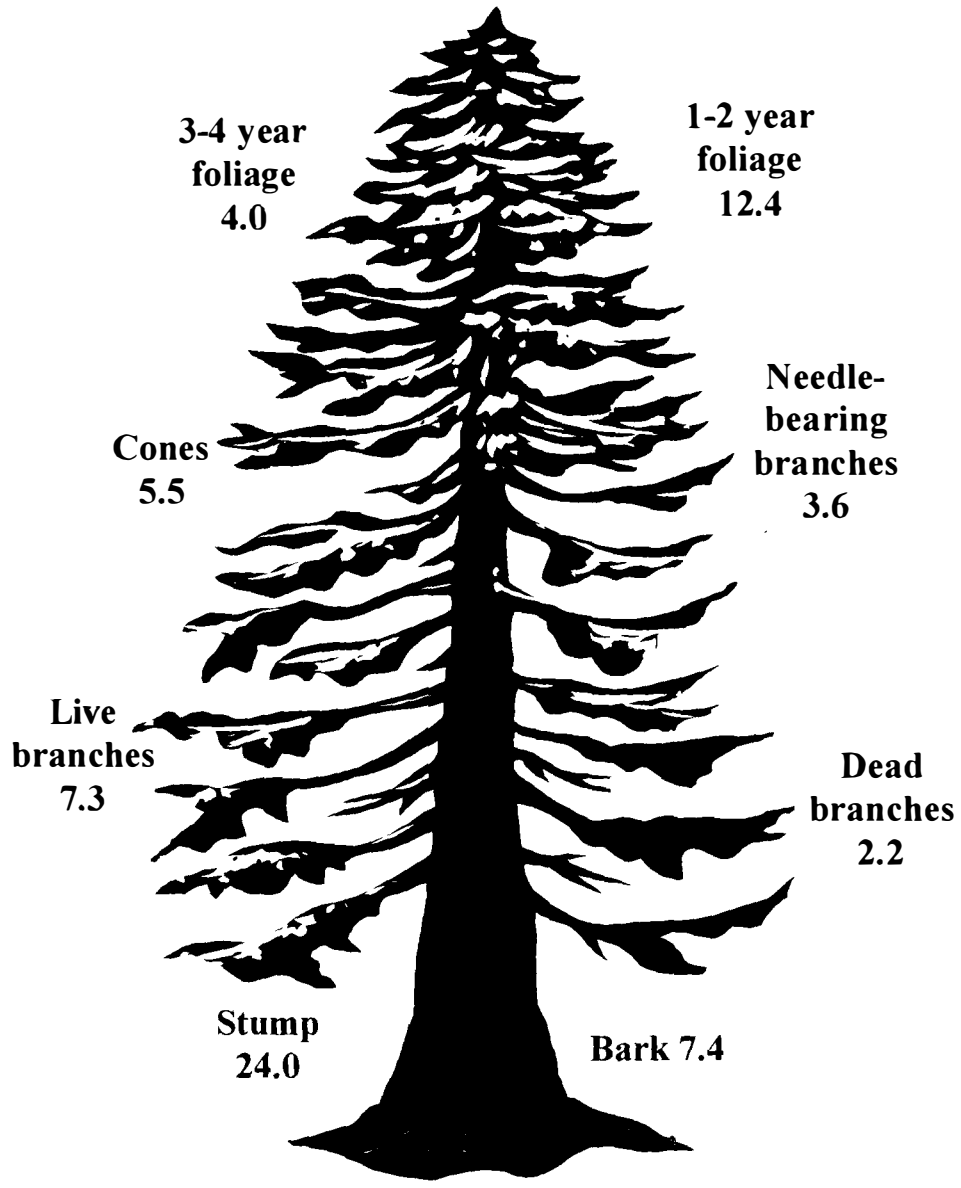

Figure 2.1 Phosphorus partitioning in a 29-year-old *Pinus radiata* tree (kg ha^{-1}) (Webber and Madgwick, 1983)

with fewer problems such as leaching, P fixation and weed stimulation. Also phosphate rock is a lower cost P source than manufactured fertiliser.

Although fertiliser P application is necessary to improve production of *P. radiata*, *P. radiata* productivity (Plate 2.1) in some P deficient soils is not always dependent on P fertiliser input (Will, 1985). In many instances low plant-available P status in some forest soils (e.g. < 4 μg g^{-1}soil of Olsen or Bray-2 P in mineral soil under 17 year-old (Sparling et al., 1994) and under 4 - 5 year-old (Chapter 4) *P. radiata* plantations) did not always lead to low forest productivity (Ballard, 1973) nor low P concentration in pine needles (an index commonly used for the diagnosis of P nutrition status of *P. radiata*) (Parfitt et al., 1994). These findings suggest that pine roots may have mechanisms for P acquisition in low P soil that are more efficient than similar rhizosphere processes of agricultural crops. In fact, fertiliser use in forestry is traditionally conservative compared to agriculture sector; with a "plant it and leave it" approach dominating until the 1970's when more intensive silvicultural regimes were adopted.

Pines were traditionally thought of as pioneer plants that could grow on even the least fertile soils (Will, 1985). Lewis et al. (1993) stated that *P. radiata* appears to have few limits in respect of soil type, although it does prefer lighter-textured soils to heavier-textured soils. These differences in soil requirement between forest and agricultural situations may be due to the longevity of the crops, differences in soil organic matter contents, rooting depth and, in particular, differing mycorrhizal populations (this will be reviewed in later sections). However, the precise mechanisms imparting P uptake efficiency to *P. radiata* are not clearly understood.

2.4 Forms and dynamics of soil P

The majority of soil P forms are poorly soluble, which can be broadly categorised as inorganic P (P_i) and organic P (P_o). The chemical nature and dynamics of P in soil have been extensively studied and reviewed by many workers (e.g. Larsen, 1967; Walker and Syers, 1976; Dalal, 1977; Anderson, 1980; Stewart and McKercher, 1982; Tate, 1985; Harrison, 1987; Stewart and Tiessen, 1987; Wild, 1988; Bolan et al., 1990; Pierzynski et al., 1990; Bolan, 1991; Sanyal and De Datta, 1991; Cross and Schlesinger, 1995;

Plate 2.1 *Pinus radiata* plantations grown on an Allophanic Soil having low plant-available P (Bray-P < 4 μg g^{-1} soil) at Kaweka Forest (Top: 4-year-old second-rotation trees; Bottom: approximately 10-year-old first-rotation trees). Note no P deficiency symptoms in tree foliage.

Frossard et al., 1995; Hedley et al., 1995; Magid et al., 1996; Frossard et al., 2000; Hinsinger, 2001; Smith et al., 2003; Trolove et al., 2003). In this section, some of the major P characteristics in soils beneath *P. radiata* in New Zealand are briefly described.

2.4.1 Soil inorganic P (P_i)

Soil inorganic P occurs at fairly low concentrations in the soil solution whilst a large proportion of it is more or less strongly held mainly by diverse, non-crystalline soil minerals (Pierzynski et al., 1990). In acidic forest soils, P anions can be strongly adsorbed onto positively charged minerals such as Fe and Al oxides, and the surfaces of some minerals, such as allophane (Parfitt, 1989). Phosphorus anions can also precipitate with Fe and Al in solution (Hinsinger, 2001). These adsorption/desorption and precipitation/dissolution equilibria are influenced by rhizosphere chemical reactions and biological processes (Hedley et al., 1995; Hinsinger, 2001; Trolove et al., 2003). The major factors that influence P_i equilibria between solid and solution phases in soils as well as the speciation of soil P_i are: (i) pH; (ii) concentrations of anions that compete with P anions for ligand exchange reactions; and (iii) concentrations of metallic ions (Ca, Fe and Al) that can coprecipitate with P ions (Hinsinger, 2001). All of these factors are influenced by the rhizosphere processes.

2.4.2 Organic P (P_o) cycling and its importance in forest ecosystem

In forest soils, the greatest percentages of a nutrient in the plant-available form, including the nutrient P, are found in the living forest and its litter layer, or previous harvest residues (Gibson and Healy, 1982; Zech et al., 1987; Parfitt et al., 1994; Firsching and Claassen, 1996). The litter-fall, harvest residues and, particularly, the soil microorganisms are the important P_o pools of forest eco-systems (Figure 2.2). Madgwick (1994) reported that more than 30 kg P ha^{-1} may be potentially available from the first harvest residues during second rotation plantation of *P. radiata* (Table 2.1). Sparling et al. (1994) estimated that the annual turnover of P through a 17-year-old *P. radiata* plantation on an Ultic soil (Typic Dystrochrept) in the Wellington region was about 5 kg P ha^{-1}, and the annual flux of P through the soil microbial fraction was up to 18 kg ha^{-1}. Parfitt (1998) reported that the microbial P (a P pool with rapid turnover

rates and the pool that releases plant-available P) in L and FH layers of a 28-year-old unfertilised *P. radiata* plantation on a coastal sand soil ranged from 231 to 526 mg kg^{-1} soil, making up 34% and 61% of the total P contents, which ranged from 673 to 863 mg kg^{-1} soil in these layers.

The cycling of P_o through soil microorganisms has a large effect on soil P availability in the forest ecosystem (Figure 2.2) (Parfitt et al., 1994; Tiessen et al., 1994; Perrott et al., 1999). Turner and Lambert (1985) suggested that P_o may be the form of P most strongly influencing long-term productivity of *P. radiata* in Australia. Saggar et al. (1998) also suggested that the release of P from organic P form during decomposition of forest floor and soil organic matter is an essential process in maintaining P supplies in these forest ecosystems.

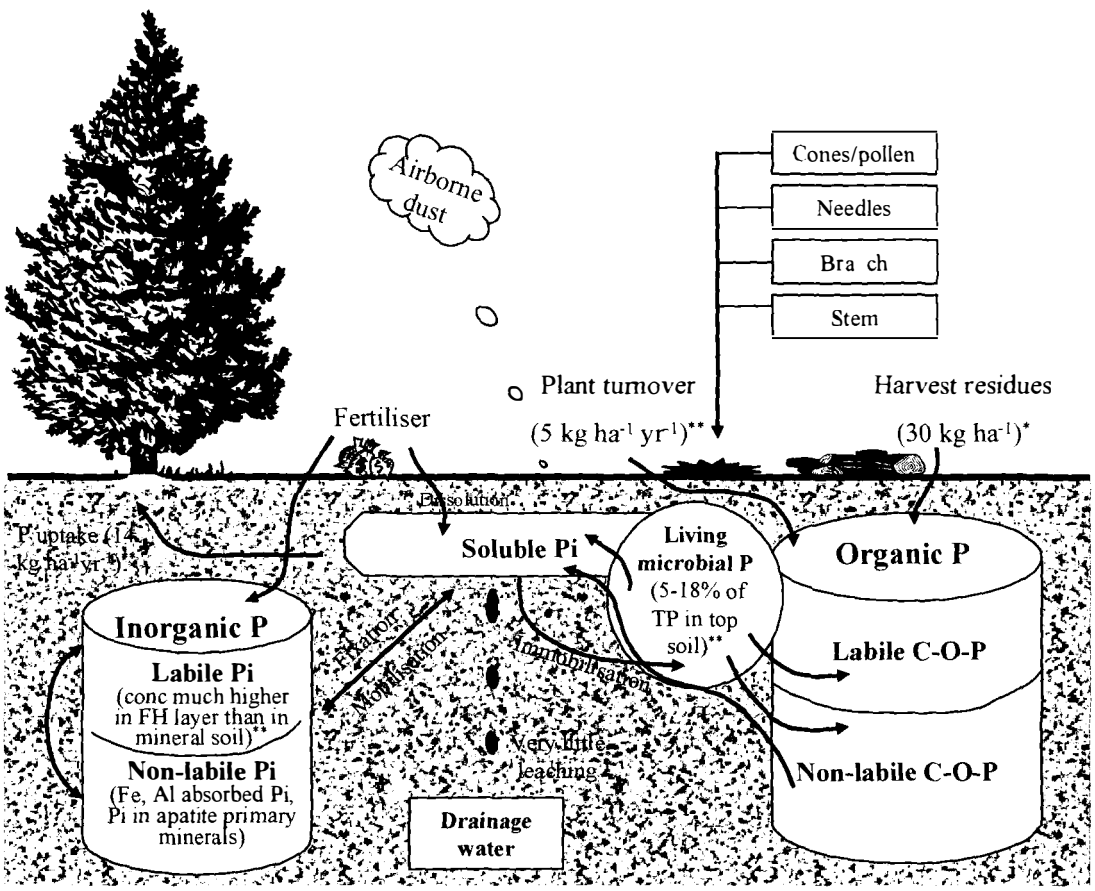

Figure 2.2 The phosphorus cycle in a second-rotation (17 to 29-year-old) *Pinus radiata* forest system (*Madgwick et al., 1977; **Sparling et al., 1994)

The availability of soil P to plants is a function of the amounts and forms of soil P present and the rate at which it can be mobilised and transported to plant roots (Hedley et al., 1995). The effect of conifer growth on the forms and availability of soil P_o has long been of interest. Soils beneath New Zealand *P. radiata* plantations have been commonly reported to have higher P_o mineralisation rates, which result in higher levels of plant-available P_i (e.g. Olsen or Bray-2 extractable P) than soils beneath adjoining grasslands (Davis and Lang, 1991; Hawke and O'Connor, 1993; Davis, 1994; Condron et al., 1996; Giddens et al., 1997; Perrott et al., 1999; Chen et al., 2000). Similar observations have also been reported overseas. For example, Fisher and Stone (1969) found that the higher organic matter mineralisation caused higher mineral N and readily soluble P levels beneath several *Larix* and *Pinus* plantations than beneath adjoining abandoned lands in New York State, U.S.A. Harrison et al. (1988) found that the P availability of soils beneath *Betula verrucosa* in northern England and Scotland was higher than in the unplanted soils. The pot trial studies conducted by others (e.g. Davis, 1995; Condron et al., 1996; Chen et al., 2002) also demonstrated that total soil P_o in the mineral soil below the litter layer was lower and P_i higher under *P. radiata* than under grass, suggesting that increased P_o mineralisation is probably an important cause for the elevated P_i availability under pines. These studies show that the rhizosphere processes responsible for either net mineralisation or net immobilisation of P_o, may affect the soil P supply for sustainable growth of trees in the forest ecosystems.

Soil P availability to trees, estimated using 'single point in time' soil tests developed for agricultural crops, are likely to provide inaccurate information because of the unique nature of soil organic P cycling in forest soils. Parfitt et al. (1994) reported that the Bray soil test, which was commonly used to predict soil P supply to *P. radiata* crops in New Zealand, was in some places over-predicting, and in others under-predicting P requirements of trees, particularly for second-rotation trees (Hunter and Hunter, 1991). It seems likely that most of the P requirement of some second-rotation *P. radiata* could be met by mineralisation of soil organic P (not measured in the Bray test). Some soils with a low Bray test value may have relatively high rates of P_o mineralisation and therefore the trees are adequately supplied with slow release P (Parfitt et al., 1994). As a result, the use of soil P tests developed for agricultural crops to estimate P availability in forestry has generally met with limited success (Parfitt et al., 1994). To develop suitable

methods for estimating P availability to trees in forest soils, an improved understanding of organic P conversion to inorganic P is needed.

Organic P mineralisation is widely believed to be controlled by the action of extracellular and periplasmic phosphatase enzymes produced by plant roots, mycorrhizae and microorganisms (Speir and Ross, 1978; Dighton, 1983; Tarafdar and Claassen, 1988) (this will be discussed in a later section). Amongst them, the ectomycorrhizal association with *P. radiata* plays an important role. However, the mechanisms involved in the mycorrhizal roots effecting organic P mineralisation in the forest soil environment remains poorly understood.

2.5 Role of ectomycorrhizas (ECM) in nutrient uptake by *P. radiata*

2.5.1 ECM fine root and its turnover

The growth of *P. radiata* is assisted by its symbiotic relations with ECM fungi (Vogt et al., 1986). The perennial root system of *P. radiata* contrasts markedly with that of annuals in that the tree has a framework of roots on which fine absorbing ECM roots are produced rapidly, which spread throughout the soil when suitable conditions occur. Mycorrhizas commonly infect the short second and later order lateral roots and form forked fine root tips (Wilson and Field, 1985; Brundrett et al., 1996) (Plate 2.2 and 2.3). The ECM roots are predominantly concentrated in, or immediately under, the forest litter layer. Here they are spatially and temporally located to provide effective uptake of soluble nutrients pools.

The nutrient and water uptake functions of the ECM fine root system, applicable to *P. radiata*, have been comprehensively reviewed by Bowen (1984). Most of the fine root system is short-lived, for a matter of only weeks or months (Bowen, 1984). Reynolds (1975) suggested that death of ECM fine roots is energy conserving in environments in which they make little contribution to tree growth. In 15 - 20-year stands of *P. sylverstris* in Sweden, Ågron et al. (1980) estimated that more than 50% of the assimilated carbon dioxide by tree shoots was used for fine root production, which accounted for 95% of the total annual root biomass production (Persson, 1980). On a

Plate 2.2 The root system of a young (approximately1.5-year-old) *P. radiata* seedling grown in Kaweka Forest, New Zealand

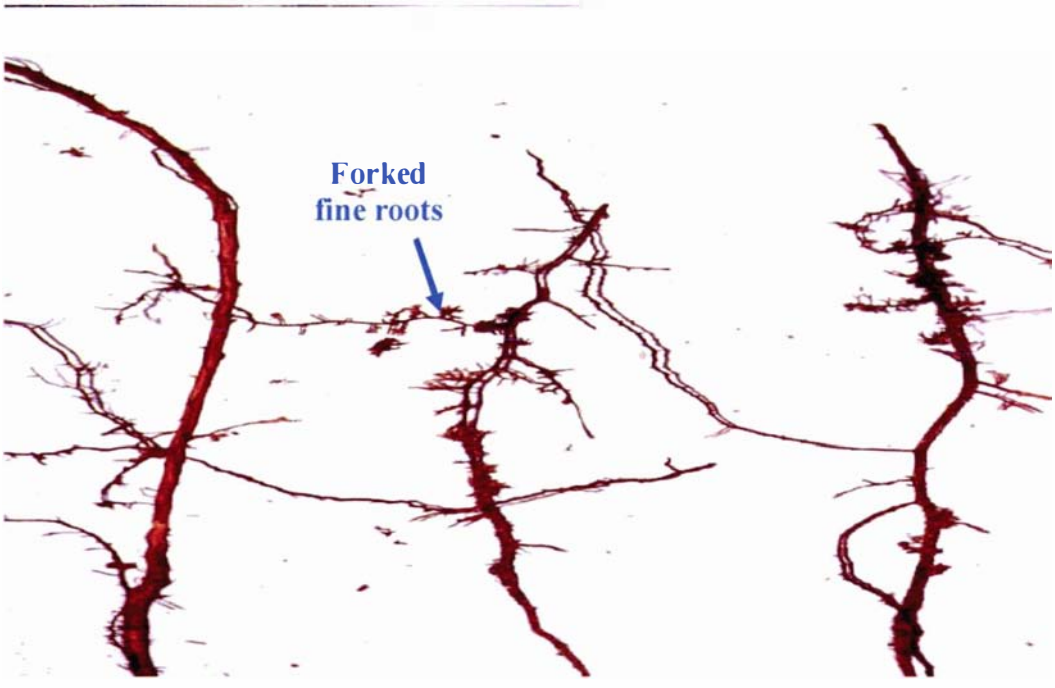

Plate 2.3 Ectomycorrhizal fine roots of *P. radiata*

high productivity stand of *Pseudotsuga menziesii* (Douglas fir), ECM fine root production accounted for 8% of total (above ground and below ground) dry matter production, and on a low productivity site, the ECM fine roots accounted for 36% (Keyes and Grier, 1981). In 23-year-old *Abies amabilis* (Pacific silver fir) in the Pacific North-west (USA), 36% of net primary productivity was used for fine-root production, and in a 180-year-old stand this was 66% (Grier et al., 1981).

The turnover of ECM fine roots and associated hyphae contributes to the accumulation of soil organic matter (SOM) in the pine rhizosphere. Sanchez and Bursey (2002) found that loblolly pine (*Pinus taeda* L.) growing on sandy and loamy sand soils for 40 years resulted in a significant build-up of labile C compounds in the rhizosphere soils (55% increase, compared to bulk soil). The accumulation of SOM influences soil moisture retention, SOM mineralisation, cation exchange capacity and microbial activity in the rhizosphere soil (Figure 2.3). Therefore, the interaction between soil, microorganisms and roots may create a mutually supportive system which has important implications for nutrient availability and supply from the mineral horizons of forest soils (Gobran and Clegg, 1996).

Bowen (1984) suggested that the turnover of ECM fine roots and the associated hyphae may have an impact on nutrient cycling in forests; in some situations ECM fine-root turnover may be a more significant factor in nutrient cycling than is litter fall. Vogt et al. (1982) estimated that ECM fine roots plus mycorrhizal hyphae in a stand of *Abies amabilis* contributes to cycle 4 times the N, 6 - 10 times the P and K, 2 - 3 times the Ca, and 3 - 10 times the Mg as compared to litter-fall. Thus, the evidence for the turnover of mycorrhizal infected fine roots in conifers being a major component of nutrient cycling is very strong. Because only a few studies have quantified the weight and P content (Vogt et al., 1991) and turnover (Bowen, 1984) of the ECM fine roots growing in the field, it is currently not possible to determine the exact extent to which fine roots influence rhizosphere P dynamics.

2.5.2 P-efficient mycorrhizal fungal tissues

Ectomycorrhizal fungi infect the short lateral roots, grow between the cortical cells and

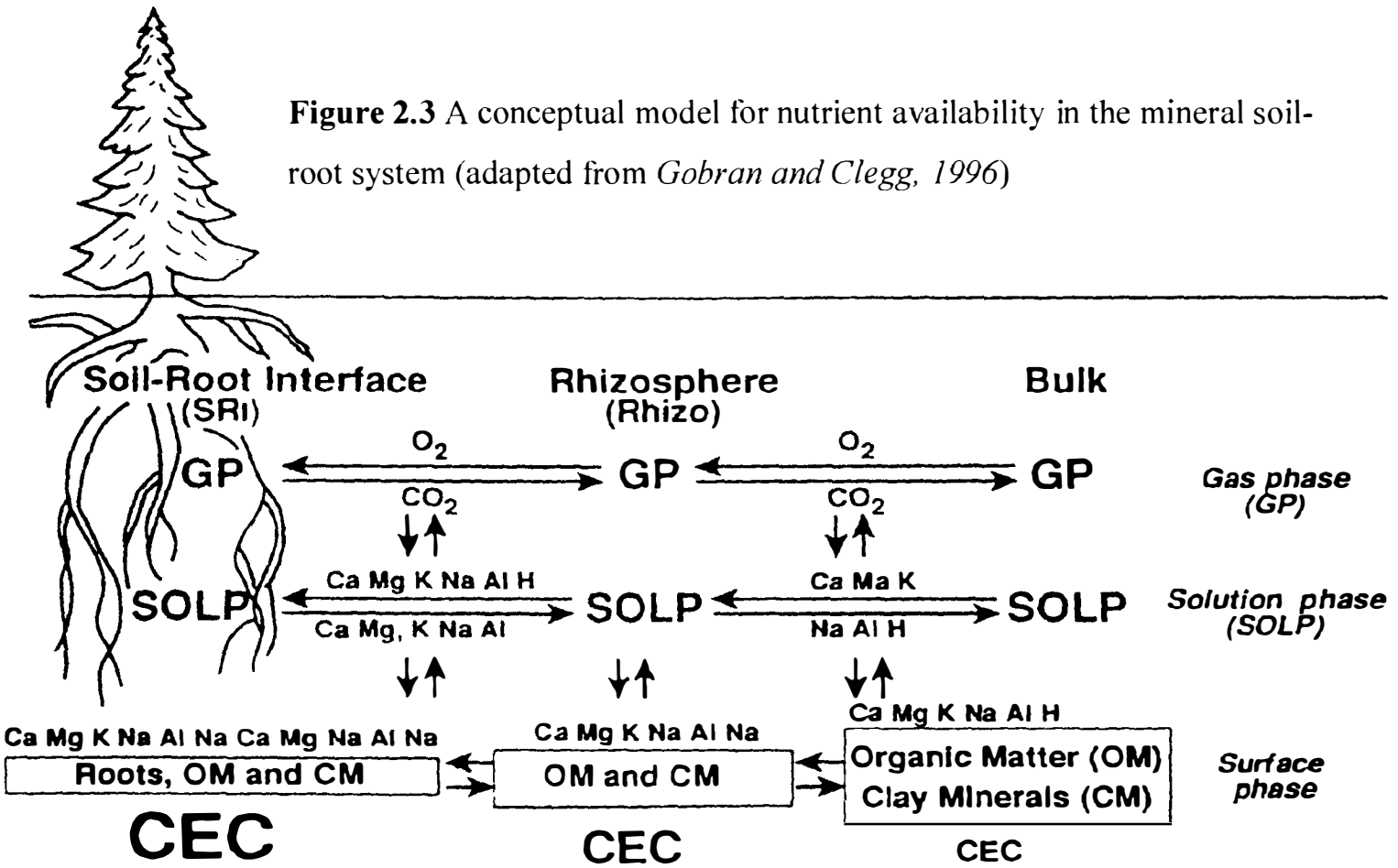

Figure 2.3 A conceptual model for nutrient availability in the mineral soil-root system (adapted from *Gobran and Clegg, 1996*)

produce a "Hartig net" enveloping the cortical cells (Plate 2.4). Outside the root they form a fungus sheath or mantle. Individual hyphae (Plate 2.5) grow from these into soil but often there are conspicuous aggregations of hyphae (mycelial strands) that branch frequently and permeate the soil and litter. The tendency for mycorrhizal hyphae to aggregate into rhizomorphs enhances their ability to explore the soil and increase their nutrient and water uptake capacity (Bowen, 1968). Root hairs complement mycorrhizae on uninfected lateral roots, including long lateral roots.

In comparison with relatively coarser root hairs (commonly larger than 10 μm diameter), the finer ectomycorrhizal fungi hyphae (2 – 4 μm diameter; Chu-Chen and Grace, 1983; Bolan, 1991) have some advantages. Firstly, it increases the surface area of the hyphae resulting in greater absorption of nutrients. Secondly, it enables the entry of hyphae into small pores in soils and organic matter that cannot be entered by root hairs and thereby increases the volume of exploration in soils. It has been suggested that mycorrhizal fungal hyphae have higher affinity for P ions and a lower threshold concentration required for absorption than do plant roots and root hairs (Bolan, 1991). Mycorrhizal tissues also appear to play a major role in the cycling and conservation of P in forest ecosystems. Mycorrhizal sheaths of beech have been shown to retain over 90% of the P absorbed from solution cultures (Harley and McCready, 1952) as polyphosphate crystals in the vacuoles of the mycorrhizal fungal cells (Chilvers and Harley, 1980). The ability to conserve P in mycorrhizal tissues when P availability in soil is high and to release it to the plant when it is in short supply may be critical in environments where P availability in soil is affected by seasonal differences (Vogt et al., 1991). In addition, the mycorrhizal hyphae are considered to be able to produce phosphatase enzymes and release significant amounts of organic anions (such as oxalate) into the rhizosphere, thereby increasing rhizosphere P bioavailability. This will be discussed in detail in later sections.

From the review of literature it can be concluded that the mycorrhizal fungal tissues are able to: (1) increase physical exploration of the soil; (2) increase P movement from soil into mycorrhizal fungus hyphae; (3) modify the root environment to increase P availability in soil; (4) increase storage of absorbed P; and (5) efficiently utilise P within

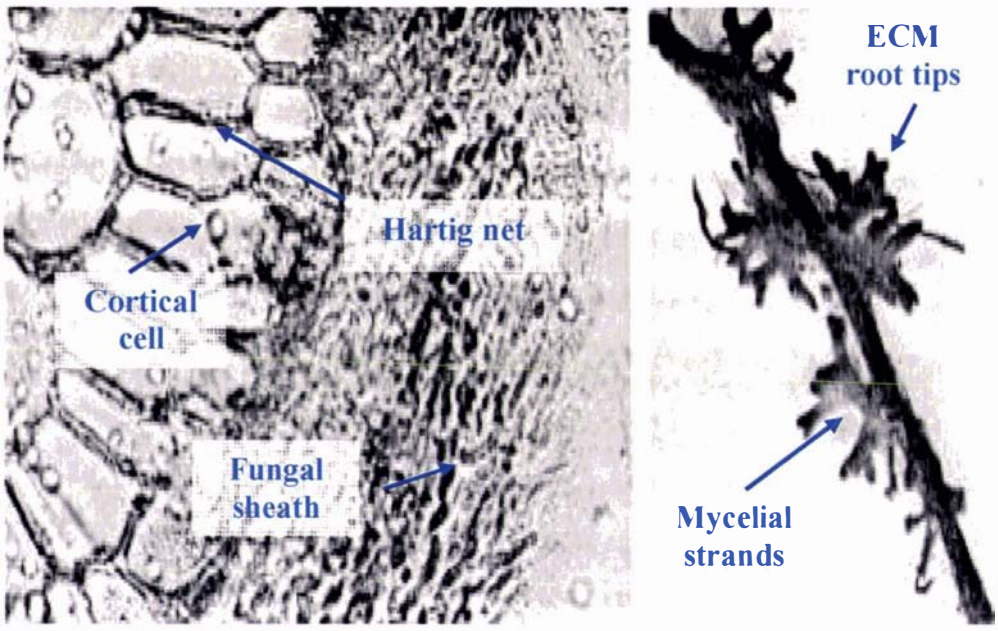

Plate 2.4 *Pinus radiata* + *Rhizopogen rubescens* mycorrhizas: right – gross morphology (1.8X); left – transverse section (350 X) (cited in Chu-Chou and Grace, 1983)

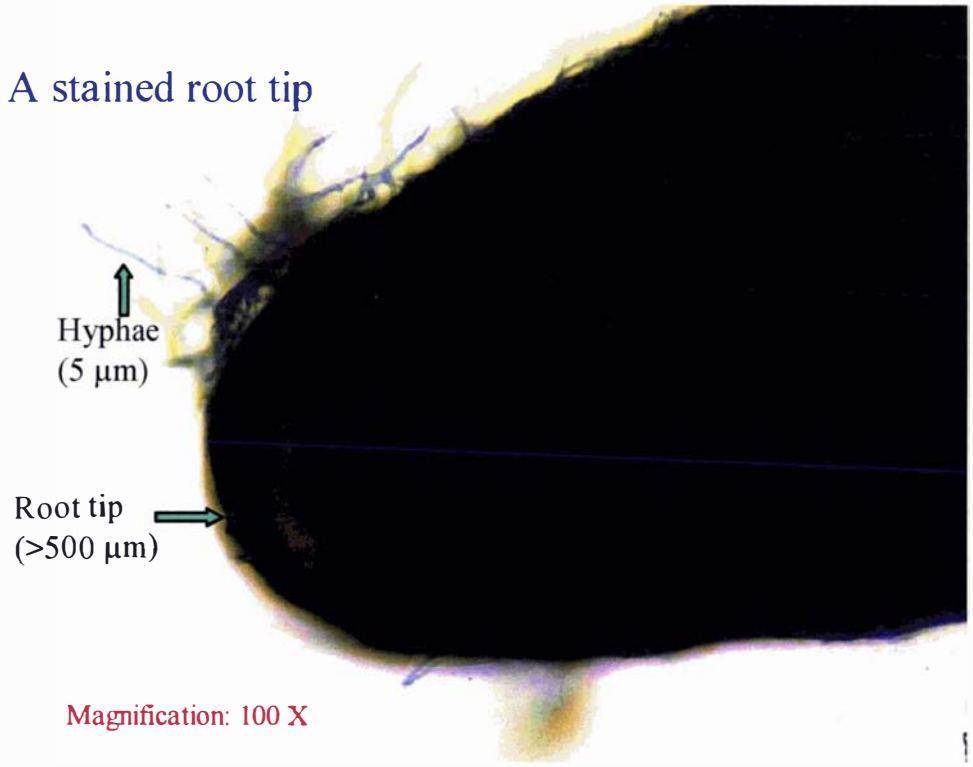

Plate 2.5 *Pinus radiata* fine-root tip associated with mycorrhizal hyphae

the plant. These processes have been extensively studied by many researchers, e.g. Skinner and Bowen (1974a), Chu-Chou and Grace (1983; 1985; 1990) and have been reviewed by Harley and Smith (1983), Bolan (1991), Vogt et al. (1991) and Smith and Read (1997).

2.5.3 Mycorrhizal fungi associated with *P. radiata* in New Zealand

In New Zealand, Chu-Chou and Grace (1983) identified 14 different fungal species capable of forming mycorrhizal associations with *P. radiata* (Table 2.3). Most of them are *Basidiomycetes* and all are restricted to the family *Pinaceae*. Compared to the known number of potential mycorrhizal fungi, the number of mycorrhizal flora associated with *P. radiata* in New Zealand is rather limited. One reason for this may be that few of the mycobionts of indigenous tree species can be shared with exotic *P. radiata*, because New Zealand has no native *Pinus* sp. and is poor in coniferous flora. Chu-Chou (1979) and Chu-Chou and Grace (1984) showed that *Rhizopogen* sp. are the most common and possibly the most important mycorrhizal symbionts of *P. radiata* in New Zealand, although *Suillus* sp., *Endogone flammicorona* and *Tuber* sp. are also common mycorrhizal fungi in certain *P. radiata* forests. For example, pot trials carried out by Chu-Chou and Grace (1985) demonstrated that *Rhizopogen* sp. are more efficient mycorrhizal fungi than *Laccaria laccata* and *Hebeloma crustuliniforme* in promoting young *P. radiata* seedling growth and nutrient uptake including P uptake. These differences in specific fungal species association with radiata roots are expected to be able to induce differences in rhizosphere environment between forests. However, most of the previous studies concentrated on the mycorrhizal effect on seedling growth but paid less attention to the influence of mycorrhizal fungi on P uptake by seedlings and P nutrient changes in the soils. Inoculating radiata roots with various ECM fungi to determine their influences on rhizosphere soil P chemistry and P uptake by *P. radiata* will increase our understanding of the role of ECM fungi on P availability to *P. radiata.*

2.5.4 Factors affecting the infection of mycorrhizas in pine trees

Mycorrhizal infection of *P. radiata* roots in plantations depends firstly on the active

Table 2.3 The mycorrhizal characteristics and their fungal symbionts associated with *Pinus radiata* in New Zealand (adapted from Chu-Chen and Grace, 1983)

Mycorrhizal characteristics	Identified fungal symbionts
I Mycorrhizas covered in mycelia	
1. Mycelia white	*Hebeloma crustuliniforme* (1)
2. Mycelia greyish	*Tricholoma pessundatum* (2)
II Mycorrhizas not covered in mycelia	
1. Mycorrhizas jet black	B 218 (3)
2. Mycorrhizas dark brown	
A. Mantle absent	*Endogone flammicorona* (4)
B. Mantle with bristles	*Tuber* sp.(5)
3. Mycorrhizas pinkish brown	*Laccaria laccata* (6)
4. Mycorrhizas creamy brown	
A. Short coralloid, sessile	
a. Pale cinnamon colour all over	*Rhizopogen luteolus* (7)
b. Pale cinnamon colour all over	*Suillus luteus* (8)
B. Long dichotomous	
a. Mantle covered with hair-like hyphae	*Thelephora terrestris* (9)
b. Mantle no attached mycelia	*Inocybe lacera* (10)
5. Mycorrhizas white	
A. Large dichotomous, mantle thick	*Rhizopogen rubescens* (11)
B. Fine dichotomous, mantle thin	
a. Peripheral hyphae of mantle and rhizomorphs with swollen tips	*Scleroderma verrucosum* (12)
b. No such structure	*Amanita muscaria* (13)
Creamy brown	*Unknown* (14)

growth of a relevant fungus in the rhizosphere, and secondly, on successful infection of pine roots by it. There are two modes of infection (Marks, 1971). Infection occurs from the existing mantle through breaks in the mantle, or it may be infected directly from the soil mycelium (Lewis et al., 1993). The first mode of infection is plant related. For the second mode of infection, the ecological adaptation of the mycorrhizal spores for its viable persistence in the soil is important. This will influence the type and number of successful associations formed (Chu-Chou and Grace, 1987; Browning and Whitney, 1992).

The type of soil where seedlings are planted and its previous management markedly influences the persistence of mycorrhizal fungi pre-established on the roots (Last et al., 1985; Chu-Chou and Grace, 1990). The effects of site-specific mycorrhizal inoculations on the degree of infection of the roots and on the growth of the seedlings have been studied mainly in sterile growth media. Very few such studies have been conducted in non-sterile soil media (i.e. on natural soils).

2.5.4.1 Effect of soil type and fertility

Mycorrhizas are highly concentrated in the top few cm of soils. A study conducted in Australia (Squire et al., 1978) by wet sieving roots from four 8-year-old stands of *P. radiata* in a wide range of sites showed that on all sites mycorrhizal roots were mainly in the top 11 cm depth of soil.

The number of mycorrhizal species and the abundance of sporocarps of these fungi vary with soil type and fertility. Chu-Chou and Grace (1988) reported that greater numbers of fungal species were found in forests established on pumice-scoria than on sandy or clay soils. Nevertheless, it appears that soil fertility has more influence on mycorrhizal infection than soil type. Chu-Chou and Grace (1987, 1990) reported that *Rhizopogen* sp. is more commonly found in low fertility soils than high fertility soils. For example, at high fertility agroforestry sites two less-common mycorrhizal fungi – *Tuber* sp. and *Scleroderma* sp had replaced the most common *Rhizopogen rubescens* Tul. found in the conventional forests of the central North Island (Chu-Chou and Grace, 1987). Also the sporocarps of *Rhizopogen* sp. were more commonly found to occur in low fertility forest soils than in high fertility nursery soils (Chu-Chou and Grace 1987, 1990). Unlike

Rhizopogen sp., *Suillus* sp. was seldom observed in nurseries (Chu-Chou and Grace, 1979).

2.5.4.2 Effect of soil P status

Little information is available on the effect of soil P level on mycorrhizal infection of *P. radiata* in New Zealand. Browning and Whitney (1992) reported that an increase of P (applied as KH_2PO_4) from 1.5 to 7.2 mg per seedling grown in peat-vermiculite in a growth chamber severely reduced the infection of *Laccaria bicolo*r ectomycorrhizae on both jack pine and black spruce 17 weeks after inoculation of this mycorrhizae. Other workers have also noted this phenomenon in oak and birch (Newton and Pigott, 1991) and spruce (Bjokman, 1942 cited in Newton and Pigott, 1991) seedlings. Very recent observation by Guadarrama et al. (2004) also showed that as levels of P increased arbuscular mycorrhizal colonisation with a tropical tree species (*Heliocarpus appendiculatus*) decreased. A possible reason for reduced mycorrhizal activity under higher P status may be related to the effect of the imbalanced nutrient level on root carbohydrates. Marx et al. (1977) reported that high levels of N and P in soil decreased sucrose content of short roots of loblolly pine (*P. taeda*) and therefore decreased their susceptibility to ectomycorrhizal development by *Pisolithus tinctorius.* Another reason for the reduced ECM infection at high P may be a decrease in total root system. However, the mycorrhizal fungi require some available P for their growth. Lamb and Richards (1974) and Skinner and Bowen (1974b) reported that there was increased mycorrhizal infection with increasing P supply in very low P status soils under *P. radiata*. These workers did not test high rates of P application to evaluate whether mycorrhizal infection decreases at high P supply as observed by Browning and Whitney (1992) and Newton and Pigott (1991). Ectomycorrhizal infection on pines may have greater importance in low P status soils or soils fertilised with insoluble phosphates that do not immediately raise the plant-available soil P status. Most of the studies on P fertiliser effect on mycorrhizal infection have been carried out using soluble P fertiliser. Very little information is available on the effect of poorly soluble P fertiliser, such as reactive phosphate rock (RPR), on the infection of ectomycorrhizae on *P. radiata.*

2.5.4.3 Effect of tree age on mycorrhizal development

The types of mycorrhizal fungi associated with *P. radiata* in New Zealand change with increasing tree age. Chu-Chou (1979) reported that *Rhizopogen* sp. were abundant in nurseries and in the stands of 3 - 5 year-old trees, but were less frequent in stands of over 10 years old. However, *Suillus* sp. were most frequently seen in stands over 5 years of age, but seldom observed in younger stands, or in nurseries. Marks and Foster (1967) also observed that in a 40 year-old *P. radiata* stand, approximately 4% of the ectomycorrhizas showed external evidence of change from one form to another over the growing period. Their studies appear to indicate that changes in mycorrhizal type and mycorrhizal succession during growth may be relative to the factors such as tree growth status, light intensity and soil conditions.

2.6 Coniferous rhizosphere processes influencing P availability

Studies on the rhizosphere processes under agricultural crops have been comprehensively reviewed by others (e.g. Jungk, 1996; Hinsinger, 2001 and Trolove et al., 2003). In this section the review of literature will focus on the studies conducted on conifers with special attention to *P. radiata*.

2.6.1 Root-induced changes in soil microbial composition

As reported earlier in this chapter, soil microbial P is an important P source in forest soils. Sparling et al. (1994) reported that annual flux of P through the soil microbial fraction was up to 18 kg ha^{-1} on Ultic soils beneath 17-year-old *P. radiata* plantation in New Zealand. The root processes influence the rhizosphere microorganism composition and their activity, and therefore the soil organic P availability.

It is generally considered that there is a gradient of microbial numbers in soil around plant roots, with numbers decreasing with increasing distance from the roots. Chen et al. (2002), using a thin layer slicing technique in a pot trial, reported that the concentration of microbial-C was greater in the rhizosphere of *P. radiata* than in the root-free bulk soils. This higher concentration of microbial-C was related to the higher dissolved

organic C (DOC) concentration in the rhizosphere. Gierasimiuk et al. (2001) isolated soil bacteria from the bulk and rhizosphere soils of nursery seedlings and 70-year-old trees of *P. sylvestris* stand in Torun, Poland, and found that the number of bacteria increased in the following order: bulk soil (old forest) < rhizosphere soil (old forest) < rhizosphere soil (nursery). Norton and Firestone (1991) studied the quantity and metabolic status of bacteria and fungi in rhizosphere and bulk soils from microcosms containing ponderosa pine (*P. ponderosa*) seedlings. Their results revealed that the highest total fungal biomass was adjacent to fine mycorrhizal roots, whereas the highest total and active bacterial biomass was adjacent to the young growing root tips. These observations suggest that fungi are enhanced adjacent to the mycorrhizal fine roots compared with the non-rhizosphere soil, whereas bacteria are more responsive than fungi to labile carbon inputs in the young root zone (Norton and Firestone, 1991).

The extent to which plant roots influence microbial populations varies widely with plant species, soil type and soil moisture. When seedlings of Scots pine (*P. sylvestris*), Norway spruce (*Picea abies*) and silver birch (*Betula pendula*) were planted into pots containing two soil types (an organic soil and a mineral soil), Priha et al. (1999) found that in the organic soil, microbial-C and microbial-N were higher in the birch rhizosphere than in the pine and spruce rhizospheres. However, the effects of the different tree species on microbial C and N did not vary in the mineral soil. One reason for the difference in soil microbial community structure between organic soil and mineral soil could be that in the organic soil there was more microbial diversity compared to that in the mineral soil.

Leyval and Berthelin (1991) suggested that rhizosphere bacteria are responsible for the solubilisation of mineral nutrients, and that the ability of mycorrhizal fungus to enhance P uptake can be explained by the greater absorptive area of mycorrhizal roots. In this aspect the interactions between ectomycorrhizas and bacteria in the rhizosphere may be important (Wallander, 2000). However, there is little research data available in literature on microbial activity in the *P. radiata* rhizosphere, although the study of Gadgil and Gadgil (1975) demonstrated that mycorrhizal fungi associated with *P. radiata* are able to suppress litter decompositors (such as *Coniothyrium* sp., *Pestalotia aloes* Trinch, *Pestalotia neglecta* Theum and *Mortierella* sp.).

2.6.2 Rhizosphere pH change

Plants are able to modify the pH around their roots and thus influence the chemical properties in the rhizosphere (Trolove et al., 2003). Considerably lower pH (as much as 2 pH units) was reported in the rhizosphere soil compared to that in the bulk soil under various plants (Hedley et al., 1982a; Smith and Pooley, 1989; Rigou et al., 1995; Zoysa et al., 1997; Chen et al., 2002). It has been shown that the difference in the pH values between the rhizosphere soils and the bulk soils varies with both plant species and mineral nutrition (Marschner et al., 1986). In a short-term (60 days) pot experiment, Chen et al. (2002) found that the soil pH decreased by 0.1 – 0.4 units near the roots of *P. radiata* seedling (within 2 mm) compared with the pH of the bulk soil. The reason for the acidification of rhizosphere was considered to be due to excess cations over anions uptake, which led to the release of H^+ ions to maintain electroneutrality inside the plant (Trolove et al., 2003).

The pH difference between rhizosphere and bulk soils depends also on the forms of N supplied to the plants. Zoysa et al. (1998a) showed that when N was supplied in the NO_3^- form ($Ca(NO_3)_2$) rhizosphere pH of tea seedling increased compared to that of the bulk soil, and when N was supplied in the NH_4^+ form ($(NH_4)_2SO_4$) the rhizosphere pH was lower than that of the bulk soil. When N was supplied in both NH_4^+ and NO_3^- forms (NH_4NO_3), the pH difference between bulk and rhizosphere was the lowest.

The relatively large amount of data available on variation in the rhizosphere pH around the roots compared to the bulk soils of herbaceous plants has been recently reviewed by Hinsinger (2001) and Trolove et al. (2003). In contrast, relatively few studies have been reported on this subject for woody species (Marschner et al., 1991; Zoysa et al., 1997; 1999; Chen et al., 2002). Moreover, most of these studies have been conducted on non-ectomycorrhizal plants. The coniferous species are often associated with ectomycorrhizal fungi and therefore the findings of the above studies may not be always applicable to coniferous species.

Mycorrhizae infected plants have been commonly reported to reduce soil pH. Wallander et al. (1997) reported that pH was lower in soils with ectomycorrhizal colonised seedlings of *P. sylvestris* than in soils under non-mycorrhizal seedlings. Their study also

showed that the changes in soil pH varied with the type of fungi species colonising the roots. Rigou et al. (1995) reported that inoculation of *P. pinaster* seedlings grown in an agar culture by *Suillus collinitus* enhanced significantly the capacity of roots to release protons. These studies indicate that the mycorrhizal fungi associated with the conifers are responsible for the acidification of the rhizosphere solution.

Differences betwcen mycorrhizal and non-mycorrhizal plants in the amounts of anions and cations taken up may lead to differences in rhizosphere pH between these plants (Bolan, 1991). Olykan and Adams (1995) reported that ECM infected *P. radiata* seedlings take up N predominantly in the cationic (NH_4^+) than anionic (NO_3^-) form from the soil. Skinner (1978) reported that non-mycorrhizal infected *P. radiata* seedlings grew better in solution containing NO_3^- than NH_4^+, suggesting that non-mycorrhizal *P. radiata* roots prefer to take up NO_3^--N than NH_4^+-N. These observations are consistent with the finding that hyphae of the VAM fungus, *Glomus mosseae,* can absorb and translocate $^{15}NH_4^+$ (Smith et al., 1985). The differences in the form and mechanisms of N uptake between mycorrhizal infected and uninfected plants are unclear. The ability to take up NH_4^+ by mycorrhizal fungi seems to be related to the increased glutamine synthetase activity in the fungi which is considered as an important enzyme in primary ammonium assimilation in land plants (Smith et al., 1985). However, Rigou et al. (1995) reported that when *Suillus collinitus* infected and non-infected seedlings of *P. pinaster* were grown for 2 months in a near neutral (pH 6.8) agar medium containing nitrate, the pH in the rhizosphere zone of the mycorrhizal infected seedling decreased approximately 1 unit compared to that in the non-infected seedling. NO_3^- -N supply commonly induces higher pH in the rhizosphere (e.g. Boxman and Roelofs, 1988; Rollwagen and Zasoski, 1988; Zoysa et al., 1998a). The lower pH obtained in the rhizosphere in the study of Rigou et al. (1995) was explained as due to the higher initial pH of the growth medium (pH 6.8) than that used by other workers. Rigou et al. (1995) stated that at this close to neutral pH, membrane ATPase activity, respiratory CO_2 release and organic acid release may have caused the acidification in the rhizosphere.

The ability of plants to solubilise inorganic P by acidifying the rhizosphere has been well documented (Hedley et al., 1982a; 1983; Gahoonia and Nielsen, 1992; Hinsinger and Gilkes, 1995; 1996; Trolove et al., 1996). Rhizosphere pH has a strong influence on the bioavailability of soil and fertiliser P, particularly the P from phosphate rock

(Hinsinger, 2001; Bolan et al., 1997). Numerous studies (e.g. Hedley et al., 1982a; Laperie et al., 1991; Trolove et al., 1996; Wallander et al., 1997; Zoysa et al., 1997; Wallander, 2000) have demonstrated that rhizosphere acidification can mobilise insoluble soil and fertiliser P. Solubilisation of mineral P by ectomycorrhizal fungi has also been observed in agar culture media (Bowen and Theodorou, 1973). Rhizosphere acidification, however, does not always result in increased P release to plant roots (Trolove et al., 2003). For example, Hedley et al. (1994) found that the amount of P uptake by upland rice from a strongly weathered Ultisol was not related directly to rhizosphere acidification. In some soils increased acidity in the rhizosphere may enhance adsorption of phosphate by hydrous iron and aluminium oxides and allophane thereby decreasing P availability to plants (Clark and McBride, 1984; Parfitt, 1989).

There is very little published information available on pine root-induced acidulation of rhizosphere soils and its effect on the solubility and mobility of insoluble soil and fertiliser P (e.g. phosphate rock) in the forest soils of New Zealand.

2.6.3 Oxalate release from roots and its effect on soil inorganic P mobilisation

The importance of oxalate anion in mobilising insoluble soil and fertiliser P has received much attention. General reviews on the oxalate release from plant roots and the factors influencing its production have been published previously by Bar-Yosef (1996), Dutton and Evans (1996), Jones (1998), Strobel (2001), Jones et al. (2003) and Trolove et al. (2003). In this section the literature relevant to conifers is reviewed.

2.6.3.1 Production of oxalate

Oxalate has been considered as the prime organic anion produced by ectomycorrhizal fungi (Lapeyrie et al., 1987; Lapeyrie, 1988) and saprophytic fungi (Connolly and Jellison, 1995) that influence the mobilisation of inorganic P in soils of conifers (Fox and Comerford, 1992a; Hinsinger, 2001). Fox and Comerford (1990) investigated the low-molecular-weight aliphatic organic anions extracted in soil solution from O, A, B_h and B_t horizons from a group of soils (Ultisols, Entisols and Spodosols) under forests in south-eastern U.S.A. These soils supported slash pine (*P. elliottii*) and longleaf pine (*P. palustris*) with slash pine or turkey oak (*Quercus laevis*). They found that oxalate

concentrations in soil solution ranged from 25 to 1000 μM and oxalates were present in the higher concentrations than other organic anions in all samples. In a greenhouse study, they also found that oxalate was the only low-molecular-weight organic acid identified in the non-rhizosphere soils of slash pine seedlings, but in the rhizosphere soils high concentration of both oxalate and formate were detected, along with trace amounts of citrate, acetate, and aconitate (Fox and Comerford, 1990).

It has been suggested that the oxalate production in ECM of conifers might have special significance in the symbiotic context (Lapeyrie et al., 1987). The oxalates extracted from soils under *P. sylvestris* seedlings were positively related to the amount of ergosterol (a fungal marker which is present only in fungal tissues) in the roots (Wallander, 2000), suggesting that oxalates in the soil solution originate from the ectomycorrhizae symbionts. Accumulation of calcium oxalate crystals around ectomycorrhizas and associated fungi rhizomorphs has been frequently observed in conifers associated with ectomycorrhizas, e.g. in the mantle of ectomycorrhizal roots of *P. radiata* and *Eucalyptus marginata* by Malajczuk and Cromack (1982); in the litter layer and soil A horizon in forests of Douglas fir in Oregon and Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*) in New Mexico by Graustein et al. (1977). Cromack et al. (1979) reported that *Hysterangium crassum* exuded large amounts of oxalate in the dense fungal mats beneath stands of Douglas fir. Casarin (1999) reported that the oxalate exuded by the mycorrhizal roots of *P. pinaster* originated mainly in the hyphae of ectomycorrhizal fungus (cited in Hinsinger, 2001).

Several pathways have been proposed for the biosynthesis of oxalates. The most likely pathway implicates the tricarboxylic acid cycle, or the glyoxylate cycle, in oxalate biosynthesis. This involves the hydrolytic cleavage of oxaloacetate to oxalate and acetate, or the oxidation of glyoxylate to oxalate (see details in Dutton and Evans, 1996; Jones, 1998).

Grayston et al. (1996) and Trolove et al. (2003) have reported that the amounts of organic anions released by various crops increases with increased root biomass (Vancura, 1988), rhizosphere microorganisms (Meharg and Killham, 1995), P stress (Lipton et al., 1987), temperature (Schroth et al., 1966) and light (Vancura, 1988).

2.6.3.2 Impact of oxalate on P mobilisation in soils

The exudation of organic anions into the rhizosphere by plant roots has been hypothesised to be one potential mechanism by which plants can enhance the mobilisation of poorly soluble nutrients in the soil. Casarin (1999) showed that the amount of oxalate exuded by some ectomycorrhizal associations with *P. pinaster* was strongly correlated with the concomitant increase in P bioavailability (Olsen-P) in the rhizosphere, relative to the non-mycorrhizal *P. pinaster* (cited from Hinsinger, 2001). In a pot trial with *P. sylvestris* (L.) seedlings growing in a sand/peat mixture, Wallander (2000) demonstrated that some ectomycorrhizal fungi were able to improve the uptake of apatite-P by ectomycorrhizal pine seedlings, and that the concentration of oxalate anions detected in soil was correlated to the concentration of P in the soil solution (Figure 2.4). Griffith et al. (1994) also showed that oxalate concentration in soil solution of ectomycorrhizal mats in a 75-80-year-old Douglas-fir stand was significantly correlated ($r = 0.985$, $P < 0.05$) with concentration of dissolved P_i in soil solutions. Fox and Comerford (1992a) demonstrated that when different amounts of oxalate (0 to 125 mmol kg^{-1} soil) was mixed with soil samples from A and B_h horizons of two forested Spodosols in U.S.A., oxalate had a large effect on both P_i and P_o release, as well as Al release from the B_h horizon soils, but a negligible effect on P and Al releases from the A horizon soils. This negligible effect was because the P_i present in A horizon soils was nearly all water soluble and there was only small amount of Al in A horizons of the soils (Fox and Comerford, 1992a). The higher pH buffer capacity in A than B horizon is probably the other reason.

The mechanism by which oxalate influences P_o mobilisation is uncertain. Fox et al. (1990) reported that oxalate was able to access pools of P_o in the B_h horizon. Oxalate in soil may be sufficient to facilitate the solubilisation of humic substances by removing the "cation bridges" hypothesised to bind negatively-charged soil organic matter (SOM) to mineral particle surface (Fox et al., 1990; Fox and Comerford, 1992a; Griffiths et al., 1994). Oxalates may also solubilise proteins and nucleic acids bound to mineral surfaces or flocculated by metal cations in a similar fashion, thereby increasing accessibility of hydrolytic enzymes to organic nitrogen and P_o (Griffiths et al., 1994).

Figure 2.5 presents a conceptual model to illustrate the action of oxalate on inorganic P

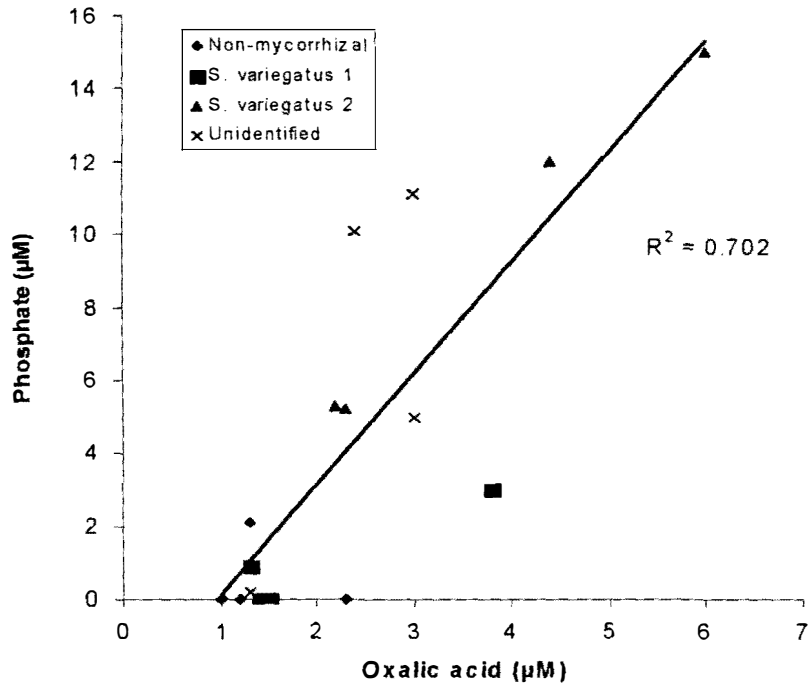

Figure 2.4 A correlation between concentration of oxalic acid and phosphate in root free bulk soil colonised by different ectomycorrhizal fungi and non-mycorrhizal control soil (Redrawn from Wallander, 2000).

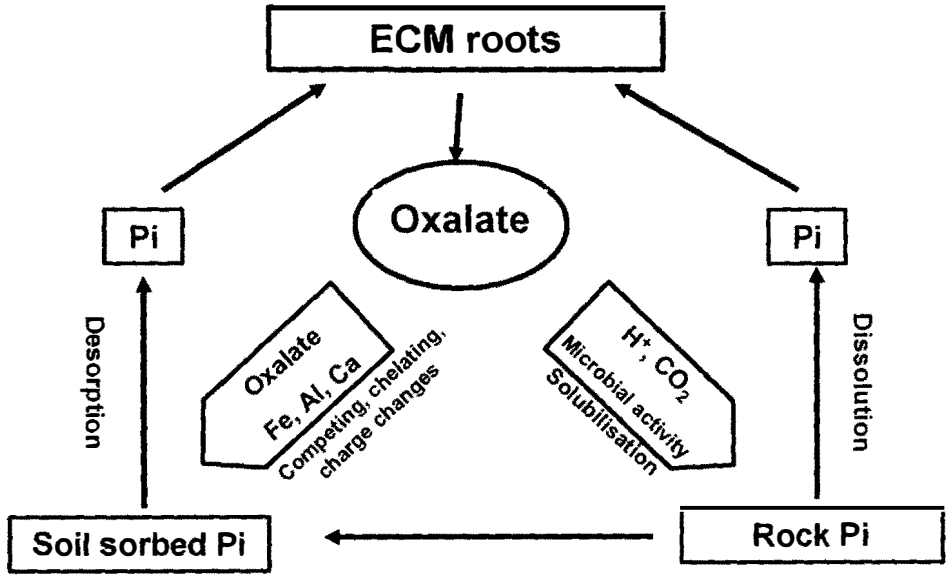

Figure 2.5 A conceptual model for the production and action of oxalate on availability of inorganic P (P_i) in the soil. Redrawn from DeLucia et al. (1997)

availability in forest soil. Three possible mechanisms of oxalate increasing the level of P in solution have been described (Traina et al., 1986; Trolove et al., 2003). They are: (1) Competition for P adsorption sites; (2) Dissolution of adsorbents and P components; and (3) Changes in the surface charge of the absorbents.

Nagarajah et al. (1970) suggested that the decrease in P adsorption caused by the presence of organic anions was due to competition of these anions with P for adsorption sites on clay minerals, especially Fe and Al oxides. This competition depends on the stability of the ferric or aluminium-organic anion complex (Table 2.4). The ability of organic anions to desorb P generally decreases with a decrease in the stability constants for the Fe- or Al-organic anion complex ($logK_{Al}$ *or* $logK_{Fe}$) in the following order: citric > oxalate > maleate > tartrate > acetate. Bolan et al. (1994) reported that the addition of organic acids caused the dissolution of soil components such as ferric and aluminium oxides and thereby decreased P adsorption by soil. They suggested that dissolution of Fe and Al oxides was caused by the complexation of these metal ions with the organic anions. Organic acids also increase the dissolution of phosphate rock, with the P solubilisation effect increasing with increased $logK_{Al}$ values. Fox et al. (1990) suggested that P release by oxalate was mainly via a ligand-exchange reaction, where oxalate forms chelates with the cations bonding the P thereby releasing the P into solution. Chelation involves the formation of two or more coordinate bonds between a molecule (the "ligand") and a metal ion, thereby creating a ring structure complex.

Although oxalate anions excreted from mycorrhizal coniferous roots are widely considered to have influence on the dissolution of insoluble P in soil, the extent to which oxalate influences soil P availability under natural conditions is uncertain, because oxalates are easily adsorbed by soil mineral surfaces, or degraded by microorganisms in the soil (Jones, 1998; Jones et al., 2003). In addition, the quantity and pattern of oxalate release by mycorrhizal fungi in natural systems has not been studied. Therefore, in-depth studies on the amount and processes of exudation of organic acids by mycorrhizal pine roots and its influence on the bioavailability of insoluble fertiliser and soil P are required to understand the root processes influencing P availability to trees.

Table 2.4 Stability constants of Al-organic acid complexes and solubility of common crystalline phosphate compounds

Stability constants for Al-organic acid complexes			
Organic acid	***Structure***	***LogK$_{Al}$***	***Reference***
Citric	$C_6H_8O_7$, tricarboxylic acid, one α-hydroxyl group	9.6	Bar-Yosef (1996)
Oxalic	$C_2H_2O_4$, dicarboxylic acid, no hydroxyl groups	7.3	Bar-Yosef (1996)
Malic	$C_4H_6O_5$, dicarboxylic acid, one α-hydroxyl group	5.4	Bolan et al. (1994)
Tartaric	$C_4H_6O_6$, dicarboxylic acid, two α-hydroxyl group	5.3	Bar-Yosef (1996)
Lactic	$C_3H_6O_3$, monocarboxylic acid, one α-hydroxyl group	2.4	Bolan et al. (1994)
Gluconic	$C_6H_{12}O_7$, monocarboxylic acid, one α-hydroxyl group	2.0	Motekaitis and Martell (1984)
Acetic	$C_2H_4O_2$, monocarboxylic acid, one α-hydroxyl group	1.5	Bar-Yosef (1996)
Formic	CH_2O_2, tricarboxylic acid, no hydroxyl group	1.4	Bar-Yosef (1996)
Solubility of common crystalline phosphate compounds				
Phosphate compound	***Equilibrium reaction***	***LogK$_{sp}$***	***Reference***	***Solubility (in water) (g/100g)****
$Ca(H_2PO_4)_2$	$Ca(H_2PO_4)_2(s) \leftrightarrow Ca^{2+} + 2H_2PO_4^-$	-1.14	Snoeyink & Jenkins (1980)	18
$CaHPO_4$	$CaHPO_4\ (s) \leftrightarrow Ca^{2+} + HPO_4^{2-}$	-6.6	Stumm & Morgan (1995)	0.14
β-$Ca_3(PO_4)_2$	β-$Ca_3(PO4)_2(s) \leftrightarrow 3Ca^{2+} + 2PO_4^{3-}$	-24.0	Snoeyink & Jenkins (1980)	0.02
$Ca_5(PO_4)_3OH$	$Ca_5(PO_4)_3OH(s) \leftrightarrow 5Ca^{2+} + 3PO_4^{3-} + OH^-$	-55.9	Snoeyink & Jenkins (1980)	b
$AlPO_4.2H_2O$	$AlPO_4.2H_2O(s) \leftrightarrow Al^{3+} + PO_4^{3-} + 2H_2O$	-21.0	Stumm & Morgan (1995)	b
$FePO_4.2H_2O$	$FePO_4.2H_2O(s) \leftrightarrow Fe^{3+} + PO_4^{3-} + 2H_2O$	-26.0	Stumm & Morgan (1995)	b

* b - Practically insoluble in 25°C; Aylward and Findlay (1994).

2.6.4 Soil phosphatase activity and its effect on soil organic P mobilisation

As discussed in section 2.4.2 of this chapter, the cycling of organic P has a large effect on P availability and long-term forest productivity. Organic P must be converted to inorganic P before it can be utilised by plants. The mineralisation of P_o in soils is mediated by phosphatase enzymes that hydrolyse C-O-P ester bonds. Plant roots, mycorrhizal fungi and bacteria produce phosphatases in soils (Haüssling and Marschner, 1989; Fox and Comerford, 1992b; Tarafdar and Marschner, 1994).

The phosphatase enzymes can be classified into five major groups (Tabatabai, 1994). These include the phosphoric monoester hydrolases (EC 3.1.3), phosphoric diester hydrolases (EC 3.1.4), triphosphoric monoester hydrolases (EC 3.1.5), enzymes acting on phosphoryl-containing anhydrides (EC 3.6.1), and enzymes acting on P-N bonds (EC 3.9). The acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) and alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) have been studied extensively (Tabatabai, 1994). The acid phosphatase activity in soil is mainly contributed by both plants roots (Dinkelaker and Marschner, 1992) and microbial action (Haüssling and Marschner, 1989; Dinkelaker and Marschner, 1992), while the alkaline phosphatase activity is mainly attributed to microorganisms (Tabatabai, 1994). The activities of both acid and alkaline phosphatases are sensitive to soil pH (Tabatabai, 1994; Staddon et al, 1998) and P concentration in soil (Hedley et al., 1982b; 1983; Kolari and Sarjala, 1995). Acosta-Martinez and Tabatabai (2000) reported that the acid phosphatase activity was negatively correlated with soil pH ($r=-0.69$) when lime was applied to an agricultural soil. Doumas et al. (1983) reported that acid phosphatase activity in the roots of *P. halepensis* seedlings was stimulated four-fold by phosphate deficiency.

Roots-induced increases in phosphatase activities in the rhizosphere soils have been widely reported. Chen et al. (2002) demonstrated that the roots of *P. radiata* seedlings, compared to ryegrass roots, induced greater alkaline phosphatase and phosphodiesterase enzyme activities. Greater phosphatase activities enhanced depletion of sodium hydroxide extractable P_o in the rhizosphere of radiata pine compared with ryegrass (Chen et al., 2002). Fox and Comerford (1992b) suggested that P_o might have

contributed to the P nutrition of slash pine growing on two forested Spodosols because significantly greater acid phosphatase activity was observed in the rhizosphere of the two soils. Haüssling and Marschner (1989) observed close correlations between the depletion of P_o in the rhizosphere soil of Norway spruce and acid phosphatase activity in an acid forest soil (Figure 2.6A).

The production of phosphatase by mycorrhizal fungi could increase the ability of host plants to obtain P directly from organic sources. Evidence for the ability of mycorrhizal fungi to produce phosphatase has been reported by many workers. Based on significant correlation between hyphal density and phosphatase activity, Tarafdar and Marschner (1994) suggested that hyphae of mycorrhizal fungi (such as *Glomus mosseae*) did produce significant extracellular acid phosphatase activity (Figure 2.6B). Koide and Kabir (2000) demonstrated that the hyphae of *Glomus intraradices* hydrolysed both 5-bromo-4-chloro-3-indolyl phosphate and phenolphthalein diphosphate. Alexander and Hardy (1981) showed that mycorrhizas of *P. sitchensis* possessed surface root phosphatase activity that was inversely correlated with the concentration of extractable P_o in the soil.

However, the production of phosphatase enzymes and their effects on organic P bioavailability are not always consistent in the literature. Dighton (1983) reported that the acid phosphatase activity in the rhizosphere soils of birch (*Betula pubescens*) seedlings was lower for mycorrhizal roots than non-mycorrhizal roots. But for pines (*P. contorta*) the phosphatase activity was not different between mycorrhizal and non-mycorrhizal roots. Cumming (1996) also reported greater acid phosphatase activity in non-mycorrhizal roots of pitch pine (*P. rigida*) than around roots colonised with any of the three ectomycorrhizal fungi, *Laccaria bicolor, Paxillus involutus* or *Pisolithus tinctorius*. The contrasting results obtained in the above studies might have been due to differences in soil type, species of mycorrhizal fungi, host plant, and plant age (Trolove et al., 2003), or due to the differences in the soil P stress. Phosphatase enzymes are inducible enzymes and their activities can be repressed by high available P concentrations in soil (Hedley et al., 1982b; 1983).

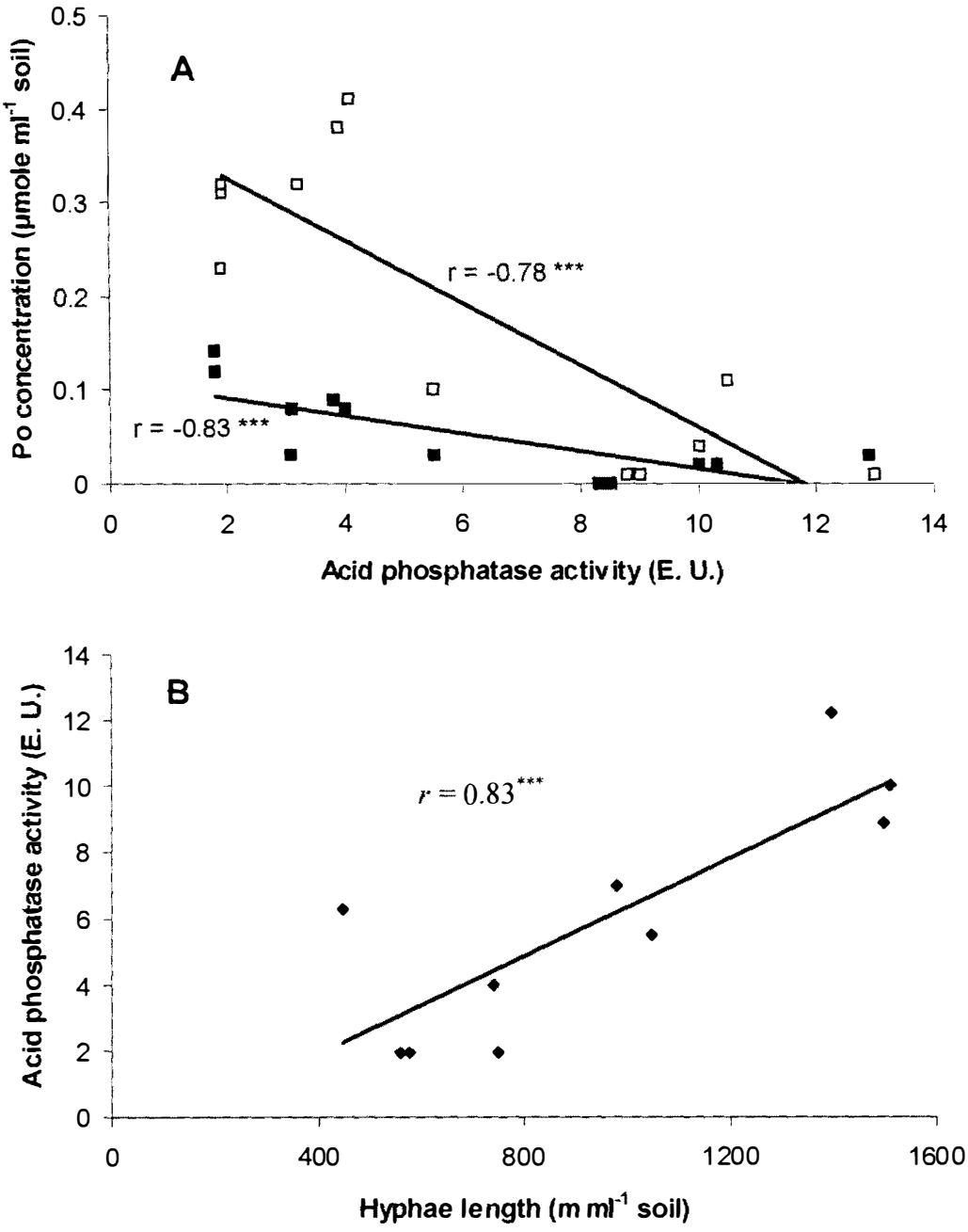

Figure 2.6 The correlation between acid phosphatase activity and organic P concentration (A) and between mycelial hyphae length and acid phosphatase activity (B) in the rhizosphere soil of 80-year-old Norway spruce [*Picea abies* (L.) Karst.]. (A) – Correlation between phosphatase activity and P_o concentration in water (□) and HCl extracts of the humus layer (■). (Redrawn from Haüssling and Marschner, 1989)

2.7 Measurement of mycorrhizal fungal hyphae density in soil

In the previous sections of this chapter it has been clearly shown that the hyphae of ECM fungi play an important role in effecting soil P bioavailability. However, there are no simple and accurate methods to quantitatively determine hyphal density in soil, particularly the active ECM hyphae. Common techniques currently being used to estimate the fungal hyphae density in soil are critically reviewed below.

2.7.1 Agar film techniques

One of the most widely used direct methods of determining fungal hyphae density is the "agar film technique" that was developed by Jones and Mollison (1948) and modified later by Nicholas and Parkinson (1967). In this technique, a soil suspension is mixed with fluid water agar and a small amount is placed on a haemocytometer and allowed to solidify with the cover slip pressed in position. The agar strip is then washed from the haemocytometer, transferred to a slide and allowed to dry. After staining the agar strip, the total length of hyphae in a defined area of the preparation is measured under a microscope. This technique allows measurement of lengths of fungal mycelium in a unit weight, or volume of soil. Nicholas and Parkinson (1967) reported that for quantitative determination of mycelium concentration, the measurement of mycelial lengths by agar film technique was the most accurate method. However, the procedure involved in this technique is very time consuming, especially when a large number of samples have to be analysed (Nicholas and Parkinson, 1967). Additionally, this method cannot differentiate between living and dead mycorrhizal mycelium (Skinner and Bowen, 1974b). Also, the hyphal length density determined would include the hyphae from saprophytes as well (Hanssen et al., 1974).

2.7.2 Membrane filter technique

The membrane filter technique developed by Hanssen et al. (1974) and modified by Abbott and Robson (1985) and Li et al. (1991) involves elaborate processing of the sample such as dispersion of the sample in liquid, blending, centrifugation, wet sieving, staining and microscopic counting. Compared to agar film method, the membrane filter

technique eliminates the procedures for the preparation of agar film, thereby reducing much of the time used for measurement. Similar to the agar film technique, the membrane filter method also has the problem that living and dead mycorrhizal mycelium cannot be distinguishable. Additionally, although this method showed promise for counting single hyphae randomly distributed in a microscopic field, it is unsatisfactory for measurements in aggregated strand material. The membrane filter is expensive, increasing the cost of measurements.

2.7.3 Counting root-tips

As the measurement of hyphal density is difficult, counting mycorrhizal root tips has been used as an indirect method to assess the degree of ectomycorrhizal infection (Smith and Read, 1997). Harley and McCready (1952) estimated that 40% of the weight of ectomycorrhizal root tip was due to the fungal sheath. This percentage has been used to calculate the fungal biomass in ECM infected roots (Marks and Kozlowski, 1973; Harley and Smith, 1983; Vogt et al., 1991). However, this value does not give quantitative information on the extent of hyphal penetration into the soil, which is important in assessing soil P uptake by plants.

2.7.4 Biochemical techniques

Biochemical methods can be used to indirectly determine the fungal biomass in soil, by assaying biochemical markers, such as chitin (Ride and Drysdale, 1972; Pacovsky and Bethlenfalvay, 1982), or ergosterol (Nylund and Wallander, 1992), which are compounds present in the fungi but not in the host. The amounts of these biochemical markers are assumed to be proportional to the total fungal biomass. Using these markers, it is possible to get a quantitative measure of the fungal biomass in mycorrhizal roots as well as in the external mycelium in the soil. For example an ergosterol assay could be used to estimate the biomass of living fungi (Nylund and Wallander, 1992). Nevertheless, there are some fundamental problems relative to the biochemical approaches. For instance, although the chitin content may be assumed to be approximately proportional to the total amount of fungal cell wall, the amount of the cell wall is certainly not proportional to the amount of cytoplasm (Nylund and

Wallander, 1992). As a result, the fungal biomass determined by biochemical techniques may over- or underestimate the real value. Also, similar to the direct approaches the biochemical techniques do not exclude the biomass of saprophytic fungi.

2.7.5 Other techniques

New techniques are being tested in laboratories to separate fungal tissues into live, senesced and dead categories (Vogt et al., 1991). DNA analysis (Haese and Rothe, 2003; Sundaram et al., 2004) seems to hold some promise as it enables hyphae to be identified according to species. However, it is required to develop this technique further especially for use in field studies.

Isotopic materials, such as ^{14}C, have been widely used to study C allocation in mycorrhizae. By labelling host plants with $^{14}CO_2$, autoradiographs may be used to determine the distribution of assimilated-^{14}C in the soil mycelium (Finlay and Read, 1986). Alternatively the ^{14}C activities in dissected fungal hyphae (Miller et al., 1989; Norton et al., 1990; Rygiewicz and Anderson, 1994) can be determined and the active mycorrhizal distribution may be visualised. However, assessing hyphal biomass by autoradiography is not quantitative, and it is very difficult to collect active fungal hyphae from soil if hyphal biomass is estimated by dissecting fungal hyphae.

Recently Turner (2001) attempted to determine the endomycorrhizal hyphae and root activities by pulse labelling the shoots of a clover/ryegrass mix with $^{14}CO_2$ and measuring ^{14}C activity in root and active endomycorrhizal hyphae by using a modified rhizosphere container study technique. The study however did not produce the conclusive results.

Considering the problems associated with the current methods, new approaches are needed to be developed in the future for satisfactory estimation of mycorrhizal hyphae activities in soils.

2.8 Summary and research needs

The review of literature indicated that conifers are different from agricultural crops in soil P acquirement. This is primarily because conifers have P-efficient ectomycorrhizal roots that are considered to play a major role in the P uptake and cycling in forest ecosystems. Nevertheless, the processes involved in ectomycorrhizal conifer roots effecting soil P release is understood less than the processes associated with endomycorrhizal roots of agricultural plants. In addition the majority of P in forest soils is found in the soil organic fraction. The mineralisation of soil P_o is probably the prime process maintaining P availability in forest ecosystem. Whereas ectomycorrhizal roots of *P. radiata* are likely to be involved in the mobilisation of soil P_o, the turnover of mycorrhizal roots, and mineralisation of P_o by phosphatase enzymes produced by mycorrhizas and rhizosphere microbial activity has received little quantitative study.

In New Zealand, soil P deficiency is reported in some *P. radiata* plantations. The application of reactive phosphate rock has been recommended to correct P deficiencies in these soils. However, the interaction of the phosphate rock and the associated mycorrhizas on *P. radiata* growth and development has not been studied in detail. The fate of the applied phosphate rock in marginally acidic, high P retention forest soils such as Allophanic Soils is also unclear. Additionally, few experimental data are currently available on the effects of rhizosphere processes on the dissolution of phosphate rock under *P. radiata.*

The solubilisation and mobilisation of insoluble soil and fertiliser P (e.g. phosphate rock) need to be understood for the efficient management of P nutrition of *P. radiata.* Some specific areas in relation to the rhizosphere processes influencing soil and fertiliser P availability to *P. radiata* need to be studied in-depth. These areas are listed below:

1) Both radiata pine roots and associated mycorrhizal fungi are able to induce changes in rhizosphere soil properties. The influence of external mycorrhizal hyphae on soil P bioavailability and changes in soil P fractions requires study.
2) Mycorrhizal infection on *P. radiata* seedlings has been proved to be able to stimulate seedling growth. The differences in specific fungal species associated with radiata pine and their influence on rhizosphere and soil properties, especially P bioavailability, need to be studied.

3) Although mycorrhizal hyphae influence soil properties and P uptake by *P. radiata*, most researchers have not measured hyphal density in soil. This is probably because there is currently no simple and accurate method available for this measurement. Therefore such techniques need to be developed.
4) Oxalate anions excreted from mycorrhizal coniferous roots are widely considered to be able to mobilise inorganic P in soil. However, the extent to which oxalate influences soil P availability under natural conditions is still uncertain.
5) Available information on the production of phosphatase enzymes by mycorrhizal fungi and the effects of phosphatases on organic P mineralisation are not always consistent. Therefore, the role of the ectomycorrhizal phosphatases of conifer in the cycling of P in forest soils warrants further investigation.

CHAPTER 3

The mobilisation and fate of soil and rock phosphate in the rhizosphere of ectomycorrhizal *Pinus radiata* seedlings in an Allophanic Soil*

3.1 Introduction

In New Zealand, *Pinus radiata* plantations on Allophanic Soils (Andosols), which contain reactive short-range order allophane capable of fixing large quantities of added P (Parfitt, 1989), have naturally low plant-available soil P status (e.g. Bray-2 P and Olsen P lower than 4 μg P g^{-1} soil at 0 – 100 mm soil depth). However, these soils commonly have more than 60% of their total P in organic form (New Zealand Soil Bureau, 1968), suggesting that considerable mobilisation of soil organic P (P_o) is taking place in the *P. radiata* rhizosphere to meet the P demand of the tree.

Reactive phosphate rock (RPR) is widely recommended as a suitable P fertiliser applied to correct P deficiency in *P. radiata* plantations (Payn et al., 1998). RPR dissolution is recognised to be slow in soils with pH > 6.0 and annual rainfall < 800 mm (Bolan et al., 1990). Allophanic Soils in New Zealand commonly have pHs in the range of 5.5 to 6.0 (e.g. Kaweka forest pH 5.8), which is close to the marginal pH range for RPR use. Several authors have reported that proton (H^+) excretion by roots is a major process by which plants can acquire P from RPR (Hinsinger and Gilkes, 1997). RPR particle size (Kanabo and Gilkes, 1988), soil P fixing capacity (Kanabo and Gilkes, 1987) and soil exchangeable Ca (Bolan et al., 1997) are also reported to be important factors influencing RPR dissolution.

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

This raises two issues worth investigating when radiata seedlings are transplanted into Allophanic Soils: (i). It is known that the excretion of organic anions (such as oxalate) by pine roots and ectomycorrhizas may play an important part in mobilisation of both soil organic P (P_o) and inorganic P (P_i), which raises the question how much native soil P can be mobilised in the seedling rhizosphere? (ii). After fertilisation of these Allophanic Soils with RPR, what is the rate of P release and does the dissolved P remain plant available?

The objective of this chapter was to test the hypotheses of that: (1) *P. radiata* rhizosphere processes are able to mobilise poorly plant-available forms of soil-P and PR-P; (2) these rhizosphere processes are associated with ectomycorrhizal root released organic acids or phosphatase activities; (3) RPRs are effective P fertiliser improving radiata growth in Allophanic Soil. To achieve these, a pot trial was designed to investigate the mobilisation of soil P in the rhizosphere of *P. radiata* seedlings by using soil P fractionation to identify changes in P forms in rhizosphere soil. Bulk soil and rhizosphere soil pH, phosphatase enzyme activities, oxalate concentration were measured to indicate the nature of P solubilisation processes. These measurements were repeated in soil fertilised with an RPR.

3.2 Materials and methods

3.2.1 Soil, fertilisers and seedlings

The soil used in the study (Orthic Allophanic Soil, Andosols; Hewitt, 1992) was sampled from an unfertilised site at Kaweka Forest, located approximately 70 km NW from Hastings, New Zealand. The soil was collected from an area of 2×2 m^2 to a depth of 100 mm after removing undecomposed particulate pine needles and tree prunings from the soil surface. The soil was air-dried and passed through a 2 mm mesh-sized sieve. Finely divided Sechura phosphate rock (SPR) (75 – 150 µm of particle size) was used as the test RPR fertiliser. Selected properties of the soil and SPR are presented in Table 3.1.

Table 3.1 Characteristics of the soil and Sechura phosphate rock used in the study

Soil properties			
Total P (μg P g^{-1} soil)	205	Total C (%)	4.2
Olsen P (μg P g^{-1} soil)	0.9	Total N (%)	0.22
Bray-2 P (μg P g^{-1} soil) (0.03 *M* NH_4F + 0.1 *M* HCl)	1.8	CEC (mol_c kg^{-1} soil) (1 *M* NH_4OAc, pH 7 extraction)	0.14
P retention (%)	90	Exchangeable Mg (mol_c kg^{-1} soil)	0.006
pH (1:2.5 soil:H_2O)	5.8	Exchangeable Na (mol_c kg^{-1} soil)	0.001
pH buffer capacity (at pH 5.5 – 6.5, mol H^+ or OH^- kg^{-1} soil pH^{-1})	0.02	Exchangeable K (mol_c kg^{-1} soil)	0.002
		Exchangeable Ca (mol_c kg^{-1} soil)	0.028
		SO_4^{2-} (μg S g^{-1} soil)	47.3
Properties of Sechura phosphate rock (g kg^{-1})			
Total P	139	Water soluble P	0.04
2 % citric acid soluble P	63.2		

One year-old seedlings of *P. radiata* (D. Don.), produced at Norcarthern Nursery (near Hastings), were used in this experiment. The seedlings were dominantly infected by the ectomycorrhizal fungus *Hebeloma crustuliniforme* (Bull. ex St. Am.) Quél., as a result of applying slurry of this fungal sporocarps to seedling beds under nursery conditions. *Laccaria laccata* (Scop. ex Fr.) Berk. & Br., *Endogone flammicorona* Trappe & Gerdemann, and *Tuber* sp. were also suspected to be present but they were not conclusively identified. The shoots of the seedlings had been trimmed to a height of approximately 300 mm and the fast growing roots of the seedlings had been cut to 50 mm length before planting in pots.

3.2.2 Experimental design and management

Soil (1 kg) was mixed thoroughly with ground SPR at the rates of 0, 50, 100 and 150 μg P g^{-1} soil. Fertiliser-grade calcium ammonium nitrate (CAN, 21%) and analytical-grade potassium chloride (KCl) were also mixed with the soil to provide 100 μg each of N and K per g soil. The soils were then transferred to 1 litre capacity pots and one seedling per pot was planted after carefully removing the original soil attached to the roots. After 5 months growth of the seedlings, another application of N and K nutrients at the same rate of CAN and KCl as the *initial* application was supplied in solution to each pot.

Treatments were replicated 4 times and arranged in a randomised complete design in a glasshouse maintained at 16 ± 2°C minimum (night) and 28 ± 3°C maximum (day) temperatures. The pots were watered using distilled water to "pot field capacity" (moisture content of soil after the free water drained from pots) at 2-day intervals by weighing the pots. The positions of the pots within the treatments were randomly changed weekly so that the plants in all treatments in each replicate received equal amounts of sunlight.

3.2.3 Soil, plant and solution sampling

3.2.3.1 Soil sampling

Seedlings were harvested after 10 months of growth (28 July 2000 – 15 May 2001). To enable sampling of soil solutions at approximately constant soil moisture content, 48 h

before harvesting, the soil moisture in all pots was adjusted to "pot field capacity".

At the harvest, the root-free soil at the surface 20 mm depth in the pots was discarded. The rhizosphere soil (approximately 110 to 130 g soil) was collected according to the method of Wang and Zabowski (1998). The whole seedling was removed from the pot with minimum injury to its roots, by shaking the roots until the soil not tightly adhering to the roots was removed and then collecting the soil closely adhering to the root system by vigorously shaking the roots. The "bulk" soil (soil not influenced by roots) was collected from upper areas in the pots where there were no roots.

3.2.3.2 Soil solution sampling and analyses

Soil solution was removed from a portion of moist soil within 6 h of sample collection, using a double-centrifuge-tubes technique (centrifuged for 0.5 h at 10000 rpm and 4°C; filtered immediately through a 0.45 μm millipore filter) (Wang and Zabowski, 1998). The pH of soil solutions was immediately determined, and oxalate concentration in the solutions was analysed within 24 h.

3.2.3.3 Seedling shoot and root sampling and measurement

The shoots of the seedlings were separated from the root system at the first lateral root level, and the heights of shoots were recorded to the stem tip. The shoots were sampled at different positions: upper part (top 50 mm of new growth from the time of planting in pots), mid part (the remaining new growth from the time of planting in pots) and lower part (original shoots before planting).

The total root length and ECM root tip numbers were calculated from the root length and ECM tips measured in a sub-sample of 3 randomly selected lateral roots. The root length was measured using an inter-gridline technique (Newman, 1966). ECM infection is associated with swollen short root tips (Brundrett et al., 1996), the majority of which are commonly forked (Chu-Chou and Grace, 1983). When randomly selected swollen short root tips were stained by trypan blue and examined under a microscope, it was found that more than 90% of the tips had ECM infection with visible hyphae. Therefore,

swollen short tip numbers were counted manually to obtain an approximate measure of the extent of ECM infection (Newton and Pigott, 1991).

3.2.4 Plant, soil and solution analyses

Plant samples were dried to constant weight at 65°C in an oven and milled to < 1 mm particle size. Herbage total P content was determined by standard procedures (Twine and Williams, 1971) after Kjeldahl digestion (Jackson, 1958). The soil pHs were determined using a pH meter with 1: 2.5 weight ratio of the soil to deionised water after equilibrating for 16 hours (Blackmore et al., 1987).

Acid and alkaline phosphatase enzyme activities in soil were determined using the methods described by Tabatabai (1994). The procedures described for assay of the phosphatase activities were based on colorimetric estimation of the *p*-nitrophenol released by phosphatase activity when soils were incubated with buffered (pH 6.5 for acid phosphatase activity and pH 11 for alkaline phosphatase activity) sodium *p*-nitrophenyl phosphate solution and toluene. The enzyme activities are expressed as µg *p*-nitrophenol released per g dry-soil per hour.

Soil P was fractionated using the method of Hedley et al. (1994) by first extracting 0.5 g soil with cation and anion exchange resin membranes to determine resin-P (plant available-P), followed by 0.1 *M* NaOH extraction and determining NaOH-P_i (inorganic P associated with positively-charged oxide surfaces) and NaOH-P_o (labile organic P) in the extract. The soil residue was further extracted by 0.5 *M* H_2SO_4 to determine H_2SO_4-P (apatite-like P) followed by heating the residue with concentrated H_2SO_4 and H_2O_2 to determine the residual P (non-extracted P).

The amount of SPR dissolution in soil was determined by the method of Tambunan et al. (1993). In this method SPR treated and untreated soils were pre-extracted with 0.5 *M* NaCl / TEA (triethonalamine), pH 7.0, and 1 *M* NaOH to remove dissolved SPR-P. The amount of undissolved SPR residues was then estimated from the difference in the amount of P extracted by 0.5 *M* H_2SO_4 between the SPR treated and untreated soils. The

percentage of dissolved P was calculated from the difference in the amounts of P added and amount of undissolved P according to the following equation (Zoysa et al., 1999):

$$\% \text{ dissolution of P} = 100 \times \left\{1 - \frac{[0.5\ M\ H_2SO_4 \text{ extractable P ((soil + PR fertiliser)-(soil alone))}]}{\text{Fertiliser P added}}\right\}$$

The oxalate concentrations in the soil solutions were analysed by the Environmental Chemistry Laboratory, Landcare Research New Zealand Ltd Palmerston North, using a Waters ion chromatographic system with electrical conductivity detection method for determining oxalate peaks. The chromatography column used was an IC-PAK A anion chromatography column (Waters, 1989).

3.2.5 Statistical analyses of data

Significant differences in seedling parameters between the P rate treatments were tested using a one-way ANOVA procedure. Differences in soil properties between treatments were analysed using a two-way ANOVA procedure (4 P rates × 2 soils - bulk and rhizosphere soils). Significant difference between treatment means were established by calculating LSDs at a significance level of $P < 0.05$.

3.3 Results and discussion

3.3.1 Effect of SPR on seedling growth and P uptake

Increasing rate of SPR significantly ($P < 0.05$) increased seedling root and total dry matter (DM), P concentrations in new shoot growth (upper and mid parts) and P uptake in the different seedling parts (Plate 3.1; Table 3.2). Only the highest rate of SPR (150 μg P g^{-1} soil) significantly ($P < 0.05$) increased the height of the seedlings (Table 3.2). Compared with the unfertilised treatment, the application of SPR produced 1.1 – 1.4-fold, 1.1 – 1.3-fold and 1.3 -1.7-fold increases in root DM, shoot DM and total P uptake, respectively. The growth and P uptake response of the seedlings to SPR application is

consistent with the low plant-available P concentration in the native soil and the measured dissolution of the SPR. The Bray-2 P concentration in the native soil was 1.8 μg g^{-1} soil (Table 3.1), which is lower than the critical Bray-2 P concentration of 12 μg g^{-1} soil suggested for mature *P. radiata* trees (Ballard, 1973). Additionally, the unfertilised seedlings had upper shoot P concentrations (0.92 ± 0.1 mg P g^{-1} DM) below 1.2 mg P g^{-1} DM (Table 3.2), the concentration considered to be the deficiency threshold for *P. radiata* trees (Will, 1978; 1985). The P concentration of the youngest (upper part, Table 3.2) shoot tops changed most with P supply. This is consistent with the current needle sampling procedure recommended by New Zealand Forest Research for diagnosis of P nutrition status of *P. radiata* in the field, where fully-grown needles in the second-order branches (i.e. the new growth) are sampled for nutrient concentration determination.

Plate 3.1 Effect of Sechura PR application rate on seedling growth

Table 3.2 The effect of different rates of Sechura phosphate rock (SPR) on the (A) growth and (B) P nutrition of *P. radiata* seedlings in the 10-months pot trial (data presented as the mean ± SE. Numbers with the same letters are not significantly different at $P < 0.05$)

A. Seedling growth parameters

P rate	Dry matter weight (g pot^{-1})			Height	Total ECM	Root length	Root tip density
(µg P g^{-1} soil)	Shoots	Roots	Total	(mm)	root tips	(m pot^{-1})	(tip m^{-1} root)
0	15.8±2.2 a	5.1±0.4 c	20.9±2.6 b	453±38 b	3999±215 b	28±0.65 c	143±4 ab
50	17.0±3.0 a	5.8±0.5 bc	22.8±3.2 ab	483±24 ab	4917±399 a	30±1.46 c	162±11 a
100	18.5±2.3 a	7.0±1.0 a	25.5±2.4 ab	525±42 ab	4366±456 ab	45±2.95 a	99±14 c
150	20.1±4.0 a	6.8±0.6 ab	26.7±2.9 a	540±47 a	4885±262 a	39±1.39 b	128±11 b

B. P nutrition in seedling tissues

P rate	P concentration (g kg^{-1})				P uptake (mg pot^{-1})		
(µg P g^{-1} soil)	Upper part shoots	Mid part shoots	Lower part shoots	Roots	Shoots	Roots	Total (shoots + roots)
0	0.92±0.10 b	0.76±0.05 b	0.45±0.05 a	0.88±0.06 a	9.92±1.69 b	4.49±0.25 b	14.41+1.84 c
50	1.25±0.10 ab	0.87±0.05 ab	0.49±0.10 a	0.96±0.15 a	12.59±2.73 ab	5.60±1.01 ab	18.19+3.14 ab
100	1.36±0.17 a	0.91±0.09 ab	0.53±0.06 a	0.88±0.09 a	14.93±3.48 ab	6.14±1.05 ab	21.07+3.33 ab
150	1.41±0.10 a	0.99±0.06 a	0.56±0.04 a	1.04±0.20 a	17.08±2.9 a	6.99±1.31 a	24.07+0.32 a

3.3.2 Effect of SPR on ECM root tips

Application of SPR significantly ($P < 0.05$) increased root length and the total number of ECM root tips (Table 3.2), but the increases in ECM tip density (tips m^{-1} root) were not consistent with the increase in the rate of SPR application. The ECM tip density was higher at the P rate of 50 μg P g^{-1} soil compared to that at the rates of 100 and 150 μg P g^{-1} soil. Decreased ECM root tip density with increasing P supply has also been observed by Newton and Pigott (1991) with oak and birch seedlings. Marx et al. (1977) reported that high levels of N and P in soil decreased sucrose content of short roots of loblolly pine (*Pinus taeda*) and therefore decreased their susceptibility to ectomycorrhizal development by *Pisolithus tinctorius*. The faster rate of root growth at high P rates could be another reason for the decrease in the ECM tip density on the roots.

Although short and split root tips are not a quantitative indicator of mycelial activity, the slightly but insignificantly lower ECM root tip density at zero P rate than at 50 μg P g^{-1} soil (Table 3.2) may indicate that P deficiency in the unfertilised soil may have interrupted the normal pattern of ECM infection (namely the root infection by hyphae from the root cortex) and the density of mycelia. A small addition of P fertiliser to P deficient soil may stimulate (rather than inhibit) the development of ECM. Lamb and Richards (1974) and Ballard (1972) also reported similar observations in extremely P deficient pine plantations.

3.3.3 Root induced changes in pH in soil and soil solution

The soil and soil solution pH in the rhizosphere soil was significantly ($P < 0.05$) lower than those in the bulk soil for all P rates (Figure 3.1). Phosphorus application rate had no influence on the pH differences.

The lower soil pH in the *P. radiata* rhizosphere soil may be due to the seedlings taking up N predominantly in the cationic NH_4^+ as opposed to NO_3^- from soil (Olykan and Adams, 1995) or H^+ may have been released as a companion cation when oxalates are excreted by ECM roots (Jones, 1998; Trolove et al., 2003; see next section).

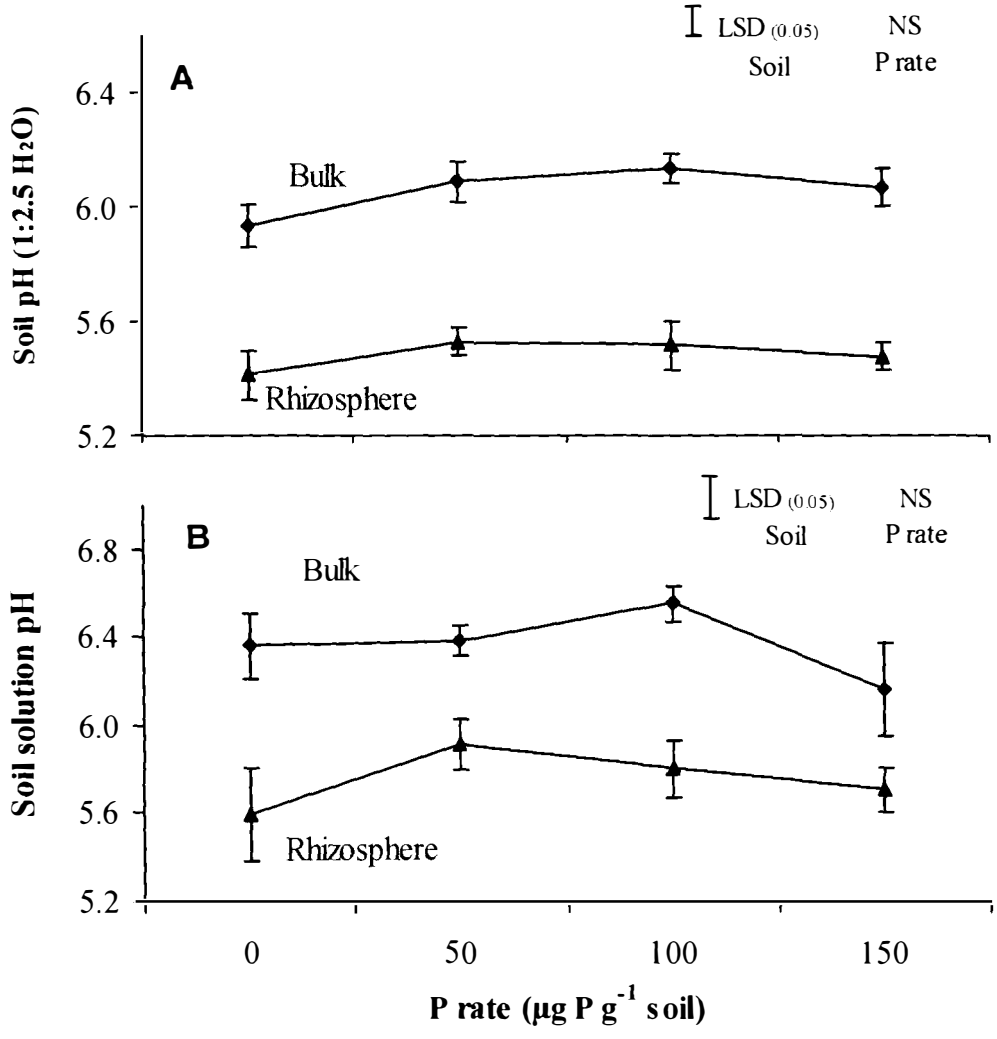

Figure 3.1 Effects of P rates on soil pH (A) and soil solution pH (B). Vertical lines across data points show SE of means.

3.3.4 Oxalate excretion by roots

Malajczuk and Cromack (1982) reported that ECM in *P. radiata* is able to release large quantities of oxalate. DeLucia et al. (1997) also reported that oxalate concentrations were highest for ponderosa pine seedlings supporting the greatest level of mycorrhizal association and speculated that fine root tips may have been the primary source of rhizosphere oxalate. Rhizosphere-released oxalate is likely to increase soil P mobilisation by chelating Fe and Al from Fe- and Al-P complexes (Jones, 1998).

In this study, no statistical difference in oxalate concentration was found between the rhizosphere and the bulk soil, although mean value of oxalate concentrations was higher in rhizosphere soil solutions (0.30 – 0.38 mg l^{-1}) than that in the bulk soil solutions (0.15 – 0.28 mg l^{-1}). This lack of any significant difference in oxalate concentrations may be explained by the variation in measured values between samples. Oxalate remaining in soil solution is a fraction of the total amount of oxalate released and is highly variable (Jones, 1998) because varying proportions of oxalate released by ECM could have been adsorbed by soil or decomposed by microorganisms. In spite of these losses of oxalate from soil solution, the measured oxalate concentrations were similar to those reported by others (Jones, 1998). The rate of application of SPR had no effect on the oxalate concentration in the rhizosphere.

Froidevaux and Kälin (1981) have reported calcium oxalate accumulation in the ectomycorrhizal fungus *Hebeloma crustuliniforme* (Bull. ex St. Am.) Quél. on roots of *Fagus sylvatica* L. However, in our study there was no relationship between ECM root tip density and oxalate concentration in the rhizosphere. In addition to the reasons given in the previous paragraph for the high variation in oxalate concentration, another reason for the absence of any relationship between ECM root tip density and oxalate concentration could be the narrow range of ECM root tip density in this study (Table 3.2). Perhaps such relationships would have become evident, had there been seedlings with no ECM fungal inoculation included in the study. It is also possible that the oxalate released by mycorrhizal roots may be more related to hyphal activity than root tip density.

3.3.5 Root induced changes in soil phosphatase activities

Both acid and alkaline phosphatase activities were significantly ($P < 0.05$) higher in the rhizosphere soil than in the corresponding bulk soil, but there was no relationship between the phosphatase activities and ECM root tip density. There was also no effect of P rate on enzyme activity as may be expected of these adaptive extracelluar enzymes that can be induced by P deficiency (Häussling and Marschner, 1989) (Figure 3.2). It must be remembered, however, that even in the SPR fertilised soil, soil P status was not excessive (see later discussion). Thus end product (P_i) repression of phosphatase synthesis may not have been fully achieved.

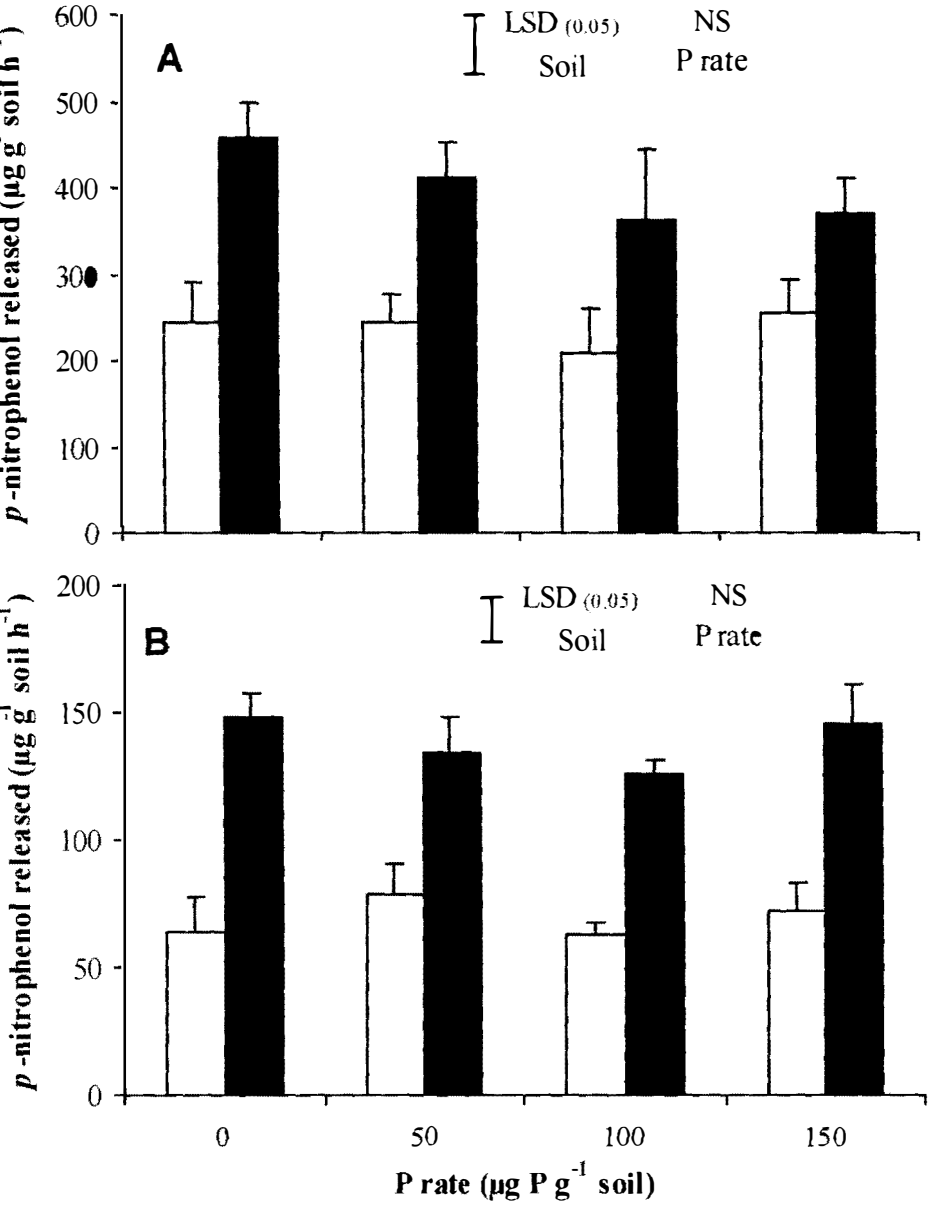

Figure 3.2 Effects of P rates on the acid (A) and alkaline (B) phosphatase activities in the rhizosphere (closed bars) and the bulk (open bars) soils where *P. radiata* seedlings were grown for 10 months. Vertical lines on top of bars show SE of means.

The higher acid phosphatase activity in the rhizosphere soil compared to the bulk soil is expected to originate from roots and soil microorganisms (Häussling and Marschner, 1989), while the higher alkaline phosphatase activity in the rhizosphere soil is mainly attributed to soil microorganisms (Tabatabai, 1994). The higher phosphatase activities in the rhizosphere were associated with a significant ($P < 0.05$) decrease in the 0.1 *M* NaOH extractable P_o fraction in the rhizosphere soil, which will be discussed later.

3.3.6 Change in soil P fractions

3.3.6.1 Addition of SPR

Addition of SPR-P caused marked increases in all soil P fractions (Figure 3.3 to Figure 3.5). The marked increases in resin-P (Figure 3.3A), NaOH-P_i (Figure 3.3B) and NaOH-P_o (Figure 3.4A) with increasing SPR application rate indicate significant dissolution and redistribution of SPR-P. For both rhizosphere and bulk soils, very high dissolution of SPR (81 - 84% in bulk soils; 84 – 91% in rhizosphere soils) was recorded 10 months after SPR application to the soil (Figure 3.6). This very high % dissolution of SPR was encouraged by the very fine particle size of SPR used (75 to 150 μm), the mildly acidic soil pH, very high soil P retention (90%), low Ca saturation of the soil (Table 3.1) and the long period of dissolution (10 months) in this trial (Kanabo and Gilkes, 1987; 1988; Bolan et al., 1997). Little dissolved SPR-P remained in the easily plant-available resin-P pool, while most dissolved P was redistributed to 0.1 *M* NaOH-P_i fraction (30 – 32% in rhizosphere soils). This shows that the dissolved SPR-P_i was sorbed to hydrous oxide surfaces of Al and Fe and allophane in the study. Hedley et al. (1994) and Zoysa et al. (1998b) also found the same fate of dissolved P from application of RPRs in weathered Ultisols.

The dissolution of SPR in the rhizosphere soil was slightly ($P < 0.05$) higher than that in the bulk soil (Figure 3.6). The higher dissolution of SPR in the rhizosphere soils may be due to the increased soil acidity in the rhizosphere soil (Figure 3.1) and the removal of dissolved products of SPR (such as P and Ca) by plant uptake. The % dissolution of SPR (y) was negatively correlated to soil pH (x) ($y = -7.13x + 126$; $r = -0.61$, $P < 0.01$) for both bulk and rhizosphere soils.

The % dissolution of SPR in the soil significantly ($P < 0.05$) decreased with increasing SPR-P rate (Figure 3.6). This was as expected for a diffusion limited dissolution process (Kirk and Nye, 1986; Zoysa et al., 1999).

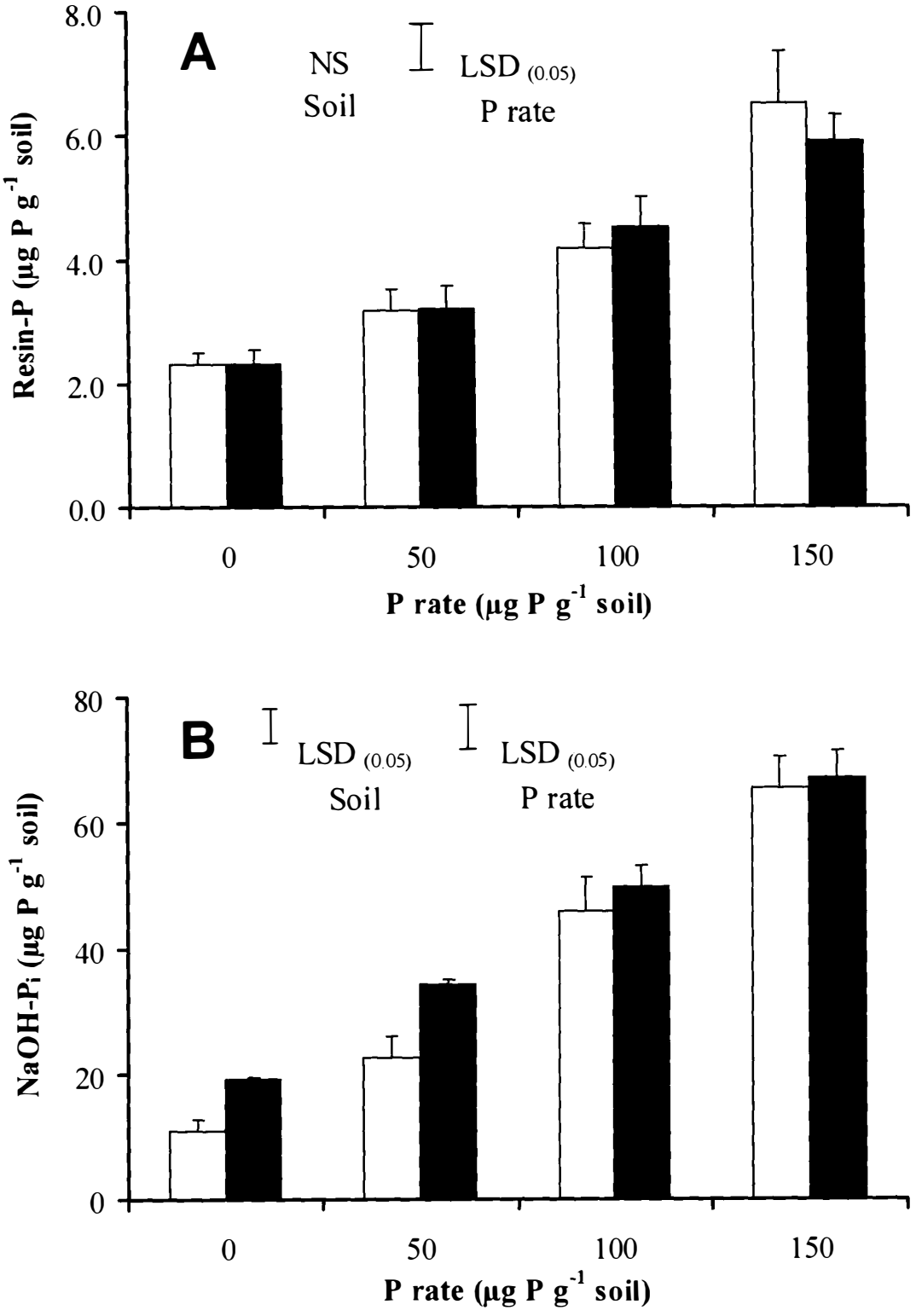

Figure 3.3 Effects of P application rates on soil resin-P (A) and NaOH-P_i (B) in the rhizosphere (closed bar) and the bulk soils (open bar). Vertical lines on top of bars show SE of means.

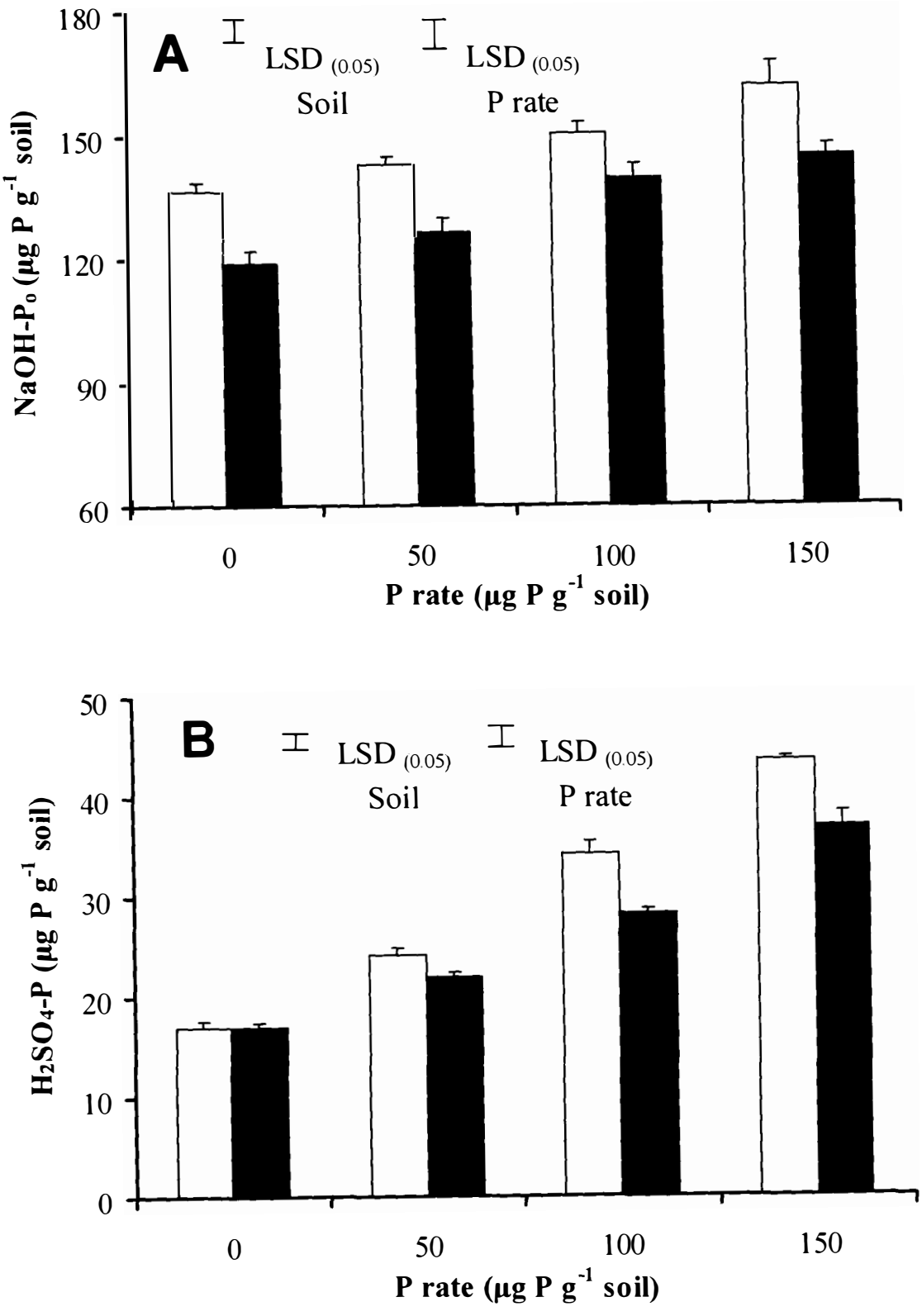

Figure 3.4 Effects of P application rates on soil NaOH-P_o (A) and H_2SO_4-P (B) in the rhizosphere (closed bar) and the bulk soils (open bar). Vertical lines on top of bars show SE of means.

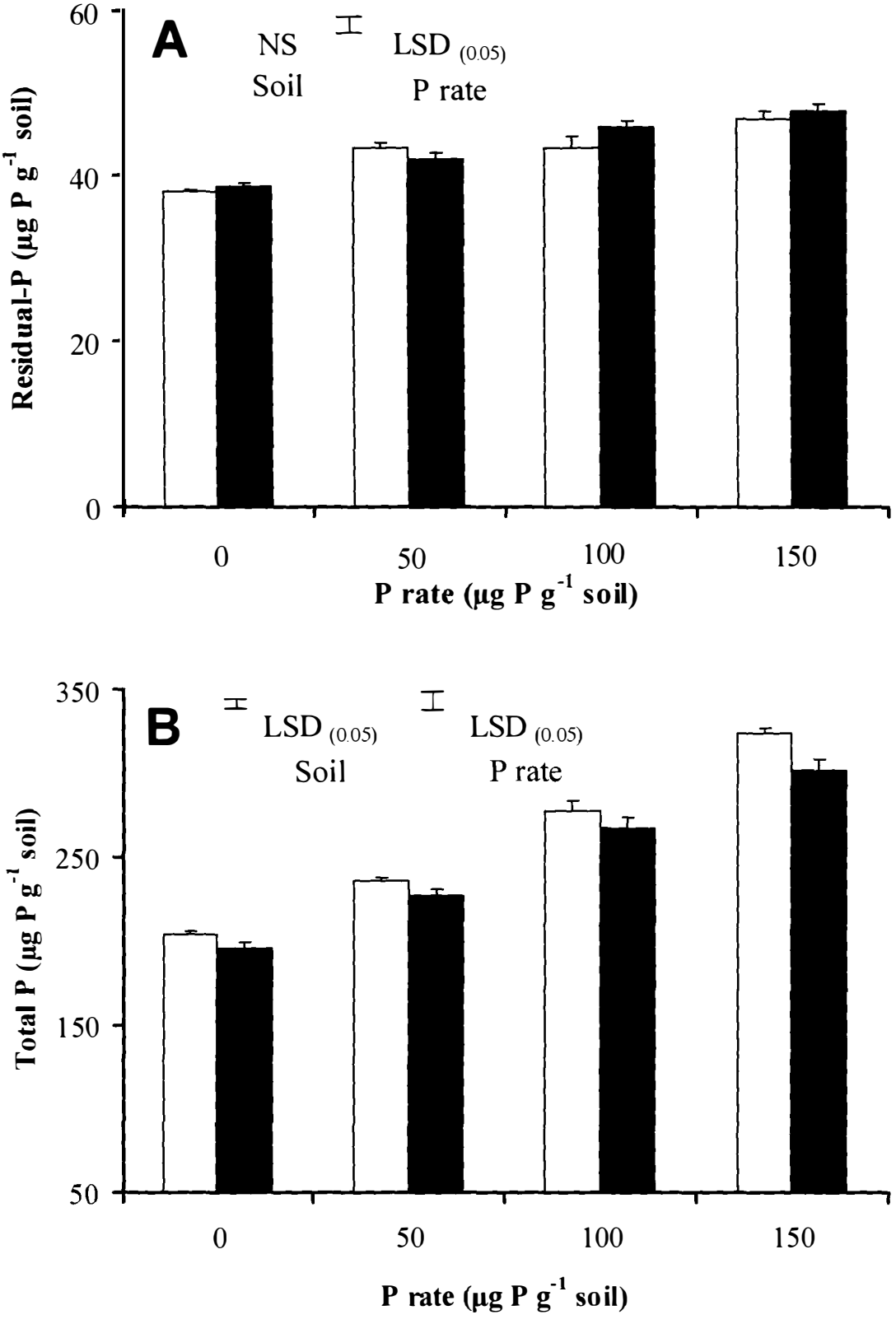

Figure 3.5 Effects of P application rates on soil residual-P (A) and total-P (B) in the rhizosphere (closed bar) and the bulk soils (open bar). Vertical lines on top of bars show SE of means.

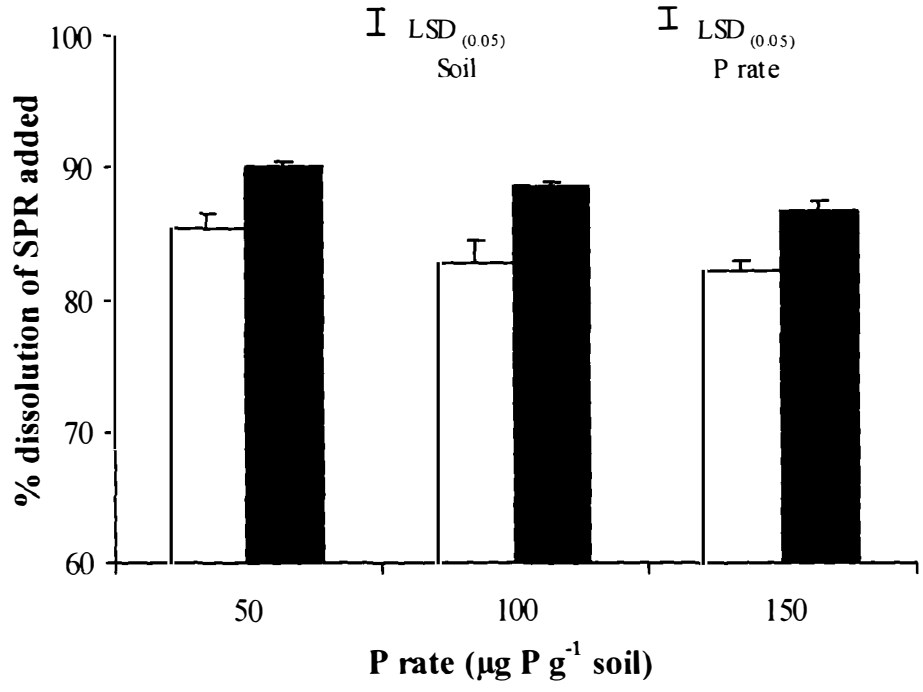

Figure 3.6 Sechura PR dissolution in soils where *P. radiata* seedlings were grown for 10 months. Open bars – bulk soil; Closed bars – rhizosphere soil; Vertical lines on top of bars – SE of means.

3.3.6.2 Root induced effects

As well as the rhizosphere soil having greater SPR dissolution rates (Figure 3.6), rhizosphere processes caused significant changes in different soil P fractions. The root-induced changes in P fractions were mainly observed in 0.1 *M* NaOH-P_i (Figure 3.3B), 0.1 *M* NaOH-P_o (Figure 3.4A) and 0.5 *M* H_2SO_4-P (Figure 3.4B); all of them are traditionally considered as sources of P not immediately available to plant.

The 0.1 *M* NaOH-P_i is considered to be moderately labile or gives rise to slow turnover of P (Hedley et al., 1982b). In this study, 0.1 *M* NaOH-P_i constitutes 5 – 20% of the total P in the bulk soils and 10 – 22% in the rhizosphere soils after 10-months of seedling growth. Compared to that in the bulk soil, the 0.1 *M* NaOH-P_i concentrations were significantly ($P < 0.05$) higher in the rhizosphere soil, particularly at the low P application rates (Figure 3.3B). The accumulation of 0.1 *M* NaOH-P_i in the rhizosphere soil might have occurred as a consequence of the higher rate of the SPR dissolution at the lower pH in the rhizosphere soil and organic P hydrolysis being faster than the rate

of P uptake by mycorrhizal roots. The net increase in solution P_i would be rapidly adsorbed by the soil into the NaOH extractable-P pool (Hinsinger and Gilkes, 1995). Additionally, the higher acidification in the rhizosphere soil would also have enhanced adsorption of P dissolved from SPR by hydrous Fe and Al oxides and allophane in the soil (Clark and McBride, 1984; Parfitt, 1989).

The labile 0.1 *M* NaOH-P_o was the largest P fraction in the soil (Figure 3.4A). It ranged from 120 to 160 μg P g^{-1} soil comprising 45 to 68% of the total soil P. This P fraction in the rhizosphere soil was significantly ($P < 0.05$) lower than that in the bulk soil (Figure 3.4A). The lower concentration of 0.1 *M* NaOH-P_o in the rhizosphere soil was associated with significantly higher phosphatase activities in the rhizosphere soil. For both the bulk and the rhizosphere soils, the concentration of 0.1 *M* NaOH-P_o (y) was negatively correlated to phosphatase activities (x) ($y = -0.0624x + 159.94$, $r = -0.55$, $P < 0.01$ for acid phosphatase activity; $y = -0.1616x + 156.81$, $r = -0.47$, $P < 0.01$ for alkaline phosphatase activity). These observations indicate that the increased phosphatase enzyme activities in the rhizosphere soil may play a role in converting 0.1 *M* NaOH-P_o to 0.1 *M* NaOH-P_i or to soluble P_i that may have been taken up by plant roots in the mean time (Häussling and Marschner, 1989). It should be noted however that rate of phosphatase enzyme activities determined using sodium *p*-nitrophenyl phosphate as the substrate is not an absolute indication of organic P hydrolysis rate in soil (Hedley et al., 1994; Chen et al., 2000).

The 0.5 *M* H_2SO_4-P concentration was significantly ($P < 0.05$) lower in the rhizosphere soil compared to the bulk soil, and the magnitude of this difference increased with the increase in P application rate (Figure 3.4B) and associated with increased root dry matter (Table 3.2). Since 0.5 *M* H_2SO_4-P represents the apatite-like P, the lower concentration of 0.5 *M* H_2SO_4-P in rhizosphere soil suggests that ECM roots of the *P. radiata* were able to induce higher SPR dissolution.

3.4 Conclusions

The majority (45 – 68%) of total P in this low P Allophanic Soil fractionated into the 0.1 *M* NaOH-P_o fraction. After 10 months growth of *Pinus radiata* seedling, the 0.1 *M* NaOH-P_o fraction (135 – 165 μg g^{-1} soil in the bulk soils) was depleted by 8 – 17% in

the rhizosphere soil compared to the bulk soil. This result supported the hypothesis that soil organic P reserves can be significantly mobilised in the *P. radiata* rhizosphere. This mobilisation of organic P to inorganic P in the rhizosphere was associated with root-induced increases in soil phosphatase enzyme activities. Both acid and alkaline phosphatase activities were found to be > 50% higher in the rhizosphere than in the bulk soils.

Finely divided Sechura PR (75 – 150 μm particle size; applied at equivalent of 50, 100 and 150 kg ha^{-1}) was an effective P fertiliser increasing *P. radiata* seedling growth and P uptake (1.3 – 1.7-times). Phosphorus application at the rate of 50 kg ha^{-1} resulted in the highest density of mycorrhizal roots; however, additional fertiliser decreased mycorrhizal root density. This suggested that application of moderate rates of P fertiliser may maximise the beneficial effect of ectomycorrhizal symbiosis on pine growth in this P deficient Allophanic Soil. More than 80% of the applied SPR dissolved in the bulk soil during the 10 month period. Additional rhizosphere processes caused further (3 – 7%) dissolution of SPR. The majority of dissolved SPR-P was not taken up by the plant, but was recovered in the 0.1 *M* NaOH-P_i fraction. Efforts to implicate rhizosphere organic acids in the soil-P concentrations and PR-P dissolution were unsuccessful because oxalate concentrations of 0.15 – 0.38 mg l^{-1} were highly variable between samples and there was no significant difference in this concentration between the rhizosphere and the bulk soils.

CHAPTER 4

Root processes influencing phosphorus availability in soils under 4 - 5 years old *Pinus radiata* plantations*

4.1 Introduction

The results of the pot trial described in Chapter 3 (Liu et al., 2004) and those of many others (e.g. Davis, 1995; Chen et al., 2002) suggested that *P. radiata* rhizosphere processes induce P_o mineralisation in soils, thereby increasing soil P availability to *P. radiata*. The short-term pot trials on repacked soils, however, may have underestimated the long-term effects of plant roots on rhizosphere environment as discussed in the literature review in Chapter 2. Improved understanding of root processes influencing soil P nutrient dynamics in *P. radiata* requires more detail investigation under field conditions.

Unfertilised soils beneath *P. radiata* forests have been reported to have commonly higher levels of plant-available inorganic P (P_i) compared with soils under adjoining unfertilised grasslands (Davis and Lang, 1991; Davis, 1994; Chen et al., 2000). This elevated P availability beneath *P. radiata* plantations has been explained as a consequence of higher mineralisation of organic P (P_o) occurring beneath *P. radiata* than under grass. Nevertheless, these field studies paid little attention to the rhizosphere properties including P chemistry. This raises an issue, namely whether the increased soil P availability under *P. radiata* plantation compared to adjoining grassland in the similar environment originated from the ecological differences in the soils or caused by differences in root characteristics and ECM association and their biochemical processes between pine and grass.

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* Liu Q, Loganathan P, Hedley MJ, and Skinner MF (2004). Root processes influencing phosphate availability in volcanic soils under young *Pinus radiata* plantations. *Forest Ecology and Management* (submitted)

Based on a field study under Norway spruce in Sweden, Gobran and Clegg (1996) reported that the soil around pine roots exhibited a widely different chemical composition favourable to the roots. They suggested that accumulation of soil organic matter in the pine rhizosphere might have raised nutrient availability by increasing microbial activity, particularly mycorrhizal activity. In P deficient *P. radiata* plantations the association of ectomycorrhizas (ECM) with roots may play an additional important role in favouring P uptake by roots. It is suggested that mycorrhizal hyphae are able to extend P exploration volume (Bolan, 1991; Smith and Read, 1997), increase the release of phosphatases (Häussling and Marschner, 1989) and low molecular weight organic chelates, such as oxalate anions (Malajczuk and Cromack, 1982). Thus the studies on radiata rhizosphere processes need to pay more attention on their ectomycorrhizal symbioses.

The study reported in this chapter was conducted to test the hypotheses of that: (1) the *P. radiata* rhizosphere processes have a greater potential to mobilise solid phase soil P than in understorey grass rhizosphere; (2) *P. radiata* rhizosphere processes in plantation associate with accumulation of organic material in the rhizosphere. In this study, the P release characteristics were investigated in soils under two second-rotation 4 - 5 years old *P. radiata* plantations, one on a Pumice Soil and the other on an Allophanic Soil, by comparing soil P fractions and selected chemical and biochemical characteristics in the bulk and rhizosphere soils of *P. radiata* and understorey grass.

4.2 Materials and methods

4.2.1 Sites description

The study was conducted at two 4 to 5 year-old, second-rotation *P. radiata* plantations in Kinleith forest (10 km SE from Tokorua) and Kaweka forest (70 km NW from Hastings) in North Island of New Zealand. No fertiliser had been applied to the first-rotation and second-rotation trees at any time. The predominant understorey species in Kinleith forest were browntop (*Agrostis capillaris*) and himalayan honeysuckle (*Leycesteria formosa*), while in Kaweka forest they were mainly browntop and bracken fern (*Pteridium esculentum*). The soils are classified as Orthic Pumice Soil (Vitricryands, Soil Taxonomy) at Kinleith forest and Orthic Allophanic Soil (Cryands

and Udands, Soil Taxonomy) at Kaweka forest (Hewitt, 1992), both developed on either Taupo or Tongariro volcanic ash materials. The Pumice Soil (0 - 150 mm depth) had a total P of 277 μg P g^{-1} soil, P retention 80%, Bray-2 P 6.0 μg P g^{-1} soil, Olsen P 4.7 μg P g^{-1} soil, pH (1:2.5 H_2O) 5.4, total carbon (C) 8.8% and CEC 24 $cmol_c$ kg^{-1} soil (methods of analyses according to Blackmore et al., 1987). The Allophanic Soil (0 - 150 mm depth) had a total P of 206 μg P g^{-1} soil, P retention 92%, Bray-2 P 3.7 μg P g^{-1} soil, Olsen P 0.9 μg P g^{-1} soil, pH 5.5, total C 8.6% and CEC 14 $cmol_c$ kg^{-1} soil. In both soils there was no obvious LFH layer but remarkable disturbance of the soils was observed at 0 – 150 mm depth, mainly due to harvest and clearance operations.

4.2.2 Root, soil and solution samplings

In Nov. – Dec. 2001 pine roots and soils were sampled from a fan-shaped soil profile (approximately 0.8 m from tree trunk) having 150 mm depth and approximately 0.2 m^2 surface area (Figure 4.1). *P. radiata* roots (< 5 mm diameter) were collected by cutting the roots using a pair of scissors. The soil closely attached to the roots (Plate 4.1) was sampled (by vigorously shaking the roots) to represent the rhizosphere soil, and the root-free soil in the same profile was sampled to represent the bulk soil (Wang and Zabowski, 1998). For collection of the grass root samples, the whole root system of the browntop was sampled. The grass rhizosphere and bulk soils were collected as described for pine roots. To obtain adequate rhizosphere soil for subsequent analyses, samples from 3 locations were combined to form a composite sample at each site. Eight sampling sites were selected to represent 8 replicates at each forest. The fresh roots and soil samples were immediately placed inside plastic bags and sealed. The bags were transported to laboratory within 8 h for Kaweka forest and 48 h for Kinleith forest (stored in a fridge before transporting).

Within 6 h of sample collection soil solution was removed from a portion of the moist soil, using a double-centrifuge-tubes technique (centrifuged for 0.5 h at 10000 revolutions min^{-1} and at 4°C; filtered immediately through a 0.45 μm millipore filter) (Wang and Zabowski, 1998). The solutions were stored frozen until ready for analyses of oxalate and dissolved organic carbon (DOC). The root lengths of both pine and grass were measured using an intercept grid method described by Newman (1966). The number of ECM tips (fine roots) on the pine roots was counted manually.

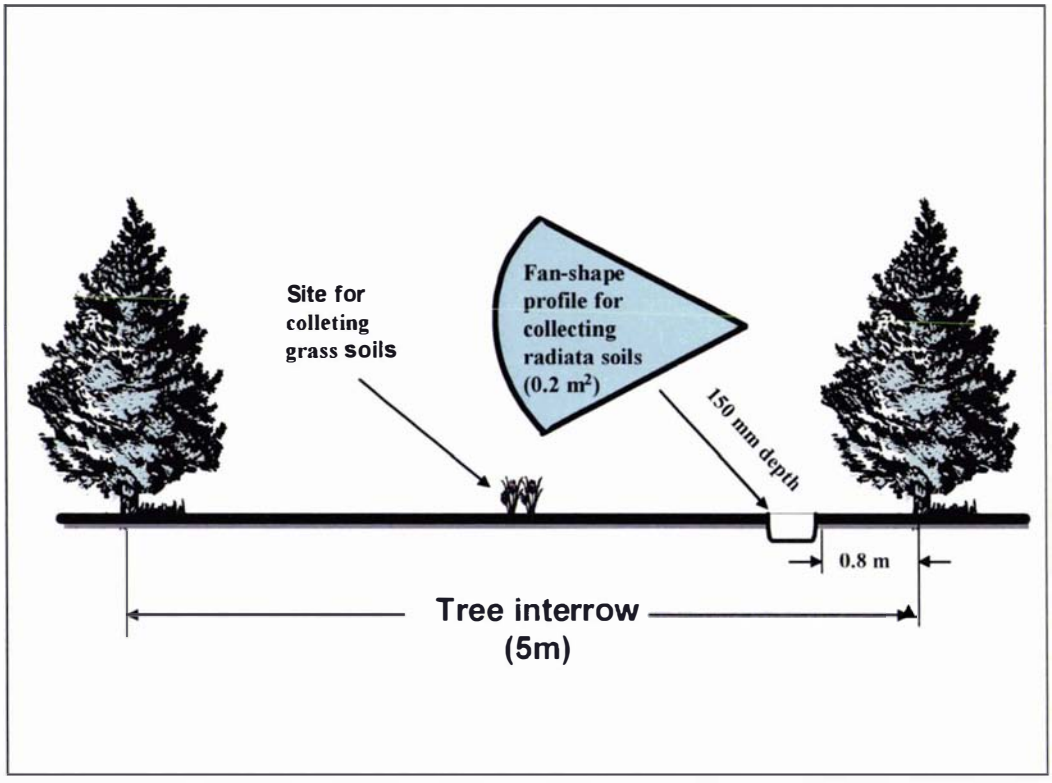

Figure 4.1 An illustration of soil profile for collecting roots and rhizosphere soil in a *P. radiata* plantation

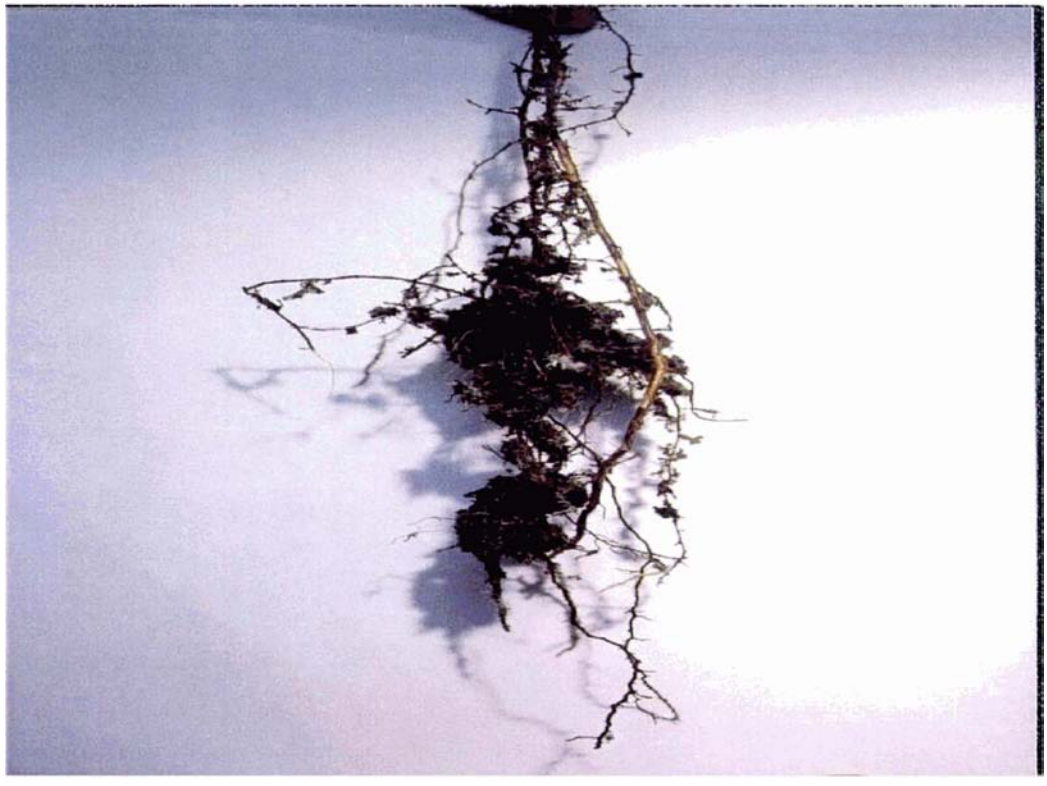

Plate 4.1 Roots of *P. radiata* with attached rhizosphere soil

4.2.3 Soil analyses

Soil P fractionation (Hedley et al. 1994), soil pH (1:2.5, w/w soil:H_2O; Blackmore et al., 1987), acid (pH 6.5) and alkaline (pH 11) phosphatase enzyme activities (Tabatabai, 1994), and oxalate concentrations in the soil solutions were analysed as described in Chapter 3. The DOC in the soil solution was determined on 4 replicates in each soil and the concentration was analysed using an Elementar Hi-TOC (Standard methods, 1998). The total P concentration in soil solution was analysed using Malachite-green method (Motomizu et al. 1983). The concentration of Al in soil solution was measured using UV spectrophotometry after 20 min reaction of soil solution Al with pyrocatechol violet (Dougan and Wilson, 1974). The soil organic matter content was determined by ignition-loss method (Ball, 1964).

4.2.4 Determination of hyphal length in soils

A simplified agar film technique (Nicholas and Parkinson, 1967) was used to measure the soil hyphal length density. About 0.5 g moist soil was ground and stained for 10 min in a mortar with 5 ml 0.05% trypan blue solution, then made to 25 ml and fully agitated. After 5 sec settlement, a small amount of the suspension was taken and placed on a haemocytometer. The length of hyphae in the suspension was measured under 100X magnification using the gridline on the haemocytometer. The average hyphal length per g dry soil was estimated from 18 observation fields, each having 1 mm^2 area.

4.2.5 Statistical analyses

A one-way ANOVA was carried out on the data for comparing the differences in soil properties. The least significant difference (LSD) was used to test the significant differences in measured parameters amongst the different sampling positions (pine-bulk, pine-rhizo, grass-bulk and grass-rhizo) and soil types (Allophanic Soil and Pumice Soil). The relationships among the soil properties were analysed using correlation procedures. Tests for significant differences were carried out at $P < 0.1$ levels.

4.3 Results and discussions

4.3.1 ECM root tips and hyphal length density in soil

In both soils, under pine and grass, the hyphal length density was generally higher in the rhizosphere than in the bulk soils (Table 4.1). The hyphal length density in the pine rhizosphere of the Allophanic Soil was significantly ($P < 0.1$) higher than in the grass rhizosphere, grass bulk and pine bulk soils, but no significant difference was found among the sampling positions in the Pumice Soil.

Table 4.1 Root induced changes in hyphal length density (HLD), soil organic matter (SOM) content and dissolved organic carbon (DOC) in soil solution

Sampling position		HLD ($m\ g^{-1}$ soil)	SOM (%)	DOC ($mg\ l^{-1}$)	DOC/Soil C**
Allophanic Soil	pine-bulk	18 ± 2b*	12 ± 0.5c	57 ± 16b	9 ± 3b
	pine-rhizo	47 ± 10a	15 ± 0.7ab	191 ± 61a	21 ± 6a
	grass-bulk	18 ± 3b	14 ± 1.2abc	39 ± 8b	5 ± 1b
	grass-rhizo	27 ± 5b	17 ± 2.3a	65 ± 17b	6 ± 1b
Pumice Soil	pine-bulk	14 ± 3b	13 ± 0.9bc	42 ± 5b	6 ± 0.6b
	pine-rhizo	22 ± 4b	17 ± 1.1a	107 ± 44ab	11 ± 4ab
	grass-bulk	19 ± 8b	15 ± 1.1abc	43 ± 4b	5 ± 0.2b
	grass-rhizo	26 ± 7b	15 ± 1.0abc	48 ± 9b	5 ± 0.6b

* Mean ± SE; same letters indicate no significant difference between the treatments at $P < 0.1$;

** Ratio of DOC ($mg\ l^{-1}$) / soil organic C (%)

In soils under *P. radiata*, there is a large diversity of fungal mycelia. Despite this fact, *P. radiata* roots are intimately associated with ECM fungi, while grasses are predominantly infected by arbuscular-mycorrhizal (AM) fungal species (Allen, 1991). Therefore, the higher hyphal length density measured in the pine rhizosphere is considered to be mainly contributed by ectomycorrhizal fungi associated with the *P. radiata* roots. In the field, a clearly visible mycelial mat was observed around *P. radiata* root tips but not around grass roots.

Compared with the highly P deficient Allophanic Soil (Olsen P 0.9 μg P g^{-1} soil), the moderately P deficient Pumice Soil (Olsen P 4.7 μg P g^{-1} soil) had lower hyphal length density in pine rhizosphere soils (Table 4.1) and lower ECM tip density (135 tips ± 40 SE m^{-1} root in Pumice Soil, 162 tips ± 39 SE m^{-1} root in Allophanic Soil), suggesting that ectomycorrhizas development may have been hindered by the higher plant-available soil P in the Pumice Soil (Newton and Pigott, 1991).

4.3.2 Oxalate concentration in soil solution

In both soils, the oxalate concentration in pine rhizosphere soil solution was significantly ($P < 0.1$) higher than that in soil solution from grass rhizosphere and grass bulk soils (Figure 4.2). Probably due to the high variation caused by previous forest harvesting disturbance in the soils and variation in the amount of rhizosphere soil surrounding ECM roots, oxalate concentration in pine rhizosphere solution was only marginally higher than that from the corresponding bulk soils. There was no significant difference in the oxalate concentration between the rhizosphere and the bulk soils under grass.

Oxalate has been reported to be the major organic anion in *P. radiata* root exudates and it is considered to be involved in the mobilisation of P in the rhizosphere (Malajczuk and Cromack 1982; Marschner, 1995). The higher oxalate concentration in pine rhizosphere solution (Figure 4.2) was associated with increased hyphal length density in pine rhizospheres, particularly in the Allophanic Soil (Table 4.1). This is consistent with the results of DeLucia et al. (1997). They reported that oxalate concentrations were highest in soil solution under ponderosa pine seedlings grown in pots supporting the highest level of mycorrhizal association and speculated that fine root tips may have been the primary source of the rhizosphere oxalate. In this study, however, oxalate concentration did not show any significant relationship with hyphal length density in either of the soils. The lack of any relationship between hyphal length density and oxalate concentration is probably due to the high variation in oxalate concentration in the soil solution, as a result of differences in the rate of decomposition of the excreted oxalate by the soil microbes or adsorption of the oxalate by soil minerals (Jones and Brassington, 1998).

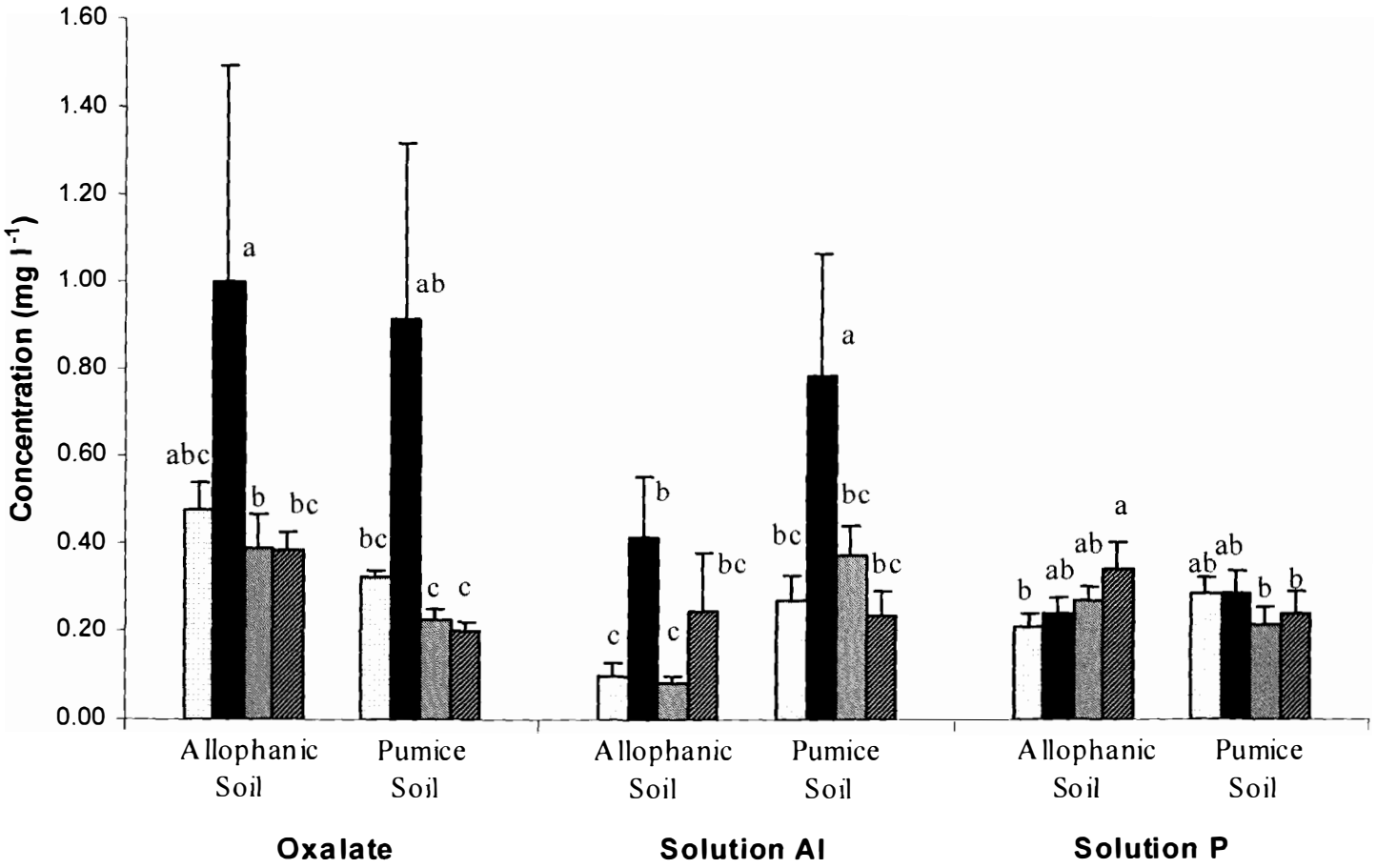

Figure 4.2 Concentrations of oxalate, Al and P in the soil solution. Same letters within the item bars indicate no significant difference at $P < 0.1$; and lines show SE of means (⊡ pine-bulk; ▩ pine-rhizo; ▨ grass-bulk; ▧ grass-rhizo)

The oxalate released by the mycorrhizal roots has been considered to complex with Al in soils thereby releasing P fixed to this metal (Fox and Comerford, 1992a). This suggestion is supported by the results in Figure 4.3 which show that Al concentration in the pine rhizosphere solution was significantly related to the oxalate concentration. However, the soil solution P concentration did not increase with the increase in concentration of either oxalate or Al in soil solution (Figure 4.2). A possible reason for the lack of any relationship between oxalate/Al concentrations and soil solution P concentration is that the P released from Al-P compounds may have undergone other reactions in soil or taken up by the plants.

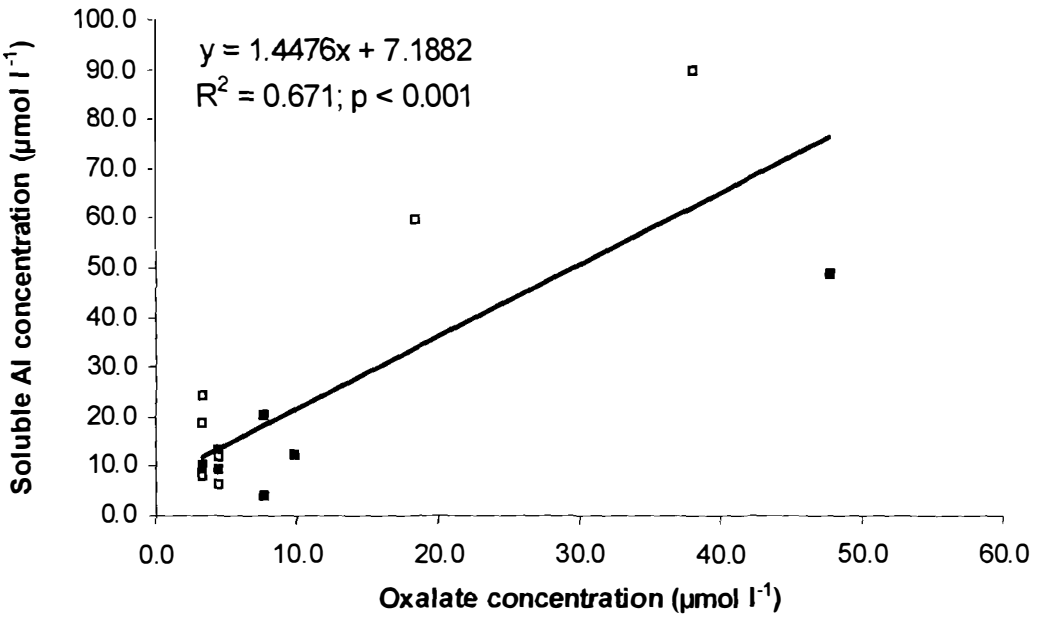

Figure 4.3 Relationship between oxalate concentration and Al concentration in the soil solution of radiata pine rhizosphere (■ –Kaweka forest; □–Kinleith forest)

4.3.3 Soil pH

No significant difference in soil pH was found between sampling positions, probably due to low nitrogen status in these two forest soils and large variation amongst the replicates. Compared to the bulk soils (mean pH ranged from 5.4 to 5.6), the means of the rhizosphere pH (5.2 -5.3) under both *P. radiata* and grass were lower by approximately 0.2 to 0.3 pH units respectively. The slightly lower pH in the pine

rhizospheres has been explained due to *P. radiata* taking up N mainly in the form of NH_4^+ with subsequent H^+ release to soil (Olykan and Adams, 1995).

4.3.4 Soil organic matter content

Soil organic matter (SOM) contents in the two soils in the respective sampling positions were not significantly different (Table 4.1). But, in both soils, there was a significant ($P < 0.1$) accumulation of SOM in the rhizospheres under pine compared to the corresponding bulk soils (Table 4.1). Similar results were also reported by others (Gobran and Clegg, 1996; Sanchez and Bursey, 2002). Organic matter input from fine root and mycorrhizal hyphae turnover, root exudates and sloughing might be the reasons for the build-up of SOM content in the pine rhizosphere. Sanchez and Bursey (2002) found that loblolly pine (*Pinus taeda* L.) growing on sandy and loamy sand soils for 40 years resulted in a significant build-up of labile C compounds (55% increase, compared to bulk soil) in the rhizosphere. The higher SOM content in the rhizospheres compared to the bulk soils is likely to create greater cation exchange capacity and microbial activity resulting in increase rates of nutrient and organic matter turnover (Gobran and Clegg, 1996).

4.3.5 Dissolved organic carbon (DOC)

As in the case of SOM, pine roots induced greater difference in the DOC concentration between the rhizosphere and bulk soils compared to the grass roots, though the difference was statistically significant only in the Allophanic Soil (Table 4.1). In a pot trial using thin soil slicing techniques, Chen et al. (2002) also observed that the concentrations of DOC were significantly greater in the rhizosphere of *P. radiata* compared with the corresponding bulk soils and ryegrass rhizosphere. Higher DOC in pine rhizosphere solution is associated with higher SOM content (Table 4.1) and soil microbial activities (Chen et al., 2002). The oxalate C concentration constitutes only 0.1 – 0.3% of the total DOC.

The DOC/Soil-C ratio (an index characterising the nature of soil organic matter) was significantly ($P < 0.1$) higher in the pine rhizosphere than in either the pine bulk, grass rhizosphere or grass bulk in the Allophanic Soil (Table 4.1). This indicates that pine roots caused a higher solubility of organic matter in the rhizosphere. This observation is consistent with the findings of Sanchez and Bursey (2002) who reported that rhizosphere region of loblolly pine had a higher relative proportion of labile organic materials than the bulk soil.

4.3.6 Soil phosphatase enzyme activities

The difference in phosphatase activities between rhizosphere and the bulk soils was found to be more striking in the Allophanic Soil than in the Pumice Soil (Figure 4.4). This might be due to the lower P status in the Allophanic soil than in the Pumice Soil. Compared to grass, radiata root processes induced greater difference in phosphatase activities. Both acid and alkaline phosphatase activities under pine soils were significantly higher in the rhizosphere soils than in the corresponding bulk soils. Under grass, however, there was no significant difference except for a significantly greater alkaline phosphatase activity found in the grass rhizosphere than in the grass bulk soil in the Allophanic Soil. The greater difference in phosphatase activities under pines is associated with greater hyphal length density in the pine rhizosphere, particularly in the Allophanic Soil (Table 4.1), suggesting that ECM fungi may have had an effect in phosphatase production.

The enhanced phosphatase activities in the rhizospheres under radiata pine might also be due to increased microbial biomass in the rhizospheres, caused by increased SOM and DOC in the rhizosphere and soil solution respectively (Table 4.1). In this study, both acid phosphatase activity ($r = 0.55$ for Allophanic Soil; $r = 0.75$ for Pumice Soil) and alkaline phosphatase activity ($r = 0.72$ for both soils) were significantly ($P < 0.01$) related to SOM content.

4.3.7 Soil P fractions

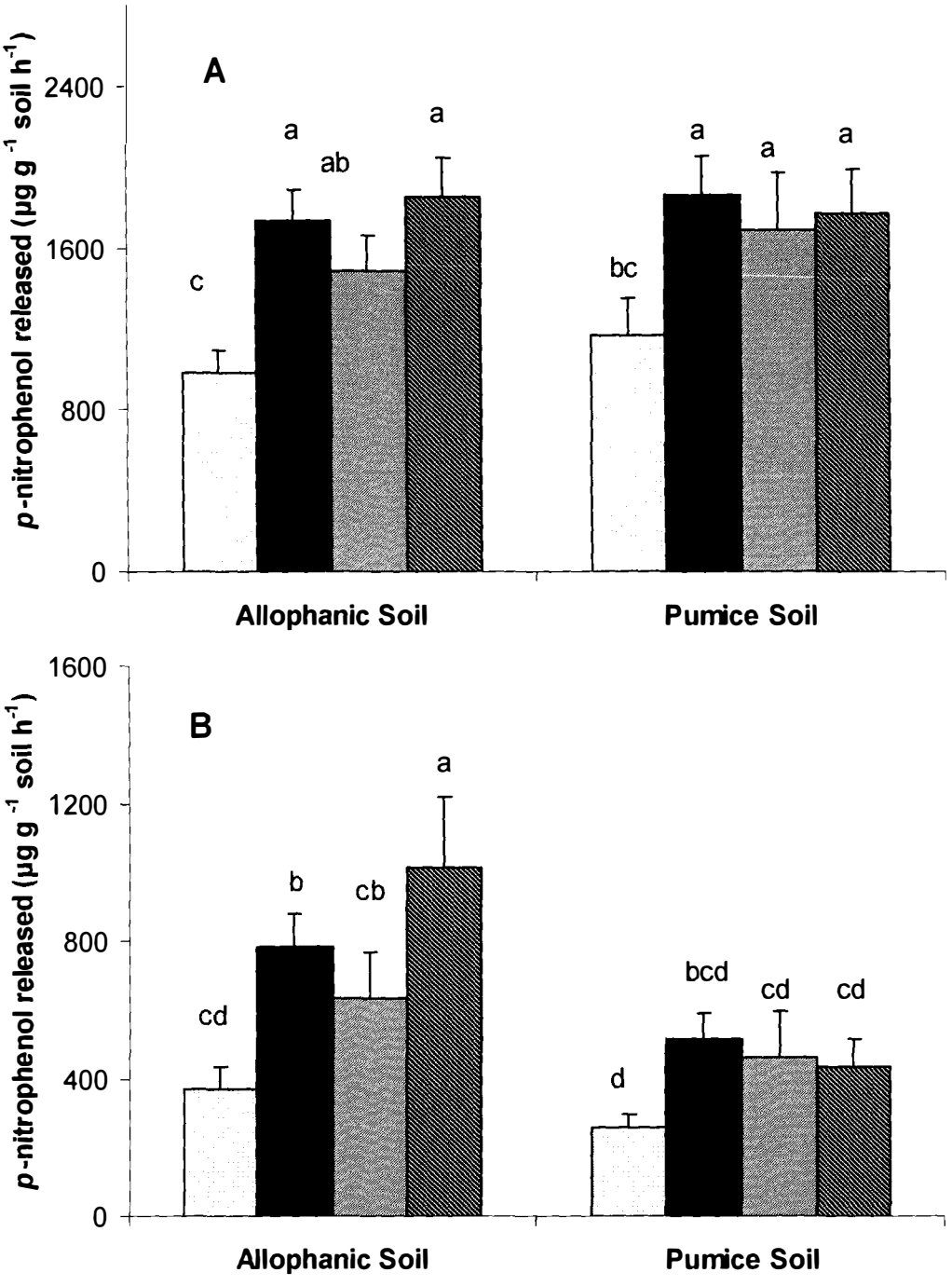

Figure 4.4 Root-induced changes in soil acid phosphatase activity (A) and alkaline phosphatase activity (B) at Kaweka forest (Allophanic Soil) and Kinleith forest (Pumice Soil). Same letters within bars indicate no significant difference at $P < 0.1$; and lines show SE of means (⊡ pine-bulk; ■ pine-rhizo; ▨ grass-bulk; ▩ grass-rhizo).

4.3.7.1 Effect of soil type

In both Pumice and Allophanic Soils, organic P, which is the sum of labile organic P (0.1 *M* NaOH-P_o) and residual-P (Chen et al., 2002), is the predominant soil P fraction, contributing to 90% and 73% of the total P in the two soils respectively (Table 4.2). The 0.1 *M* NaOH-P_o concentration and its proportion in the total 0.1 *M* NaOH-P concentration were significantly ($P < 0.1$) lower in Allophanic Soil (90 – 120 μg P g^{-1} soil, 73% of NaOH-P) than in Pumice Soil (180 – 210 μg P g^{-1} soil, 93% of NaOH-P), but the 0.1 *M* NaOH-P_i concentration and its proportion in the 0.1 *M* NaOH-P_o concentration were higher in Allophanic Soil (Table 4.2). This lower proportion of NaOH-P_o and higher proportion of NaOH-P_i in Allophanic Soil may have been due to higher rate of P_o mineralisation and adsorption of the mineralised P_i onto allophane in this soil.

Compared to Pumice Soil, the Allophanic Soil had lower resin-P concentration, which was associated with a higher P retention in this soil (92% compared to 80% in the Pumice Soil). The H_2SO_4-P concentrations in both soils were very low (4 - 6% of total P), indicating that there are very little apatite-like minerals remaining in these soils.

4.3.7.2 Effects of root processes

There was no significant difference observed in any P fractions between pine bulk (beneath tree rows of *P. radiata*) and grass bulk (interrow zone) soils (Figure 4.5 to Figure 4.7). This indicates that there was no microsite effect on bulk soil P availability under young second rotation *P. radiata* plantations, caused by the current generation of trees.

For both pine and grass soils, the concentrations of resin-P (Figure 4.5A), NaOH-P_o (Figure 4.6A), residual-P (Figure 4.7A) and total P (Figure 4.7B) were positively correlated with SOM content in both soil types (Table 4.2), while NaOH-P_i (Figure 4.5B) in Allophanic Soil was negatively related to SOM content (Table 4.2). This suggests that the changes in the above P fractions were influenced by the accumulation and the rate of mineralisation of SOM, both of which are higher in the rhizosphere (see section 4.3.8).

Table 4.2 Relationships between soil P fractions and organic matter content and the P fractions as a percentage total P in Allophanic and Pumice Soils (0 - 150 mm depth)

P fractions	P form	Allophanic Soil			Pumice Soil		
		relation with SOM		%	relation with SOM		%
		r	*P*	in total P *	*r*	*P*	in total P
Resin-P	plant-available P	0.69	< 0.001	0.8 ± 0.2	0.74	< 0.001	1.7± 0.3
0.1 *M* NaOH-Pi	Fe, Al and allophane associated P	-0.42	0.016	20.1 ± 1.7	-0.10	NS	4.9 ± 1.5
0.1 *M* NaOH-Po	labile organic P	0.65	< 0.001	53.7 ± 2.4	0.75	< 0.001	72.4 ± 2.3
0.5 *M* H_2SO_4-Pi	apatite P	0.07	NS	6.1 ± 0.8	-0.07	NS	3.9 ± 0.4
Residual-P	non-extracted P	0.66	< 0.001	19.3 ± 0.9	0.74	< 0.001	17.1 ± 2.8
Total-P	-	0.68	< 0.001	-	0.84	< 0.001	-

* Mean of 32 samples ± SE (8 replicates of pine-bulk, pine-rhizo, grass-bulk and grass-rhizo)

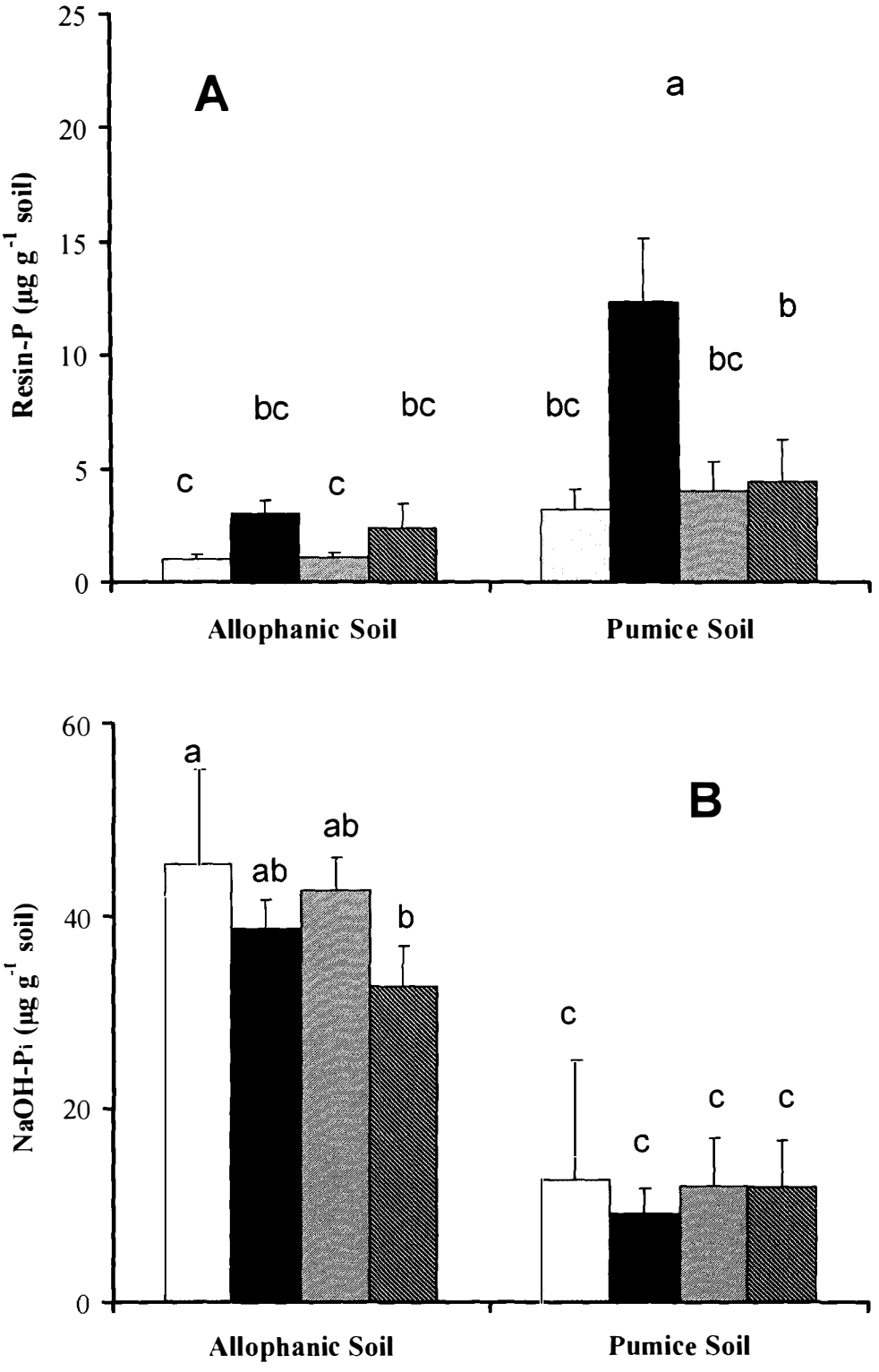

Figure 4.5 Root-induced changes in concentrations of resin-P (A) and 0.1 *M* NaOH-P_i (B) at Allophanic and Pumice Soils. Same letters within bars indicate no significant difference at $P < 0.1$; and lines show SE of means (□ pine-bulk; ■ pine-rhizo; ▨ grass-bulk; ▩ grass-rhizo).

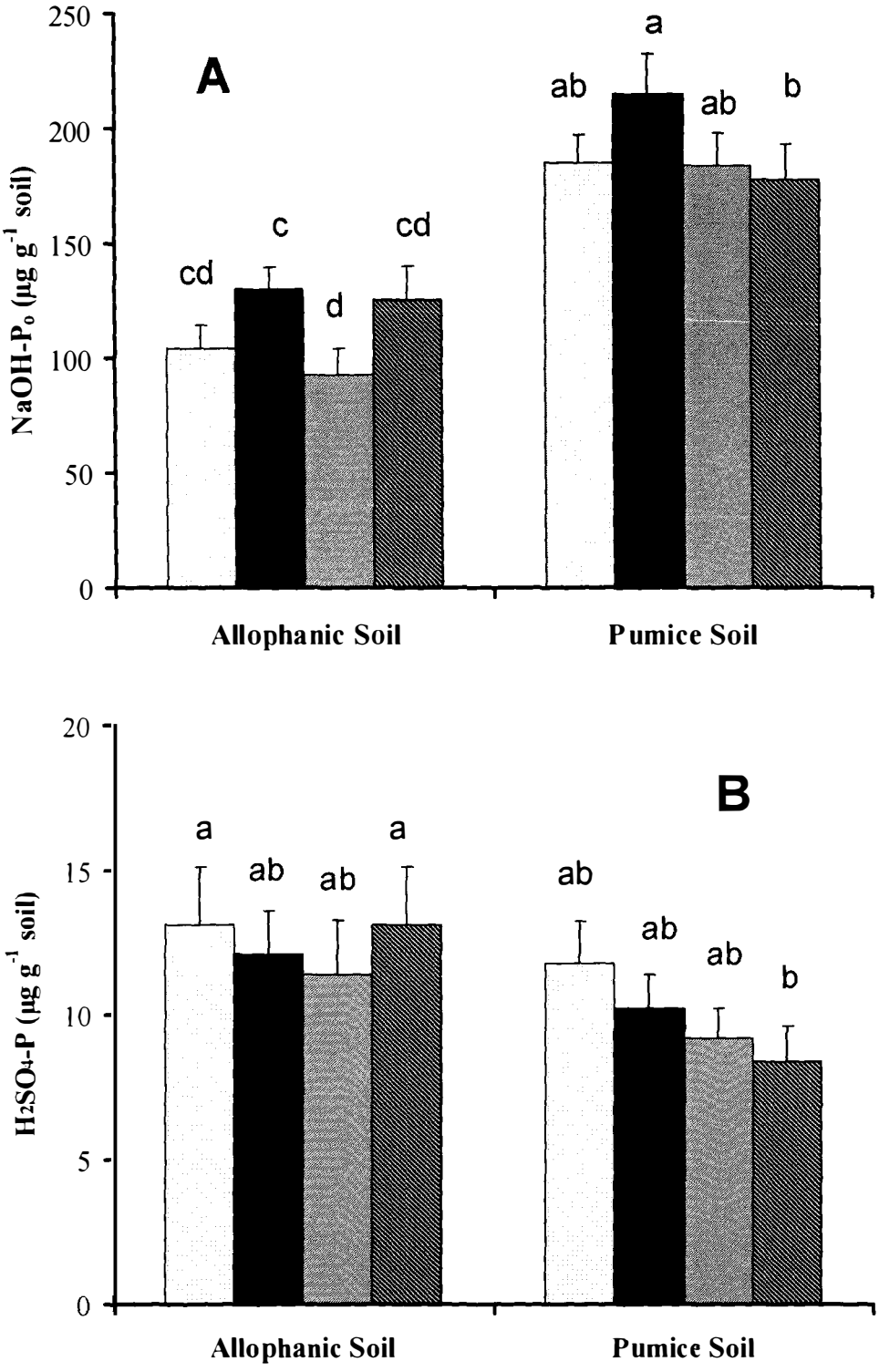

Figure 4.6 Root-induced changes in concentrations of 0.1 *M* NaOH-P_o (A) and H_2SO_4-P (B) at Allophanic and Pumice Soils. Same letters within bars indicate no significant difference at $P < 0.1$; and lines show SE of means (⊡ pine-bulk; ▩ pine-rhizo; ▨ grass-bulk; ▧ grass-rhizo).

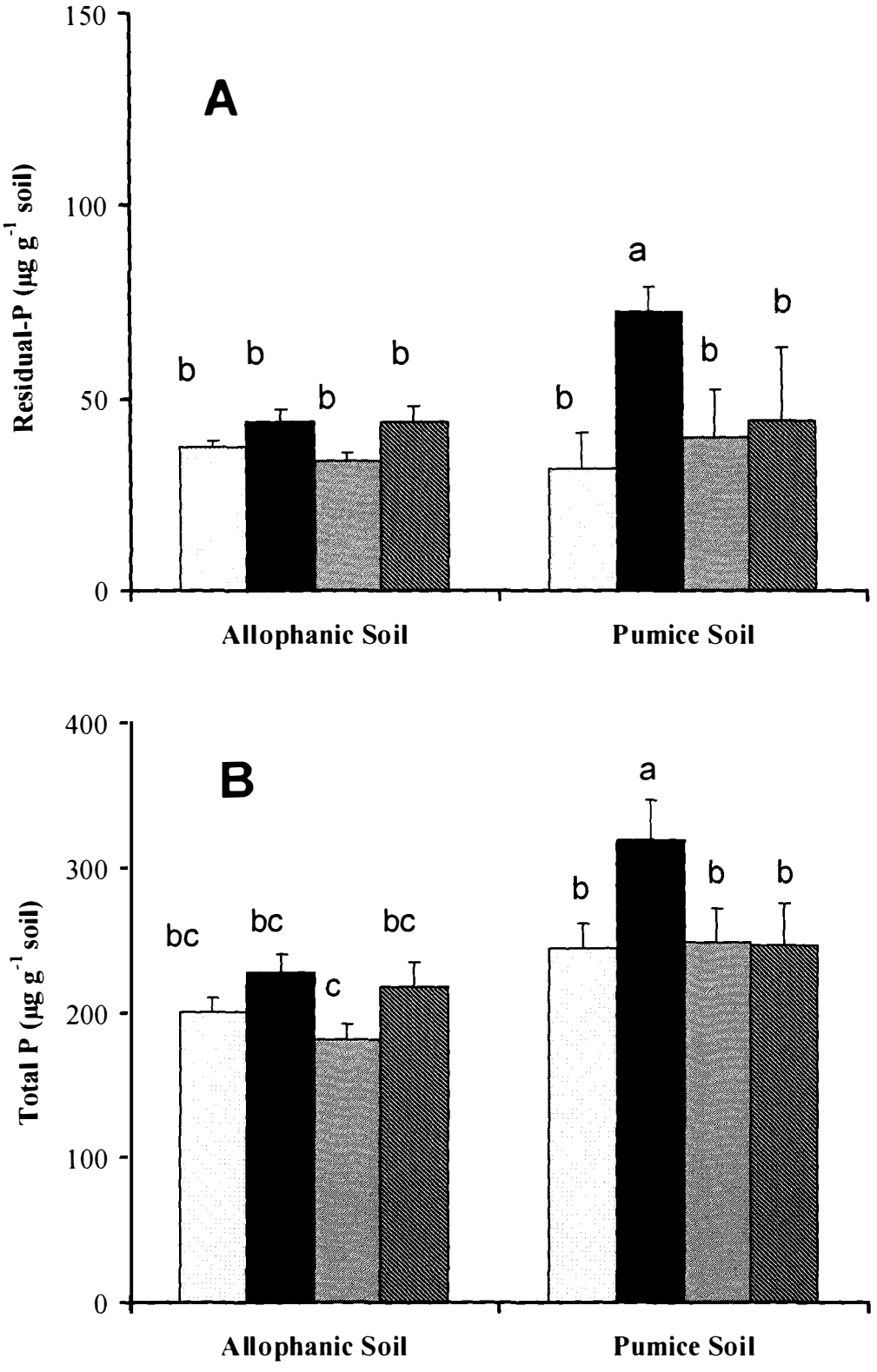

Figure 4.7 Root-induced changes in concentrations of residual-P (A) and total-P (B) at Allophanic and Pumice Soils. Same letters within bars indicate no significant difference at $P < 0.1$; and lines show SE of means (□ pine-bulk; ■ pine-rhizo; ▨ grass-bulk; ▩ grass-rhizo).

Significant difference in P fractions between the rhizosphere and the bulk soils was only found in concentrations of resin-P (Figure 4.5A) and residual-P (Figure 4.7A) under pine soil in the Pumice Soil. It should be noted that resin-P concentration measured in this study may have been overestimated due to air-drying the soils prior to P extraction which probably has resulted in the release of P_i from microbial-P_o (Sparling et al., 1987). This overestimation of resin-P is expected to be higher in the pine rhizosphere compared to the bulk soils due to higher microbial biomass including ECM hyphae in the pine rhizosphere (Table 4.1). The higher resin-P concentration in pine rhizosphere compared to the bulk soil in the Pumice Soil could also be associated with higher total P (Figure 4.7B) and NaOH-P_o (Figure 4.6A) concentrations and their rate of mineralisation in the rhizosphere soil. If the higher resin-P measured in rhizosphere is real, then it is likely that the pine root processes might have induced a greater rate of P_i supply in soil than the rate of P_i uptake by the tree and P_i adsorption by rhizosphere. The effect of pine root processes on soil P availability will be discussed at section 4.3.8.

4.3.7.3 Effects of plant type

No significant difference was found in any P fractions between pine and grass rhizosphere in Allophanic Soil, but in the Pumice Soil significantly ($P < 0.1$) higher resin-P (Figure 4.5A), NaOH-P_o (Figure 4.6A), residual-P (Figure 4.7A) and total-P (Figure 4.7B) were observed in the pine rhizosphere than in the grass rhizosphere. This may be due to the higher rate of ECM roots turnover under pine soils. As discussed in Chapter 2, the ECM root turnover under pine rhizosphere may have resulted in accumulation of labile organic materials and reduced P fixation and increased P_o mineralisation, causing relatively higher P availability in the rhizosphere.

4.3.8 Soil P availability model for *P. radiata*

Based on the results obtained in this study and data in the literature, a conceptual model illustrating the influences of ECM root processes on P availability in secondary *P. radiata* plantation is developed (Figure 4.8). This model emphasises that (i) turnover of ECM fine roots and labile P_o cycling in pine rhizosphere; (ii) P exploitation from bulk

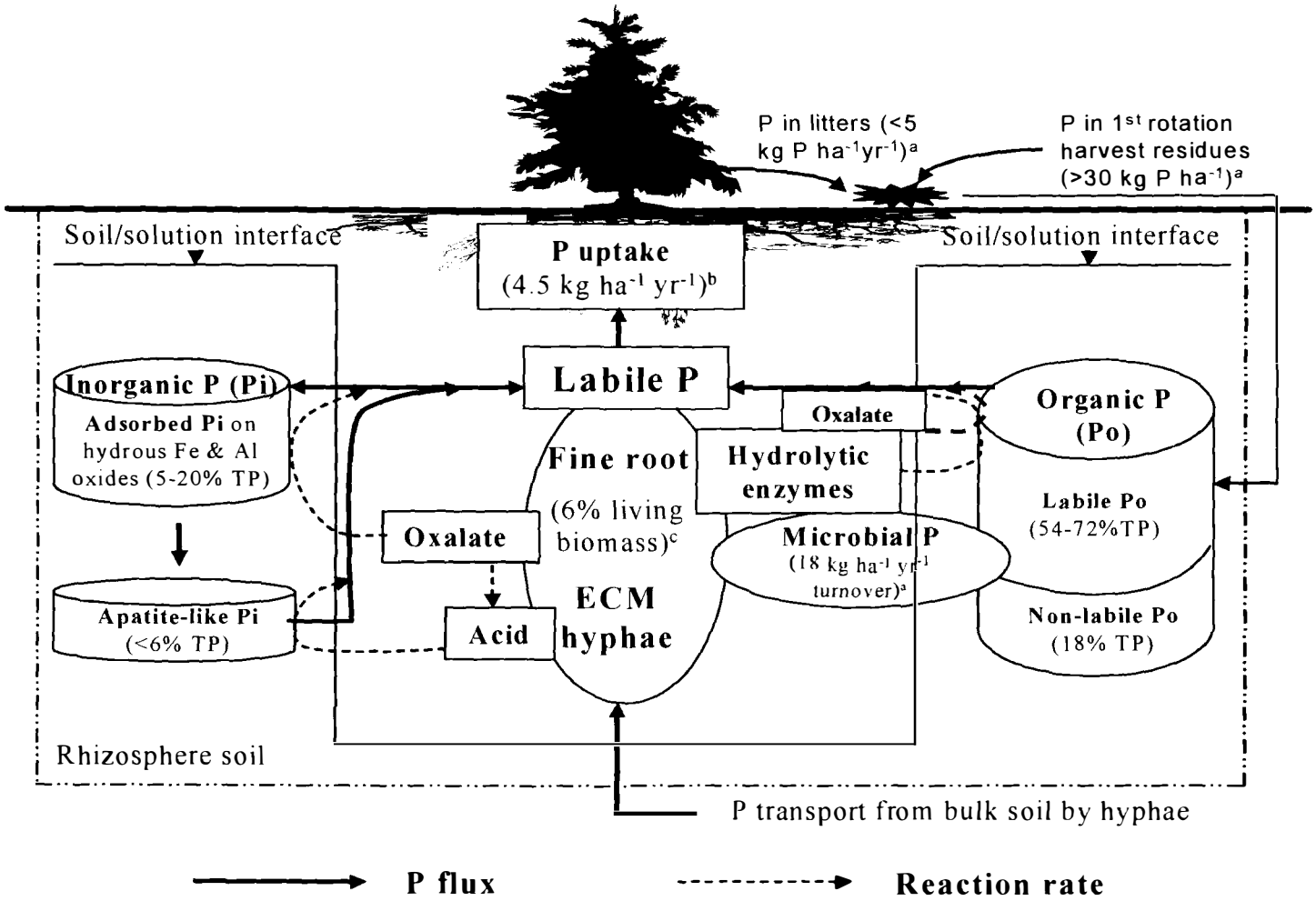

Figure 4.8 A conceptual model showing the effect of root processes on soil phosphorus availability under second rotation *P. radiata* plantation (data citation: [a] Sparling et al., 1994; [b] Will, 1964; [c] Fogel and Hunt, 1983)

soil by ECM hyphae; and (iii) P_i mobilisation by oxalate anion exudates from the roots are important processes maintaining P availability to pine tree.

In *P. radiata* plantations, continued root growth and fine root turnover may be more important in maintaining P availability in pine rhizosphere. Fogel and Hunt (1983) estimated that ECM roots in a Douglas-fir stand constituted 6% of the living biomass while they contributed to 48% of the organic matter in the soil. Our previous observation also showed that the ECM fine roots consisted 3% of the total dry weight in approximately 1-year-old *P. radiata* seedlings. The turnover of these labile materials may have provided a relatively high-quality C source (such as higher DOC concentration, Table 4.1) in the rhizosphere to stimulate higher microbial biomass (Chen et al., 2002). With the substantial transport of P from bulk soil by fungi hyphae, which has low P uptake threshold and are able to extend P exploitation zone to bulk soil region (Skinner and Bowen, 1974a, b; Marschner, 1995; Smith and Read, 1997), the increased microbial biomass and their turnover could result in higher microbial-P level in the pine rhizosphere than in the bulk soils. Sparling et al. (1994) estimated that annual P turnover by microbial-P in bulk soils of a 17-year-old *P. radiata* was up to 18 kg ha^{-1} (equivalent to 15 μg P g^{-1} soil for 0 - 150 mm soil depth, bulk density 0.8 g cm^3). This value is expected to be higher in the pine rhizosphere and may have a markedly effect on P supply to trees, particularly in the second-rotation plantations because of enrichment of C resource in harvest residues of first rotation trees. The P availability in pine rhizosphere may further be improved by pine root exudates. Pine roots were able to induce higher phosphatase activities (Figure 4.4). The increased phosphatase activities in the rhizosphere are considered to hydrolyse ester linkages between P and C (Häussling and Marschner, 1989), thereby releasing P_i from organic P which is the largest P pool in these soils (Table 4.2). Additionally, the oxalate released by ECM roots and measured in greatest concentration in pine rhizosphere (Figure 4.2) could have increased P_i mobilisation by chelating Al and Fe, thereby reducing P_i fixation by Al and Fe complexes (Fox and Comerford, 1992a). Such reduction in P_i fixation was reflected in the decreased 0.1 *M* NaOH-P_i in the rhizosphere (Figure 4.5B). The processes described above were likely to result in the dynamic cycling of organic P and mobilisation of inorganic P, thereby be able to maintain P_i availability to pine tree even in soils having low soil test P.

In this study, the concentration of 0.1 *M* NaOH-P_o in the pine rhizosphere was marginally higher than that in the bulk soil, but this was not statistically significant. In the pot trial results presented in Chapter 3 and other pot trial studies (Condron et al., 1996; Chen et al., 2002) the concentration of 0.1 *M* NaOH-P_o was observed to be lower in pine rhizosphere than in bulk soils due to its conversion to inorganic P. This disagreement between this field study and the pot studies is considered to be associated with the underestimation of long-term organic material cycling (e.g. ECM roots turnover) in these pot trials. The SOM differences between rhizosphere and bulk soils in this study made it difficult to compare the rhizosphere effects on P_o mineralisation in the soils. However, when the concentrations of P_o (0.1 *M* NaOH-P_o + residual-P) was divided by organic C and then the ratios (P_o:C) were compared, it was found that pine rhizosphere of the Pumice Soil had significantly higher ($P < 0.1$) P_o:C ratio compared to that in the bulk soil (2.9×10^{-3} for bulk soil; 3.4×10^{-3} for rhizosphere soil). More interestingly, the P_o:C ratios were consistently higher under pine soils ($1.9 - 3.4 \times 10^{-3}$) than under grass soils ($1.5 - 2.5 \times 10^{-3}$) in both soils.

High P_o:C ratio in substrate is considered to promote P_o mineralisation (Saggar et al., 1998). Therefore, the larger P_o:C ratios in the pine rhizosphere soils indicate that the SOM accumulated under pine rhizosphere may have potentially larger rate of mineralisation, thus being able to release more P_i from organic bound P pools. This is consistent with the increase in plant-available P_i (resin-P) in rhizosphere soils, especially under *P. radiata*.

4.4 Conclusions

The results obtained in this study supported our hypothesis that beneath forest plantations, *P. radiata* rhizosphere processes have a greater potential to mobilise soil P than the understorey grass rhizosphere processes. Compared to the understorey grass roots, ECM roots of *P. radiata* markedly increased rhizosphere organic matter content (> 4% higher in the rhizosphere than in the bulk soils for *P. radiata*; no difference for the grass soils), both acid and alkaline phosphatase enzyme activities (> 50% increase in radiata rhizosphere soil; < 25% increase in most grass rhizosphere soil) and the concentrations of dissolved organic carbon and oxalate in both the Allophanic and Pumice Soils. These rhizosphere processes have raised resin-P concentration (9 μg g^{-1}

soil) in the radiata rhizosphere, compared to the bulk soil (3.5 μg g^{-1} soil) in the Pumice Soil, but not in the understorey grass rhizosphere. The greater potential to mobilise soil P in *P. radiata* rhizosphere than in the understorey grass rhizosphere appeared to be interacted by ECM root turnover and long-term accumulation of organic materials hence organic P.

CHAPTER 5

Influence of ectomycorrhizal hyphae on phosphorus fractions and dissolution of a phosphate rock in the rhizosphere soils of *Pinus radiata*[*]

5.1 Introduction

Ectomycorrhizal (ECM) fungi association with *Pinus radiata* roots helps to enhance phosphorus (P) uptake from soil (Skinner and Bowen, 1974a; Chu-Chou and Grace, 1985). The experiments reported in Chapter 3 and 4, and studies by others have demonstrated that ECM roots can induce many changes in rhizosphere chemistry, such as acidification (Cumming and Weinstein, 1990), production of phosphatase enzymes (Häussling and Marschner, 1989) and metal-chelating organic substances (Malajczuk and Cromack, 1982; Lapeyrie, 1988). Häussling and Marschner (1989) reported that acid phosphatase activity was positively correlated with hyphal length in soils under 80-year-old Norway spruce trees (*Picea abies* (L.) Karst.). Malajczuk and Cromack (1982) suggested that ECM hyphae were responsible for the exudation of oxalate by *P. radiata* roots. However, it was not possible to clearly distinguish the influence of the ECM external hyphae from that of the host roots on the above rhizosphere properties.

The objective of this study was to test hypothesis of that *P. radiata* rhizosphere processes are largely associated with ECM hyphae. This study was conducted under a 1-year-old *P. radiata* plantation.

[*] Q. Liu, P.Loganathan and M. J. Hedley (2004) Influence of ectomycorrhizal hyphae on phosphorus fractions and dissolution of a phosphate rock in the rhizosphere soils of *Pinus radiata*. *Journal of Plant Nutrition* (accepted).

5.2 Materials and methods

5.2.1 Experimental design and techniques

The modified rhizosphere study container (RSC) technique developed by Hedley et al. (1994) and Zoysa et al. (1997) was used in this trial. The RSC consisted of a PVC cylinder with two compartments (Figure 5.1). The upper compartment had an internal diameter of 82 mm and an effective depth of 25 mm, and the lower compartment had an internal diameter of 74 mm and a depth of 50 mm. The two compartments were separated by a 26 μm pore-diameter polyester mesh or by two 10 μm pore-diameter meshes placed one on top of the other respectively to represent the two treatments of the trial – 26 μm mesh treatment and < 10 μm mesh treatment. The intention was to use a mesh with 26 μm opening and another one with < 10 μm opening. Since meshes with < 10 μm pore diameter were not available, it was decided to use two meshes with 10 μm opening placed one on top of the other to produce openings of < 10 μm. The 26 μm mesh treatment was designed to stop pine roots but allow ECM hyphae to penetrate into the lower compartment; and the double 10 μm meshes in the other treatment were expected to stop the penetration of roots and minimise the penetration of hyphal strand into the lower compartment.

The lower compartment of RSC was packed with a mixture of 155 g soil (oven-dried based, sieved < 3 mm), 6 g pine needle powder (< 0.3 mm size) and 0.118 g Ben Guire phosphate rock (BGPR) (particle size 150 – 250 μm; total P 131 g kg^{-1} soil; 2 % citric acid extractable P 27.5 g kg^{-1} soil; supplying 100 μg P g^{-1} soil). The soil used was an Orthic Allophanic Soil (Andosol; Hewitt, 1992) and was taken from 0 – 150 mm depth in the interrow of a 5-year-old second-rotation *P. radiata* plantation located at Kaweka forest, approximately 70 km NW from Hastings in the North Island of New Zealand. The pine needle-powder was mixed with the soil to provide carbon (C) and create a favourable condition for development of soil microorganisms. BGPR was mixed with the soil to study the effect of radiata roots and ECM hyphae on PR dissolution. The bulk density of the packed soil was 0.73 g cm^{-3} which was similar to the bulk density of the soil at 0 - 150 mm depth in the forest. The upper compartment of the RSC was packed with the same soil mixtures but without BGPR addition.

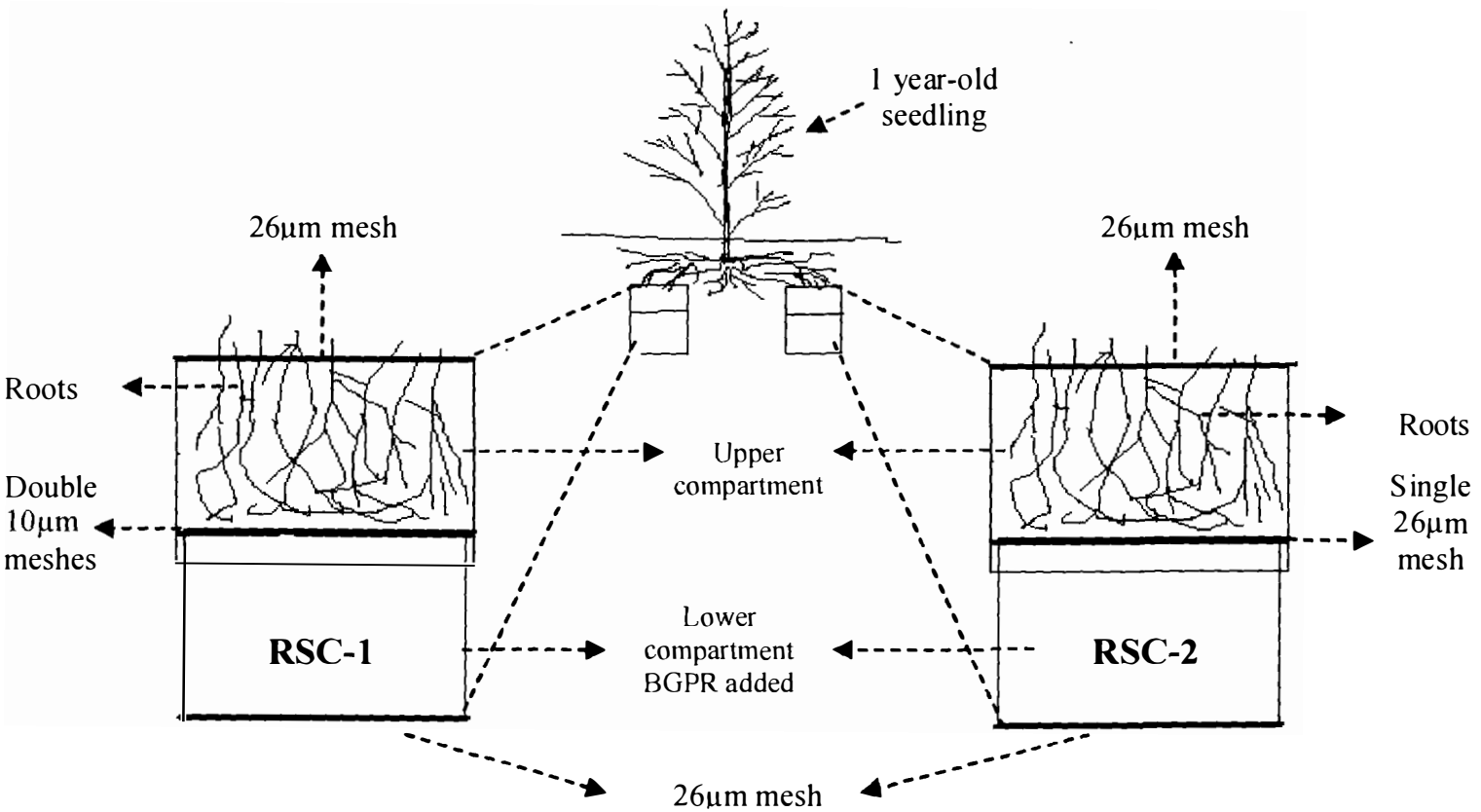

Figure 5.1 An illustration of the rhizosphere study container installation in a one-year-old *Pinus radiata* plantation.

The soil used in the trial had a pH (1:2.5, soil:H_2O) 5.8, P retention 92%, Olsen P 0.9 μg g^{-1} soil, pH buffering capacity (at pH 5.5 - 6.5) 0.02 mol H^+ kg^{-1} soil pH^{-1}, and organic C 4.2% (all measured according to Blakemore et al., 1987). The exchangeable Ca, Mg and K (mol_c kg^{-1} soil) in the soil were 0.028, 0.006 and 0.002, respectively, and CEC was 0.14 mol_c kg^{-1} soil (Blakemore et al., 1987). The concentration of P fractions in the soil, pine needle-powder, and the mixture of soil and pine needle-powder are presented in Table 5.1.

Table 5.1 P fractions (μg g^{-1} soil) in soil, pine needle-powder, and mixtures of soil and pine needle-powder used to pack the RSCs

Material		Resin P	0.1 *M* NaOH-P_i	0.1 *M* NaOH-P_o	0.5 *M* H_2SO_4-P	Residual P	Total P
Soil	P fraction	0.4	44.5	141.1	15.0	41.3	242.3
	% in TP	0.2	18.4	58.3	6.2	17.0	
Needle powder	P fraction	237.8	52.6	454.8	3.8	71.3	820.1
	% in TP	29.0	6.4	55.5	0.5	8.7	
Mixture	P fraction	10.0	44.8	152.9	14.6	42.4	264.6
	% in TP	3.8	16.9	57.8	5.5	16.0	

The RSCs with the soils were vertically buried approximately 100 mm below the soil surface under one-year-old *P. radiata* seedlings having similar growth characteristics in a second rotation plantation at the Kaweka forest (Figure 5.1; Plate 5.1). The site selected for the trial had minimal spatial variability as a result of harvesting disturbance. Approximately equal number of roots was placed inside each upper compartment of the two RSC treatments. The bottom and the top of the RSCs were firmly covered by a layer of 26 μm pore-diameter mesh to prevent any weed roots penetrating into the containers. The mesh cover on the bottom of the RSC was fixed by a PVC ring with 10 mm thickness and an internal diameter of 82 mm. The mesh on the top of the RSC was tightly twisted on the RSC and the gaps around roots were sealed by silicon glue. The RSCs were left in the field for 10 months (from 20 May 2002 to 24 March 2003) for the roots and hyphae to induce changes in soil properties in the lower RSC compartments. Each of the two treatments had 5 replicates. One more pair of RSCs (spare RSCs)

Plate 5.1 Installation of rhizosphere study containers (RSCs) in the one-year-old *P. radiata* plantation at Kaweka Forest (Top - setting up the RSCs; Bottom – a view of trial site after RSCs installation).

were buried under a seedling for the purpose of destructively sampling at 6 months to examine whether the double 10 μm mesh prevented any hyphae penetration to the lower compartment of the RSC cylinder.

At the end of 6 months, it was found that the double 10 μm meshes in the spare RSC failed to completely prevent ECM hyphae penetrating into the lower compartment. At this point of time, all RSCs from the 10 μm mesh treatment were carefully dismantled at the boundary between upper and lower compartments and glass filter papers with 0.3 μm pore-size diameters were placed one for each RSC between the two 10 μm meshes. This operation was carried out with minimal disturbance to the soil inside the RSCs. The RSCs were re-buried and the trial was continued for four more months.

5.2.2 Harvest and sampling

After a total of 10 months of experimental period, the RSCs were removed from the field by cutting the roots entering the RSC. The RSCs were taken to the laboratory and the upper and lower compartments of the RSCs were separated. The soils in the lower compartments were sliced in thin sections with a piston microtome starting at the inter-compartment boundary (Zoysa et al., 1997) (Plate 5.2). The first two slices were at 0.5 mm thickness, and each of the next set of four slices had 1.0 mm thickness. A slice at 14 – 19 mm from the inter-compartment boundary was selected as the bulk soil, which was assumed to have had minimal influence of the roots and hyphae. A 2.0 mm thick soil slice in the upper compartment immediately above the inter-compartment boundary was also sampled, and the total length of all roots in this slice was measured by the line intercept method described by Newman (1966). The root volume was determined by the amount of water displaced when the roots were immersed in water. The surface area of the roots was determined using the formula, surface area $=2\sqrt{3.14LV}$ where L is the length of roots and V is their volume, and assuming that each root is a cylindrical tube with a constant radius.

5.2.3 Soil analysis

Soil pH (1: 2.5 soil:H_2O, Blackmore et al., 1987), acid (pH 6.5) and alkaline (pH 11)

Plate 5.2 The microtome to slice the soil in the lower compartment of RSC

phosphatase enzyme activities (Tabatabai (1994), P fractionations (Hedley et al., 1994) and BGPR dissolution (Tambunan et al., 1993) were analysed as described at Chapter 3. The hyphal length density in soils was measured using a simplified agar film technique (Nicholas and Parkinson, 1967) detailed at Chapter 4.

5.2.4 Statistical analyses of data

Significant differences in soil properties were analysed according to a two-way ANOVA procedure (mesh treatment × soil section distance - slices). Significant difference between treatment means were established by calculating LSDs at $P < 0.05$.

5.3 Results and discussion

5.3.1 Mycorrhizal tips and root area

There was approximately the same root surface area (41 cm^2 ± 4 SE in 26 μm mesh treatment and 38 cm^2 ± 6 SE in < 10 μm mesh plus 0.3 μm filter treatment) and density of ECM infected root tips (mean: 328 tips m^{-1} root ± 20 SE in 26 μm mesh treatment and 334 tips m^{-1} root ± 24 SE in < 10 μm mesh plus 0.3 μm filter treatment) in the inter-compartment zone (0 - 2 mm section in the upper compartment just above the inter-compartment boundary) in the two treatments. Thus, any observed difference in the soil properties in the lower compartment did not result from different root densities in the upper compartment.

5.3.2 ECM hyphal length density

At harvest, the hyphal length density in the lower compartment was significantly (P < 0.05) higher in the 26 μm mesh treatment than in the < 10 μm mesh plus 0.3 μm filter treatment at distances up to 4 mm from the roots (Plate 5.3; Figure 5.2). The results suggest that the addition of the 0.3 μm pore-size glass filter to the double 10 μm meshes at 6 months from the trial commencement appeared to have reduced the hyphae penetration into the lower compartment. The differences in hyphal length density between treatments ranged from 12 to 46 m g^{-1} soil in the rhizosphere (0 - 4 mm from roots) compared to approximately 4 m g^{-1} soil in the bulk soil (> 14 mm from roots), suggesting that the ECM fungal hyphae extend to at least 4 mm away from the root surface under this experimental condition. The greater difference in hyphal length density at distances closer to the roots was associated with changes in some soil properties within this distance. These changes are discussed in later sections.

The hyphal length density in both the treatments significantly decreased with increasing distances from the roots. The higher hyphal length density in the rhizosphere of the < 10 μm mesh plus 0.3 μm filter treatment, compared to the bulk soil (Figure 5.2), may have resulted from the hyphal penetration through the < 10 μm mesh during the first 6 months of the experiment (before the 0.3 μm glass filters were inserted) (see method section).

Plate 5.3 An overview of the interfaces between upper (top) and lower (bottom) compartments after dismantling of the RSCs (Left: RSCs at 26 μm mesh treatment in which visual ECM hyphae appearance on top of soil in the lower compartment; Right: RSCs at < 10 μm mesh plus 0.3 μm filter treatment)

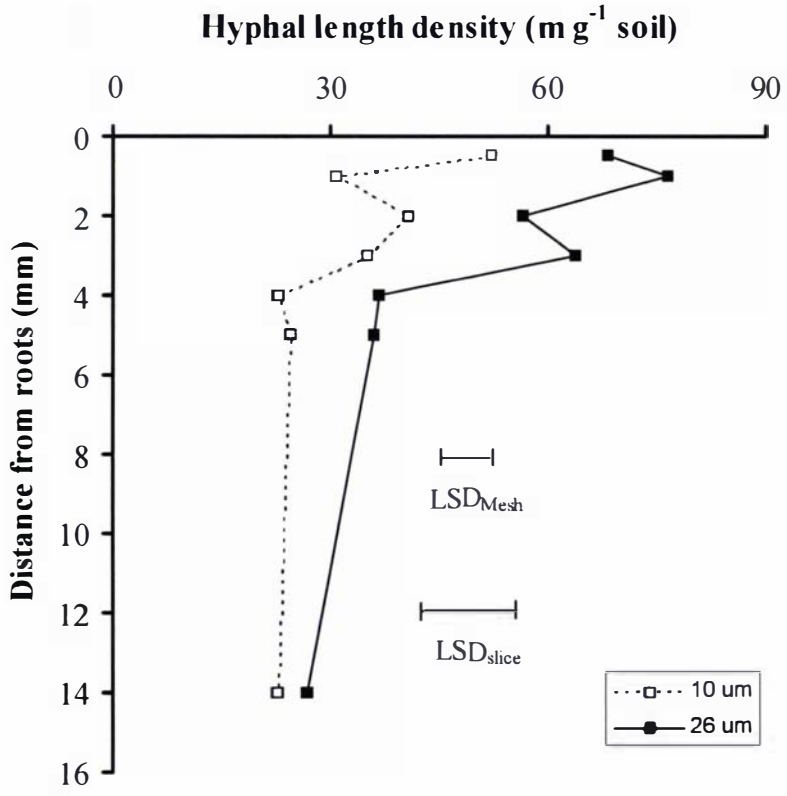

Figure 5.2 Hyphal length density in soil

5.3.3 Soil pH

The soil pH in both treatments was significantly ($P < 0.05$) lower in the rhizosphere soils compared to that in the bulk soil (Figure 5.3). The bulk soil pH remained approximately the same as the pH in the soil at the start of the trial (pH 5.8). There was no significant difference in soil pH between the treatments, but changes in mean pHs with distance from the roots presented a markedly different pattern between the two treatments. In the < 10 μm mesh plus 0.3 μm filter treatment, soil acidification mainly occurred down to 0 – 3 mm from the roots. In contrast, in the 26 μm mesh treatment, soil acidification extended to beyond 5 mm soil depth.

The lower soil pH in the rhizosphere compared to the bulk soil (< 3 mm from root surface) in the < 10 μm mesh plus 0.3 μm filter treatment is considered to be due to the rhizosphere processes occurring at the interface of the RSC compartments (Chen et al., 2002; Trolove et al., 2003), while the extensive acidification of soil beyond 3 mm from the interface in the 26 μm mesh treatment may be associated with the fungal hyphae penetration through the wider openings in this mesh into the lower compartment. This is consistent with the significant negative correlation between hyphal length density and soil pH in the lower compartment (Figure 5.4). These results suggest that external ECM hyphae may have similar role as plant roots on soil acidification.

5.3.4 Phosphatase enzyme activities

The acid phosphatase activity in the rhizosphere was significantly ($P < 0.05$) higher in the 26 μm mesh treatment than in the < 10 μm mesh plus 0.3 μm filter treatment with an exception in the soil slice closest to the roots (0 - 0.5 mm from root surface) (Figure 5.5A). Adjacent to the mesh boundaries the root effects may have been more dominant and this may have caused a relatively smaller difference in the enzyme activity between the treatments. The higher acid phosphatase activity in the < 26 μm mesh treatment indicates that in addition to roots, ECM hyphae also produced acid phosphatase enzyme, as suggested by Häussling and Marschner (1989). Acid phosphatase activity significantly decreased with increasing distance from the root surface. This declining pattern in acid phosphatase activity was associated with a decrease in hyphal length density (Figure 5.6).

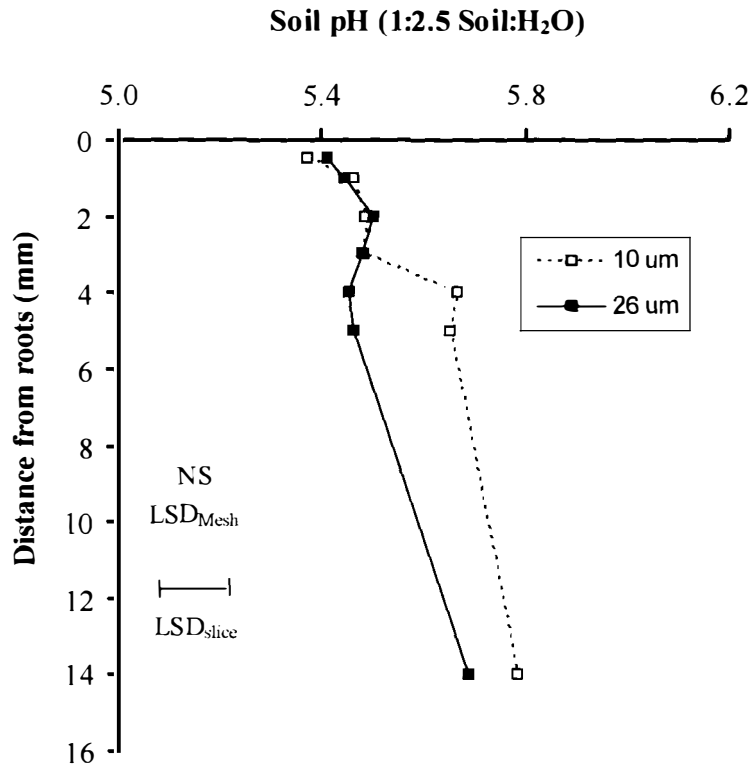

Figure 5.3 Roots/hyphae-induced changes in soil pH

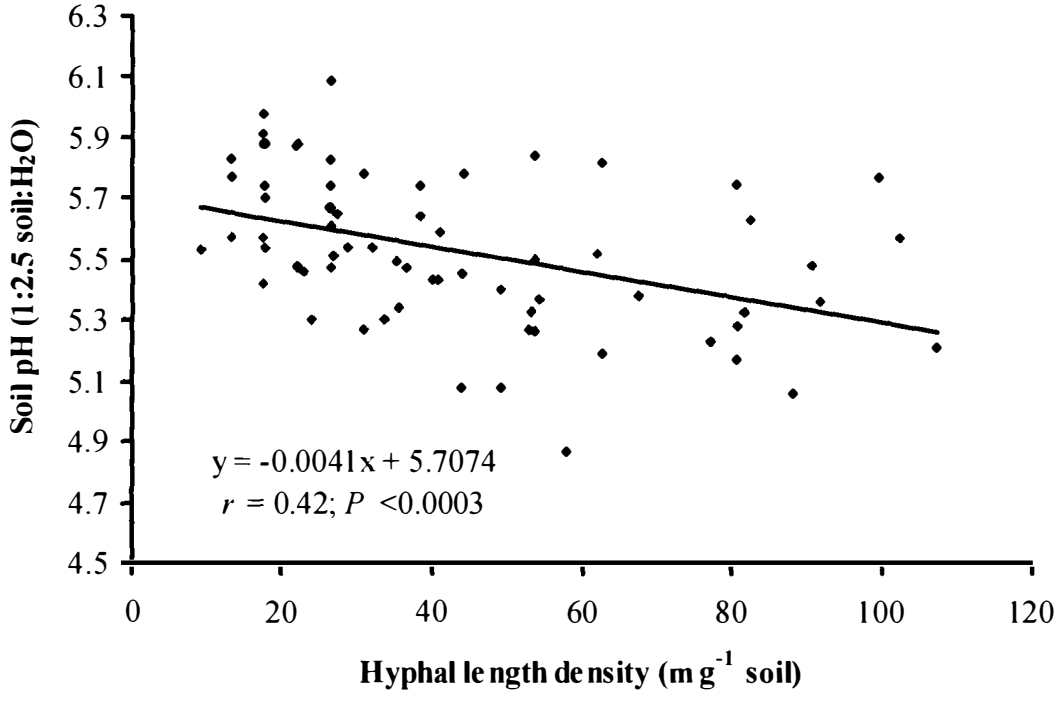

Figure 5.4 Relationship between hyphal length density and pH in the soils of the RSC lower compartment.

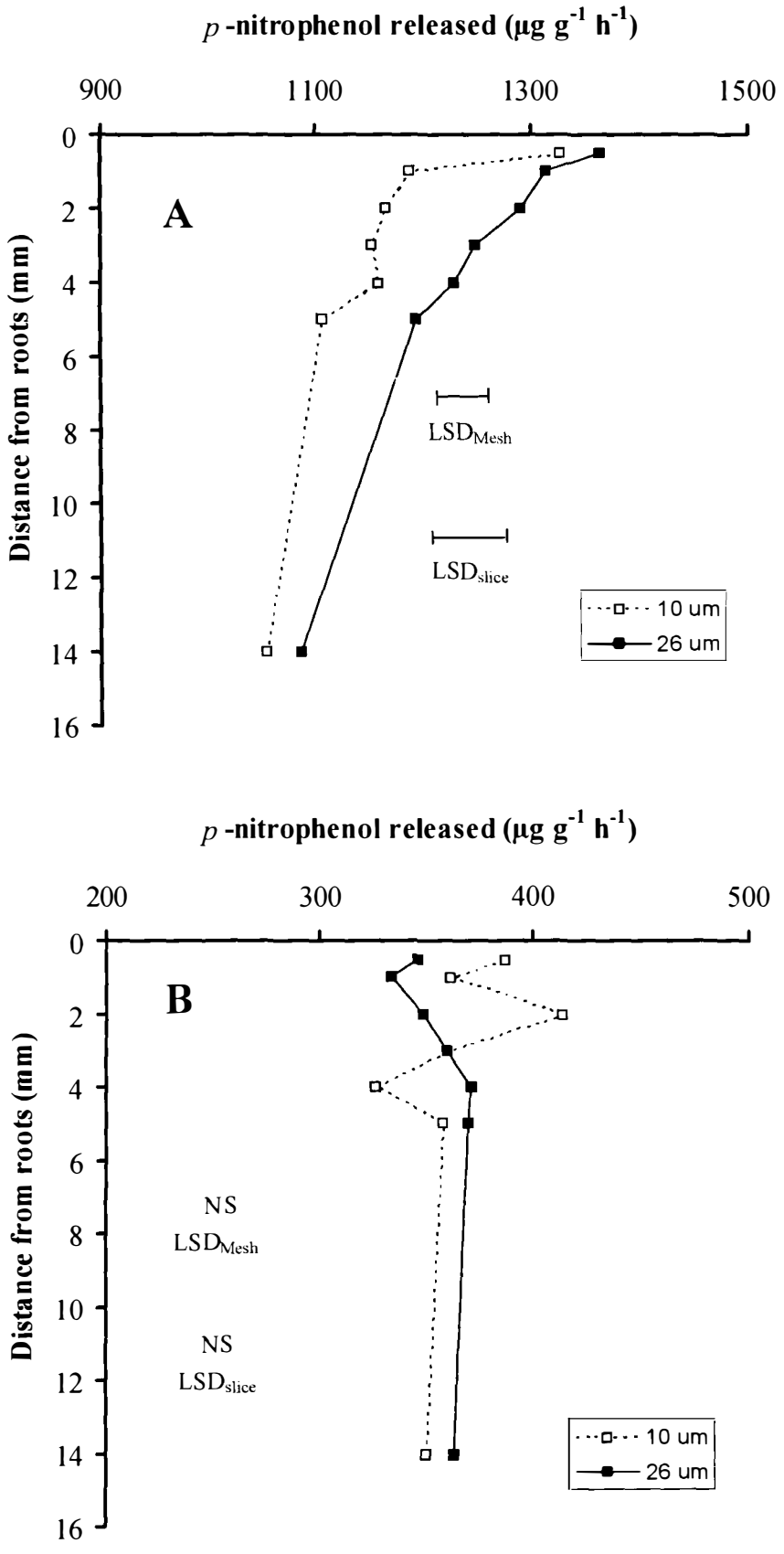

Figure 5.5 Roots/hyphae-induced changes in soil acid (A) and alkaline (B) phosphatase enzyme activities.

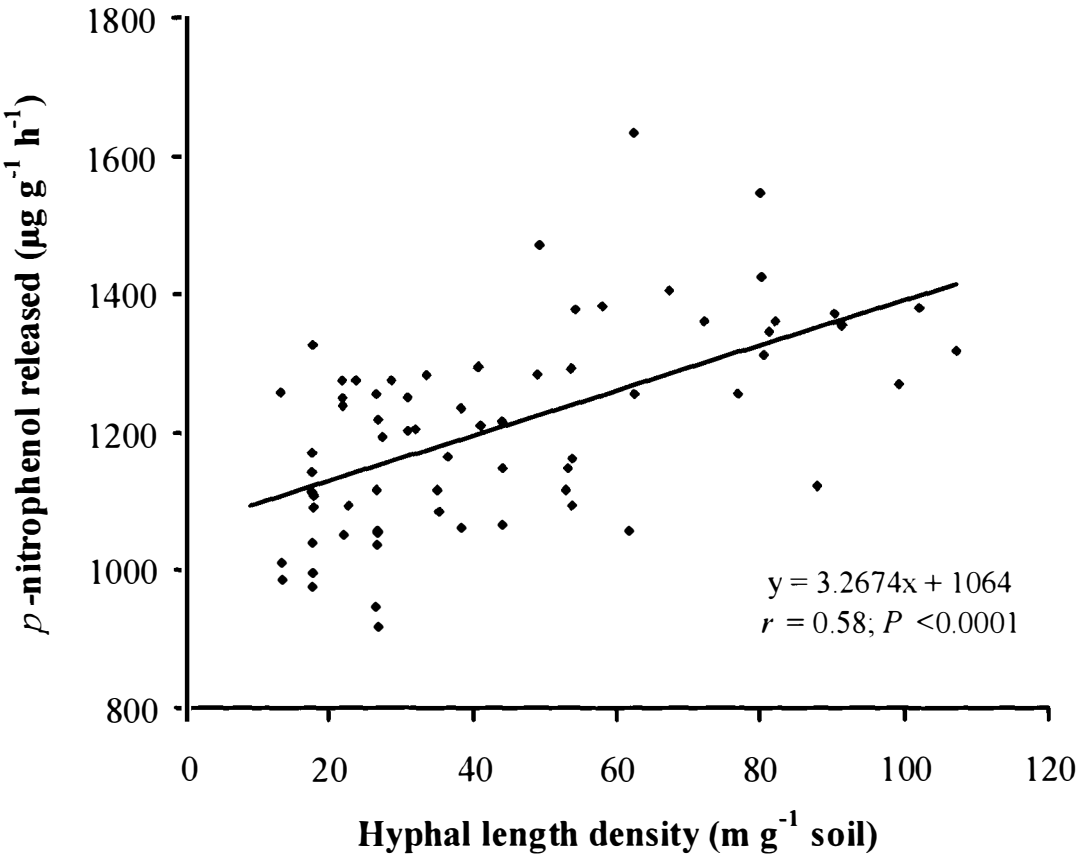

Figure 5.6 Relationship between hyphal length density and acid phosphatase enzyme activity in the soils of the RSC lower compartment.

Alkaline phosphatase activity was much lower than the acid phosphatase activity and was variable, particularly in the < 10 µm mesh plus 0.3 µm filter treatment (Figure 5.5B). There was no significant ($P < 0.05$) difference observed between the treatments or the distances from the roots. However, it should be noted that differences in alkaline phosphatase activity were observed between rhizosphere and bulk soils in the studies reported in Chapters 3 and 4. The differences in experimental techniques and conditions used in this study and in the previous studies might have been the reasons for these inconsistent observations.

5.3.5 BGPR effect on soil P fractions

A sequential P fractionation scheme, which uses resin extraction as the first step was used to characterise the initial P fractions in soil and pine-needle powder and the

redistribution of dissolved BGPR-P into these fractions after 10 months. More than 70% of P in the unfertilised initial mixtures of soil and needle-powder existed as organic P ($NaOH\text{-}P_o$ + residual-P) (Table 5.1).

A second P fractionation scheme, designed to measure PR dissolution in soil by using the pH 7.0 buffer TEA/NaCl as the first step rather than resin (Tambunan et al., 1993), estimated BGPR dissolution to be > 67% (Table 5.2). This high % dissolution of BGPR was due to the finer particle size (150 – 250 µm) of BGPR used, high soil P retention (92%), mildly acidic soil pH (5.8), low Ca saturation (0.028 $mol_c\ kg^{-1}$ soil) of the soil, and the long period allowed for the dissolution (10 months) as discussed in Chapter 3.

Table 5.2 % of Ben Guire phosphate rock dissolution (data presented as mean ± SE)

Distance from roots (mm)	**BGPR dissolution (%)***		
	< 10 µm mesh plus 0.3 µm filter treatment	26 µm mesh treatment	Difference in treatments
0 - 0.5	72.4 ± 0.8 **	77.0 ± 0.8 **	4.5
0.5 - 1	69.8 ± 0.3 **	79.2 ± 2.1 **	9.4
1 - 2	72.0 ± 0.8	73.5 ± 0.4	1.5
2 - 3	71.7 ± 0.8	73.5 ± 0.8	1.8
3 - 4	67.3 ± 1.3	71.0 ± 0.7	3.7
4 - 5	68.9 ± 2.7	71.4 ± 1.5	2.5
> 14	68.6 ± 0.7	70.5 ± 0.4	1.9
	$LSD_{(mesh)}$ = 1.51;	$LSD_{(slice)}$ = 2.82	

* % PR dissolution = 100 × {1- [0.5 *M* H_2SO_4 extractable P ((soil + PR fertiliser)-(initial soil mixture alone))]/ PR-P added}(Zoysa et al., 1997).

** Data were not available for TEA/NaCl method due to inadequate soil samples for analyses. Therefore, BGPR dissolution was calculated from P fractionation method using resin strips as the first step. There was a very good 1:1 correlation between the two methods for soil slices from 1 to > 14 mm.

The extensive dissolution of BGPR-P and redistribution of soluble P applied in the needle powder markedly increased concentrations of all P fractions except the resin-P concentration at the end of 10 months (Table 5.1; Figure 5.7 to Figure 5.9). The resin-P concentration (1.0 to 1.7 μg P g^{-1} soil) (Figure 5.7A) at the end of the trial was significantly lower than that in the mixtures of soil and pine needle-powder (10 μg P g^{-1} soil) at the start of the trial (Table 5.1). The higher resin-P concentration in the initial soil mixtures originated from the pine needle-powder which had very high resin-P concentration (238 μg P g^{-1} soil) (Table 5.1). The resin-P derived from the needle-powder in the soil + needle powder mixtures would have been fixed as inorganic P or immobilised as organic P by soils or taken up by the trees during the 10 months trial period and hence the lower resin-P concentration observed at the end of the trial. At the end of 10 months, the proportion (18 – 23%) and concentration (64 – 81 μg g^{-1} soil) of NaOH-P_i (Figure 5.7B) increased due to fixation of P derived from the needle-powder and dissolved BGPR. The labile NaOH-P_o (Figure 5.8B) still constituted the highest P fraction (50 – 56%). It is estimated that at least 26% and 38% of the applied BGPR-P were respectively recovered in the NaOH-P_i and NaOH-P_o fractions, indicating that majority of the added BGPR-P was quickly immobilised by allophane and the Fe/Al oxides (NaOH-P_i) or by microorganisms (NaOH-P_o) in the soil.

5.3.6 Effect of roots and ECM hyphae on P fractions

5.3.6.1 Effect on resin-P

Compared to the bulk soil at distances greater than 14 mm from the root surface, the soils closer to the roots (< 5 mm) had significantly lower resin-P concentrations for both the mesh treatments (Figure 5.7A), possibly because the rate of plant-available P depletion by plant uptake was higher than the rate of plant-available P replenishment by other processes in this highly P deficient soil. The 26 μm mesh treatment resulted in greater depletion of resin-P in the 0 - 5 mm zone from the root surface, compared with the < 10 μm mesh plus 0.3 μm filter treatment. But this was not statistically significant. The mean of resin-P concentration in the 26 μm mesh treatment was approximately 0.8 μg P g^{-1} soil lower compared to that in the bulk soil, but only approximately 0.3 μg P g^{-1} soil lower in the < 10 μm mesh plus 0.3 μm filter treatment. A higher depletion of

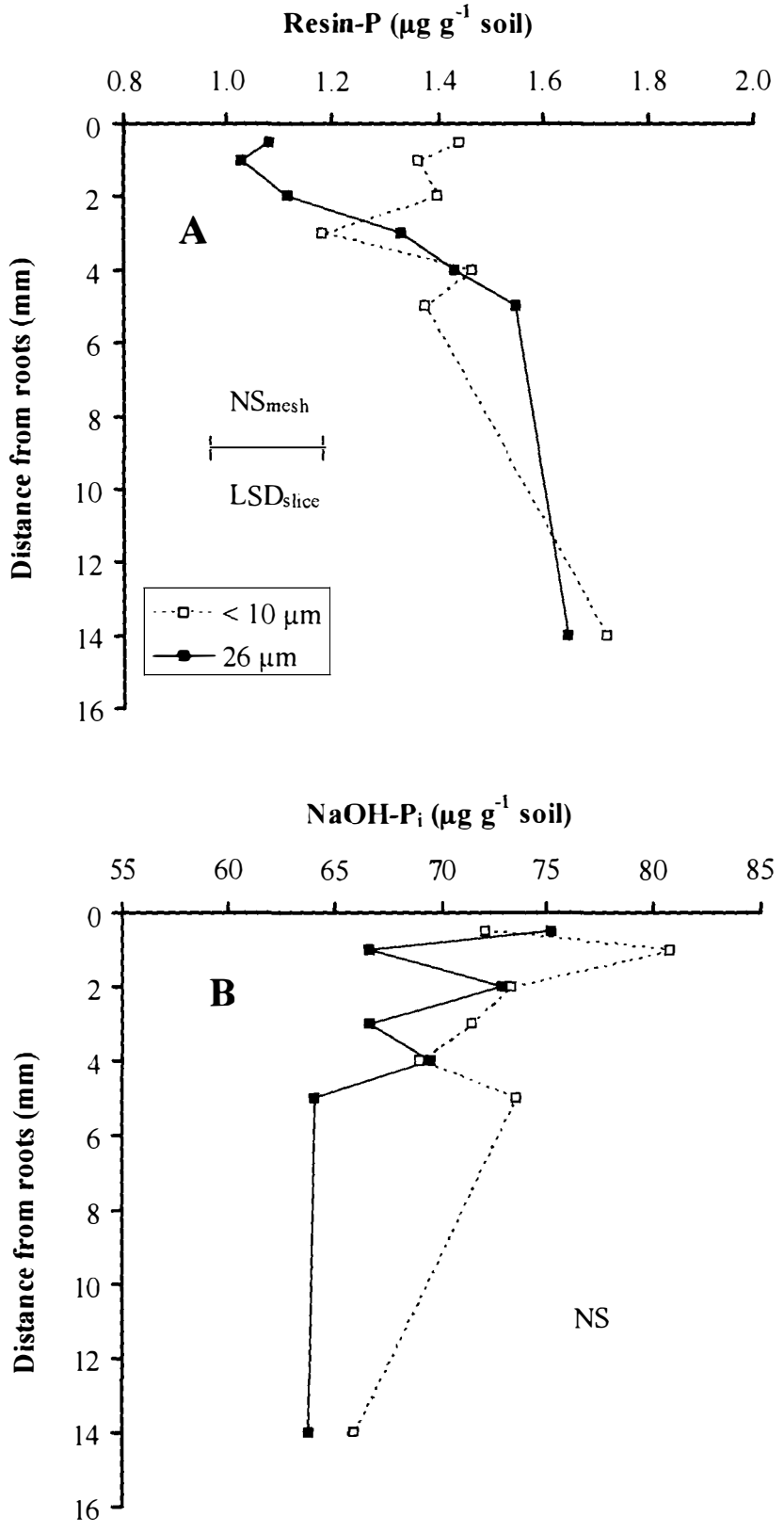

Figure 5.7 Concentrations of resin-P (A) and 0.1 *M* NaOH-P_i (B) in the RSC lower compartment soils.

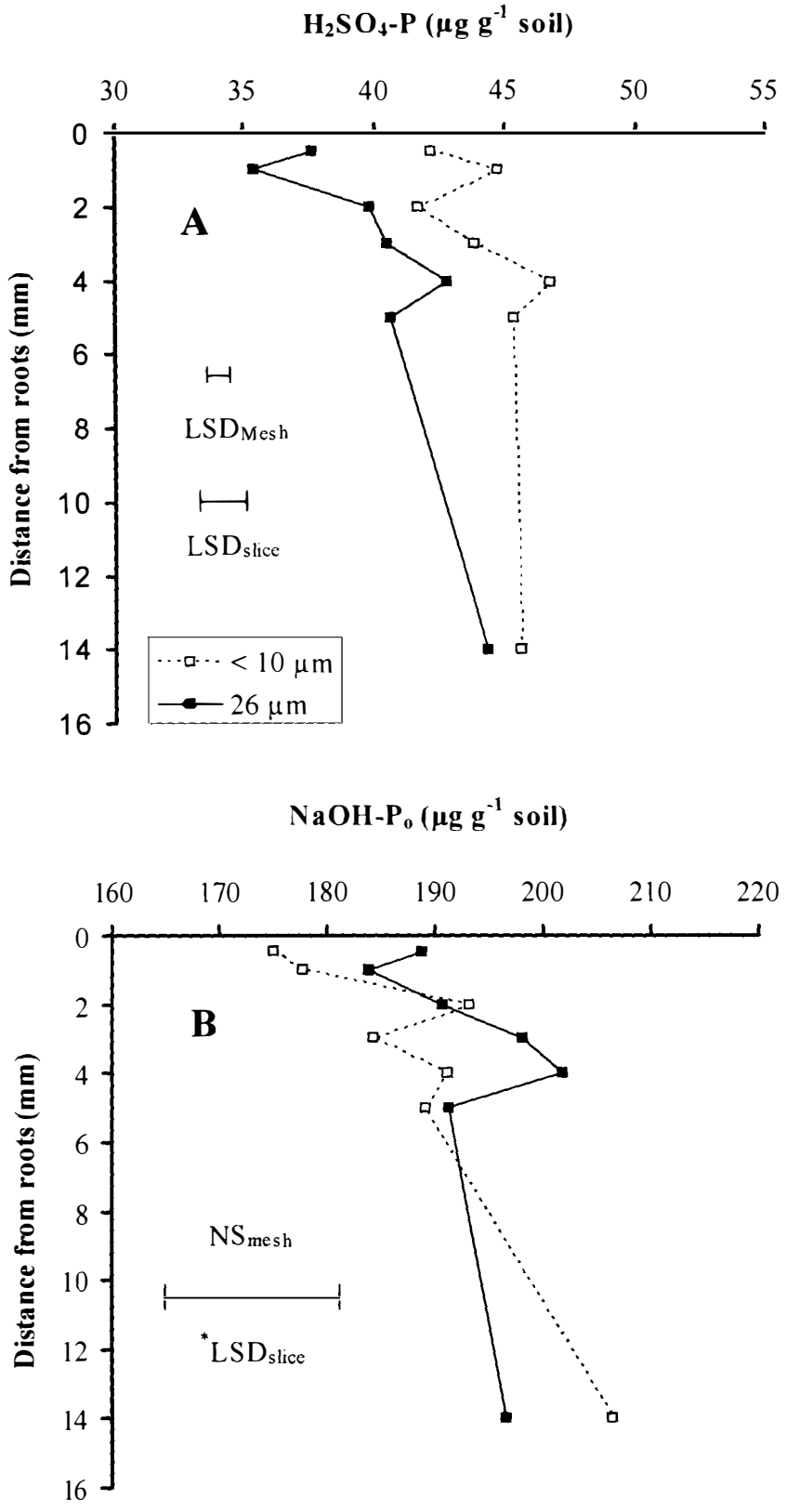

Figure 5.8 Concentrations of H_2SO_4-P (A) and 0.1 *M* NaOH-P_o (B) in the RSC lower compartment soils (* represents significant ($P < 0.05$) differences only between < 1mm (first two slices) and > 14 mm distance from the root interfaces).

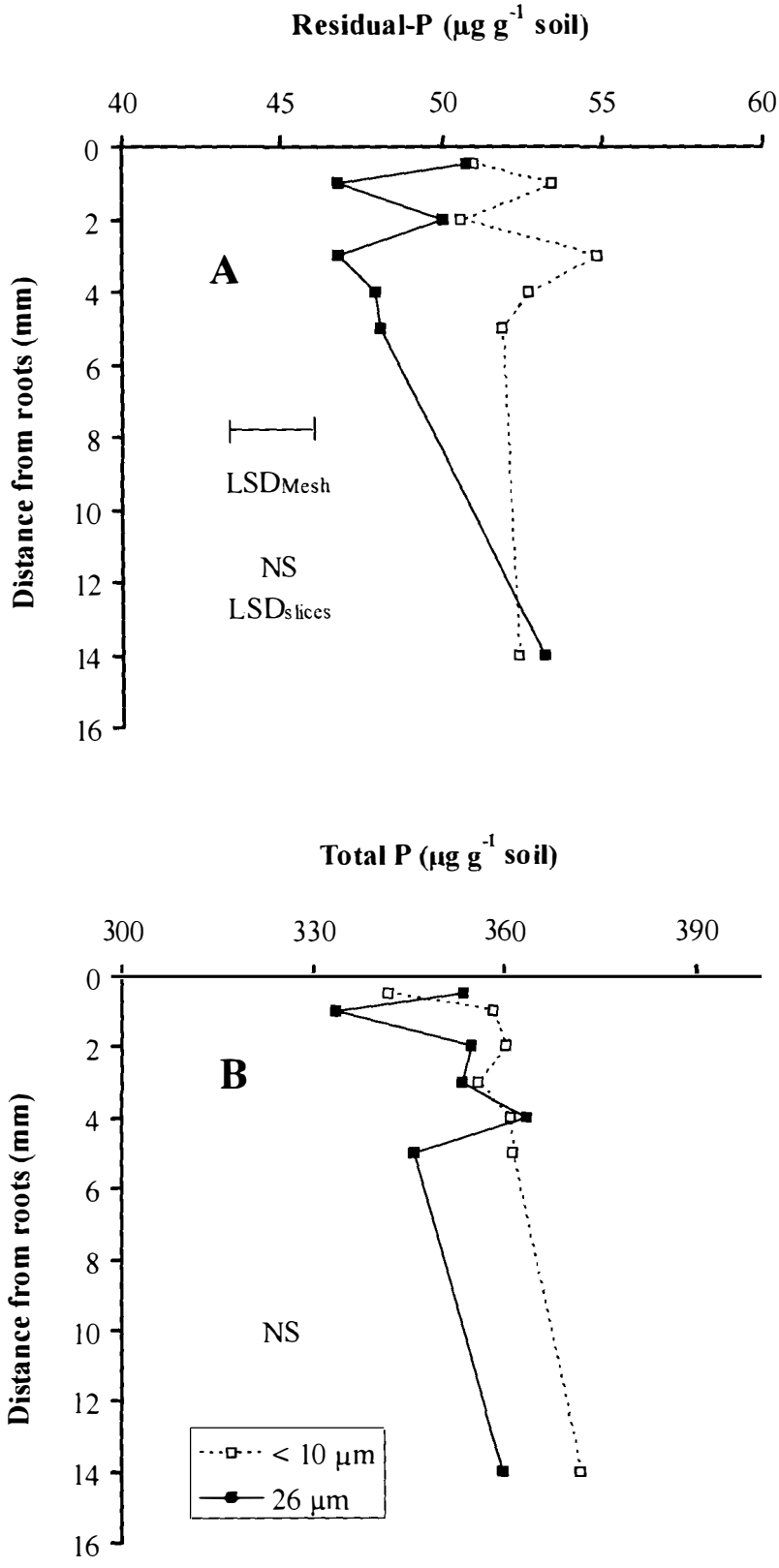

Figure 5.9 Concentrations of residual-P (A) and total-P (B) in the RSC lower compartment soils.

resin-P in the 26 μm mesh treatment is expected because of the possibility of higher amount of P uptake from the lower compartment of the RSC as a result of higher hyphal density.

The decrease of resin-P concentration in the rhizosphere soils agreed with the observations made by others (e.g. Zoysa et al., 1997; Chen et al, 2002), but not with a previous study (Chapter 4) on the same soil as the one used in this study where the resin-P concentration was higher in the rhizosphere soil than in the bulk soil under 4 - 5 year old *P. radiata* seedlings. This disagreement may be due to the difference in the techniques of sampling rhizosphere soil as well as the age of the trees. The technique employed to collect rhizosphere soil by vigorous shaking of roots to detach soils adhered to roots in the previous study may have overestimated the resin-P as a result of contribution of P from broken roots and hyphae. Also under older trees, ECM roots turnover may have resulted in higher total C and P in the rhizosphere, thereby increasing resin-P concentration.

5.3.6.2 Effect on NaOH-P_i

The NaOH-P_i constituted 18 to 23% of the total P in BGPR treated soil compared to 17% in BGPR untreated soil at the end of 10 months of trial. There were no significant differences in NaOH-P_i between treatments or with distance from the mesh boundaries (Figure 5.7B).

5.3.6.3 Effect on H_2SO_4-P

The 0.5 *M* H_2SO_4-P fraction constituted 10 - 13% of the total P in the BGPR treated soil compared to 5.5% in the BGPR untreated soil at the end of 10 months of trial. The higher percentage of H_2SO_4-P in the BGPR treated soil is due to undissolved BGPR in the soil. The root/hyphae induced significantly ($P < 0.05$) lower concentration of H_2SO_4-P in the rhizosphere in both the treatments, particularly to 26 μm treatment (Figure 5.8A). The depletion zone of H_2SO_4-P extended to at least 5 mm from the root surface. The lower H_2SO_4-P concentration in the rhizosphere is due to higher dissolution of the BGPR caused by the lower pH in the rhizosphere (Figure 5.3) as

reported by others (Zoysa et al., 1997; Trolove et al., 2003). The exudation of low molecular weight organic anions, especially oxalate by *P. radiata* (Malajczuk and Cromack, 1982), has also been suggested to be the important solublising-agents improving mobilisation of poorly soluble P sources by ECM hyphae (Wallander et al., 1997). Additionally, it is known that the ECM hyphae have a high affinity for P at low P availability (Ekblad et al., 1995). Thus, it is possible that the ECM hyphae acted as a more efficient sink for P which would have stimulated the dissolution of BGPR (Wallander et al., 1997).

Compared to the < 10 μm mesh plus 3 μm filter treatment, the H_2SO_4-P concentration was significantly ($P < 0.05$) lower in the 26 μm mesh treatment, suggesting that the hyphae penetration may have induced a higher amount of BGPR dissolution in the 26 μm mesh treatment (Table 5.2). This is consistent with the significantly ($P < 0.05$) negative correlation between hyphal length density and the 0.5 *M* H_2SO_4-P concentration (Figure 5.10).

5.3.6.4 Effect on NaOH-P_o

The 0.1 *M* NaOH-P_o which is considered to be the labile organic P (Hedley et al., 1994) was the major P fraction in the soils with (50 – 56% of total P) and without (58% of total P) BGPR addition at the end of 10 month of the trial. The NaOH-P_o in both the mesh treatments was depleted in the rhizosphere region (Figure 5.8B). The concentration of NaOH-P_o in the two soil slices nearest to the meshes were significantly ($P < 0.05$) lower than that in the bulk soil zone (> 14 mm from roots). Of the various P fractions, depletion of the labile NaOH-P_o was the highest, constituting 13 to 31 μg g^{-1} soil. This observation was consistent with the results obtained in Chapter 3. The larger depletion of NaOH-P_o in the rhizosphere suggests that soil P_o may be an important source of P for radiata pine (Davis, 1995; Condron et al., 1996; Chen et al., 2002). The lower concentration of 0.1 *M* NaOH-P_o in the rhizosphere soil compared to the bulk soil is associated with increased acid phosphatase activity in the rhizosphere soil (Figure 5.5A). This suggests that the roots/hyphae-induced acid phosphatase activity had a role in converting 0.1 *M* NaOH-P_o to 0.1 *M* NaOH-P_i or to soluble P_i, a part of which may have been taken up by plant roots (Häussling and Marschner, 1989).

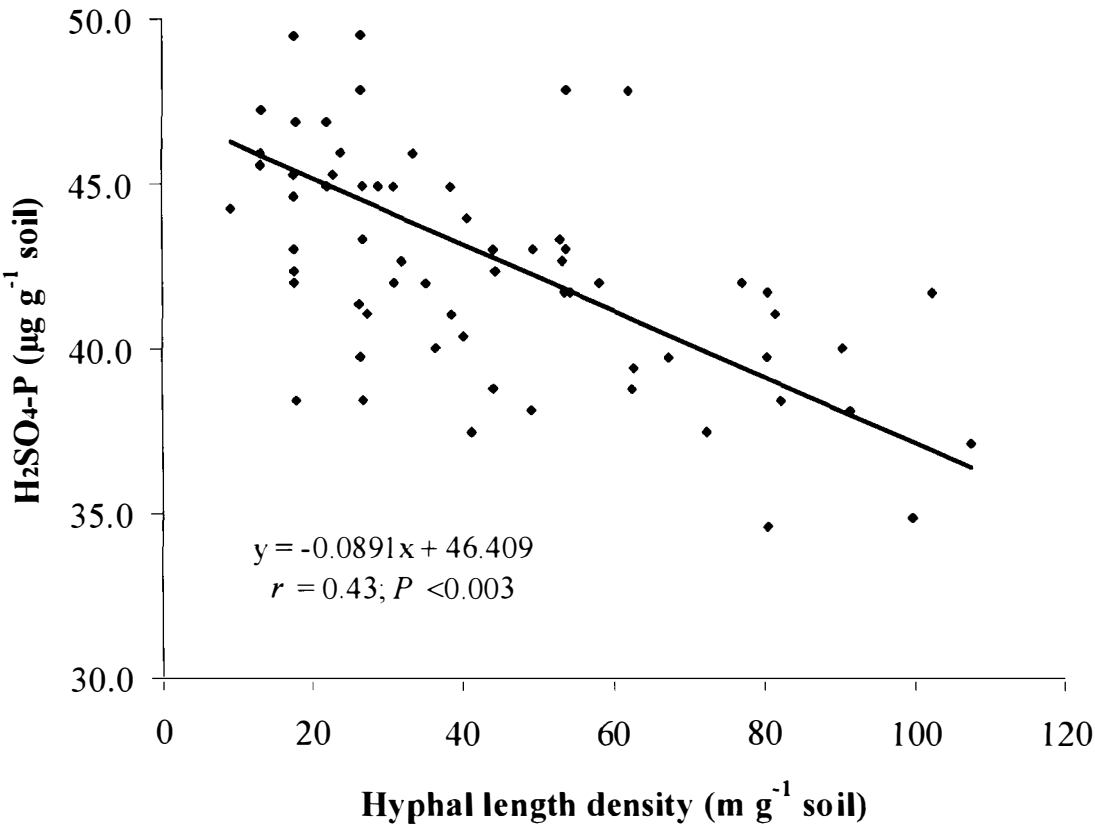

Figure 5.10 Relationship between hyphal length density and pH in the soils of the RSC lower compartment.

There was no significant difference in NaOH-P_o concentration between the two treatments, suggesting that the hyphae-induced higher acid phosphatase activity in the 26 µm mesh treatment (Figure 5.5A) did not cause a significant decrease in NaOH-P_o concentration in this treatment. This lack of significant difference in NaOH-P_o between the treatments could have been due to organic P hydrolysed by the phosphatase partly getting resynthesised into microbial-P_o forms, resulting in no net decrease in NaOH-P_o in the 26 µm mesh treatment (Hedley et al., 1994). This was evidenced by the relatively higher percentage of BGPR-P recovery in the NaOH-P_o fraction (mean of 38%), suggesting that P_i released from BGPR was immobilised by soil microorganisms. The absence of any difference in NaOH-P_o between the treatments could have been also due to the complex interactions of many factors including tree site variations (such as soil moisture, microbial activities etc.) under field conditions.

5.3.6.5 Effect on residual-P

The residual-P which is considered to be the non-labile organic P (Hedley et al., 1994; Chen et al., 2002) consisted of 13 - 15% of the total P in the BGPR treated soils. In the 26 μm mesh treatment, the concentration of residual-P was significantly ($P < 0.05$) lower than that in the < 10 μm mesh plus 3 μm filter treatment in the rhizosphere region (Figure 5.9A). This suggests that the higher acid phosphatase activity in the 26 μm mesh treatment resulting from the higher hyphal density may have induced higher mineralisation of non-labile P_o compared to that in the < 10 μm mesh plus 3 μm filter treatment.

5.4 Conclusions

The results demonstrated that the novel technique using rhizosphere study containers with different pore-sized nylon meshes to allow (mesh opening diameter 26 μm) or reduce (< 10 μm mesh plus 3 μm filter) hyphae penetration into the rhizosphere soil can be successfully applied to study the *P. radiata* mycorrhizal hyphae effect on rhizosphere properties in an Allophanic Soil. By creating high and low soil hyphae activity in the treatments, it was shown that acid phosphatase activity in soil was associated with hyphal length density, indicating that the external ECM hyphae are able to promote acid phosphatase activity in soil and increase the rate of mobilisation of soil organic P. The ECM hyphae also caused depletion of plant-available P and encouraged further dissolution of finely-divided (150 – 250 μm pore-size) Ben Guire phosphate rock. These results demonstrated that the ECM hyphal strand has a significant role in mobilising low solubility P forms for utilisation by the tree.

CHAPTER 6

Mycorrhizal inoculation of *Pinus radiata* seedlings and its effect on the growth and rhizosphere properties of seedlings grown in an Allophanic Soil with and without phosphorus fertiliser application*

6.1 Introduction

Inoculation of *P. radiata* seedlings with ectomycorrhizal (ECM) fungi has been considered necessary to improve tree growth on low fertility soils (Bowen, 1968; Skinner and Bowen, 1974a, b; Chu-Chou and Grace, 1985; 1990; Smith and Read, 1997). Results reported in Chapter 5 showed that the natural ECM hyphae induced soil acidification and promoted phosphatase activity, causing changes in soil P availability. There may be differences in the effectiveness of ECM fungal species or isolates in inducing changes in rhizosphere properties. In this respect, it will be interesting to investigate the differences in *P. radiata* associated ECM fungi for their capacities to mobilise rhizosphere soil and fertiliser P.

The soil conditions where forest seedlings are transplanted markedly influence the persistence of mycorrhizal fungi in pre-inoculated seedling roots (Last et al., 1985; Chu-Chou and Grace, 1990). Studies in New Zealand (e.g. Chu-Chou and Grace, 1990) and elsewhere (Last et al., 1985) have shown that in some instances the mycorrhizal fungi

* Q. Liu, P. Loganathan, M. J. Hedley and L.J. Grace (2004) Effect of mycorrhizal inoculation on rhizosphere properties of *Pinus radiata* seedlings. In *SuperSoil 2004*: Proceedings of the 3rd Australian New Zealand Soils Conference, University of Sydney, Australia, 5 – 9 December 2004. www.regional.org.au/au/asssi/

species associated with the roots changed dramatically when seedlings are transplanted from nursery to forest soil. A selection of site-adaptive ECM fungi for inoculating seedlings in the nursery is expected to have longer persistence in the field.

In Chapter 3 it has been shown that application of a high rate of P fertiliser reduced ECM infection. Others also have shown that application of P fertilisers may reduce the degree of mycorrhizal infection on tree roots (Abbott et al., 1984; Newton and Pigott, 1991; Browning and Whitney, 1992; Smith and Read, 1997). However, little information is available in extremely P deficient soils on the degree of ECM infection. Most of the earlier studies examined the efficiency of mycorrhizal fungi in exploiting P from non-rhizosphere soil (Bowen, 1968; Skinner and Bowen, 1974; Smith and Read, 1997). Knowledge on the effect of ECM fungal type on the biochemistry and the P availability in the rhizosphere of *P. radiata*, the soil zone in the immediate vicinity of ECM fungi in the roots, is limited (Trolove et al., 2003).

The study reported in this chapter was based on a hypothesis of that the type of fungal species involved in ECM association with *P. radiata* influences the nature of biochemical change in the rhizosphere and seedling growth. This study was designed to compare the efficiency of *P. radiata* seedlings inoculated with two ECM fungi, *Rhizopogen rubescens* Tul. and *Suillus luteus* (L. ex. Fr.) S. F. Gray, with that of uninoculated *P. radiata* seedlings (associated with ECM in natural soil) in promoting growth and P uptake of *P. radiata* seedlings under P-unfertilised and P-fertilised conditions. *R. rubescens* and *S. luteus* were selected for this study because these are two common ECM fungi species associated with *P. radiata* forests in New Zealand. Attempt was also made to determine the ECM root-induced changes in the rhizosphere properties, particularly P chemistry, of the *P. radiata* seedlings.

6.2 Materials and methods

6.2.1 Experimental design

A factorial experiment with 2 levels of phosphate rock (PR) fertilisation and 5 soil treatments was conducted on *P. radiata* seedlings in a pot trial under glasshouse conditions. The two PR fertilisation levels were: P-unfertilised soil (0 μg P g^{-1} soil) and

P-fertilised soil (100 μg P g^{-1} soil applied as Ben Guire phosphate rock (BGPR)). The soil treatments were: (1) autoclaved soil (autoclaved forest soil); (2) soil treated with fungicide (natural forest soil treated with fungicide); (3) natural soil (soil collected from forest); (4) *S. luteus* inoculated soil (natural forest soil inoculated with *S. luteus* spores); and (5) *R. rubescens* inoculated soil (natural forest soil inoculated with *R. rubescens* spores). Each treatment was replicated five times, giving a total of 50 experimental pots. A major consideration for using natural soil, instead of sterile soil for inoculation of ECM, was that soil sterilisation processes may impact on the nutrient status and microorganisms in the natural soils to the extent of affecting the P chemistry of the soil that is being investigated in this study.

6.2.2 Soil preparation

The soil material used in this study came from the 0 - 100 mm horizon of an Orthic Allophanic Soil (Andosol, Hewitt, 1992) which is described in Chapter 3 (Table 3.1). The autoclaved soil was prepared by autoclaving the air-dried soil for 30 min at 120ºC temperature and 15 P In^2 pressure using a steam autoclave; the soils were then placed in a tray and left in a room isolated from possible contamination with ECM spores for a week. This process is expected to allow the entry of air-borne microorganisms into the soil. The fungicidal treatment consisted of soils leached with a dilute solution of commercial Terrazole 35WP at the rate of 0.05 ml fungicide per pot containing 0.5 kg soil (applied the fungicide after soils were potted). The soils for the P-fertilised treatments were prepared by thoroughly mixing BGPR (particle size 150 – 250 μm; total P 13.1%; and citric acid extractable P 2.75%) with the soil at a rate of 100 g P μg^{-1} soil. The nitrogen (N) and potassium (K) were applied to all treatments as solutions of NH_4NO_3 and KCl (both are chemical grade) at a rate of 100 g N or K kg^{-1} soil. At 5 months of age the seedlings received another addition of N and K at the same rate.

6.2.3 Seed germination

Seeds of *Pinus radiata* Don D. were soaked overnight in distilled water; then sown in moist perlite contained in 2 L plastic containers. The containers were left in the dark at 22 – 24ºC temperature for seed germination. After germination of the seeds (7 days after

sowing), the young seedlings were allowed to grow in perlite under normal light for another week. At 2 weeks of age (on 28 March 2001) the seedlings were transplanted into pots containing 500 g air-dried soils (equivalent to 462 g oven dried soil) packed to a bulk density of 0.85 g cm^{-3}, at the rate of 5 seedlings per pot. The pots had holes at the bottom to allow drainage of excess water. A filter paper (Newman No.31) was placed at the bottom of the pot to prevent soil loss through the pot drainage holes and another two covered on the surface of the soil to minimise cross infection by air-borne inocula.

6.2.4 Seedling inoculation

Sporocarps of *R. rubescens* and *S. luteus* were collected (by Ms L. Grace, Forest Research Ltd. New Zealand) from a Forest Research Institute nursery and beneath a *P. radiata* plantation at Rotorua, New Zealand (Plate 6.1), respectively. After transporting the sporocarps to the laboratory, their skins were removed and spore suspensions were prepared by squashing the hymenial tissue of the fresh sporocarps (gills of *S. luteus* and glebal tissue of *R. rubescens*) in autoclaved water until the spores had dispersed. The spore concentration in the suspension was estimated by counting the spores using a haemocytometer plate under 200X magnification and the suspension was diluted with autoclaved water to 3 - 4 × 10^7 spores ml^{-1}.

Each seedling was inoculated with 5 ml of the diluted spore suspension by pipetting the suspension into holes made to 40 mm depth in soils inside the pots near the stem base. Seedlings were inoculated again at 4 weeks after transplanting to ensure that inoculation was successful. Seedlings in uninoculated treatments, namely the seedlings in the autoclaved, fungicide treated and natural soil treatments, received the same amount of inocula suspension but after autoclaving for 30 min under 120°C temperature and 15 P In^2 pressure using a steam autoclave to sterilise the suspension. By this process the nutrient added in the suspension was maintained the same for all treatments.

6.2.5 Trial management and harvesting of plants

Treatments were arranged in a randomised complete design inside a glasshouse maintained at 16°C - 28°C temperatures (Plate 6.2-Top). The pots were watered using

Plate 6.1 Sporocarps of *Rhizopogen rubescens* Tul. (top) and *Suillus luteus* (L. ex. Fr.) S. F. Gray (bottom)

Plate 6.2 *Pinus radiata* seedlings inside the glasshouse. Top – one month old seedlings; Bottom – 10 months old seedlings before harvest (Front: P unfertilised seedlings; Back: BGPR-P fertilised seedlings; Left to right: autoclaved, fungicide treated, natural, *S. luteus* inoculated and *R. rubescens* inoculated treatments).

distilled water to "pot field capacity" at 2-day intervals by weighing the pots. The direction of the bench on which the pots were arranged was changed weekly so that the seedlings in all treatments received equal amounts of sunlight.

To enable sampling of soil solutions (described in section 6.2.7) at approximately constant soil moisture content, forty-eight hours before harvesting of the seedlings the soil moisture in all pots was adjusted to "pot field capacity". Seedlings were harvested on 9 January 2002 after 10 months growth. The seedling height was recorded as the mean of the five seedlings in each pot. The total root length and ECM root tip numbers were calculated from the root length and ECM tips measured in a sub-sample of 5 randomly selected lateral roots per pot (1 lateral root per seedling). The root length was measured using a gridline technique described by Newman (1966). The number of ECM root tips (forked fine root) was counted manually.

6.2.6 Soil sampling

At plant harvest, the soil at the top 10 mm depth in the pots was discarded to minimise the influence of the atmosphere on the soils. The rhizosphere soil was collected as described in Chapter 3. The whole seedling was removed from the pot with minimum injury to its roots, by shaking the roots until the soil not tightly adhering to the roots was removed and then collecting the soil closely adhering to the root system (rhizosphere soil) by vigorously shaking the roots. The root growth in the pots was very high and it was difficult to clearly separate bulk soils from rhizosphere soils. As much as possible the "bulk" soil (soil less influenced by roots) was collected from areas in the pots where there were fewer roots.

6.2.7 Soil solution sampling

Soil solutions were sampled from two of the five replicates of each treatment as the subsequent analyses of these solutions are expensive (see section 6.2.9). Solutions were removed from a portion of moist soil within 6 h of sample collection, using a double-centrifuge-tubes technique (centrifuged for 0.5 h at 10000 rpm and 4°C; filtered

immediately through a 0.45 μm millipore filter) (Wang and Zabowski, 1998). The solutions were stored frozen for the analyses of oxalate and phosphate concentrations.

6.2.8 Mycorrhizas identification and ECM hyphal length measurement

The ECM fungal species which infected the roots were identified by visibly examining the fine root characteristics as described by Chu-Chou and Grace (1983). This identification was assisted by Ms L. Grace of the Forest Research of New Zealand. The ECM hyphal length density in the soils was measured using a simplified agar film technique described in Chapter 4.

6.2.9 Plant, soil and solution analyses

The herbage total N and P contents (Kjeldahl digestion; Blackmore et al., 1987; Jackson, 1958), soil pH (1:2.5 of soil:H_2O; Blackmore et al., 1987), acid (pH 6.5) and alkaline (pH 11) phosphatase enzyme activities (Tabatabai, 1994), soil P fractions (Hedley et al, 1994) and BGPR dissolution (Tambunan et al., 1993) were analysed as described in Chapter 3.

The oxalate concentrations in the soil solutions were determined on 2 replicates of each treatment, using a Waters ion chromatographic system at the Landcare Research Ltd. Palmerston North (see Section 3.2 of the Chapter 3). The inorganic P (P_i) concentrations in the soil solutions were analysed using malachite-green method described by Motomizu et al. (1983).

6.2.10 Statistical analyses of data

Significant differences in seedling parameters between the treatments were tested using a two-way ANOVA procedure. Differences in soil properties between treatments were analysed according to two factorial design (2 soil positions - bulk and rhizosphere soils × 5 soil treatments) and three factorial design (2 P fertilisation levels × 2 soil positions × 5 soil treatments). Significant differences between means were established by calculating LSDs at a significance level of $P < 0.05$.

6.3 Results and discussion

6.3.1 Effects of ECM inoculation and P fertilisation on seedling growth and P uptake

6.3.1.1 Effects of ECM inoculation

The seedling roots in non-autoclaved natural soils (Plate 6.3) had a mixture of ECM fungal species. The main species present were *R. rubescens* and *Laccaria laccata*. In the *S. luteus* and *R. rubescens* inoculated treatments, the seedling roots were predominately infected by the corresponding mycorrhizal fungus in the inoculums. The application of fungicide to the soil did not completely eliminate the mycorrhizal infection on the roots. The mycorrhizas present in the fungicide treated soil were dominated by *R. rubescens* and *L. laccata*, which were originally present in the natural soil. The seedlings in autoclaved soil were rarely infected with any fungi, but a few seedlings, particularly in the P-fertilised treatment, were slightly infected with the original fungi *R. rubescens* and *L. laccata* in the roots. Mycorrhizal infection caused a change in colour of seedling shoots from yellow or yellowish-brown to green (Davis et al., 1997). Since there were only a very few interactions between P fertilisation and soil treatments, only the main effects of the treatment are presented in this chapter.

The effect of soil treatments on seedling growth parameters was more striking in P-fertilised soils than in P-unfertilised soils. In P-unfertilised soils, soil treatments did not result in any significant difference in seedling height, dry matter (DM) and P uptake in both shoots and roots (Table 6.1). In P-fertilised soils however, compared to the autoclaved treatment, the non-autoclaved treatments (i.e. the treatments with *S. luteus* and *R. rubescens* inoculated, natural, and fungicide soil) significantly ($P < 0.05$) increased seedling height, shoot dry matter and P concentration and P uptake in both shoots and roots of the seedlings. In both P-unfertilised and fertilised soils significantly lower root:shoot ratio and higher ECM tip density were found in the non-autoclaved treatments than in the autoclaved treatments (Table 6.1). The highest ECM tip density was observed in the P-fertilised, *R. rubescens* inoculated treatment. The N concentration in seedling shoots and roots did not show any statistically significant difference amongst soil treatments.

Autoclaved soil – P

Autoclaved soil + P

Fungicided soil - P

Fungicided soil + P

Natural soil - P

Natural soil + P

S. luteus - P

S. luteus + P

R. rubescens - P

R. rubescens + P

Plate 6.3 *P. radiata* root systems at various mycorrhizal inoculations and BGPR fertiliser treatments

Table 6.1 Effects of P fertilisation and ECM inoculation on growth and P uptake of *P. radiata* (numbers associated with same letters are not significantly different at $P < 0.05$; upper case letters – difference between BGPR-P fertilised and unfertilised soils; lower case letters – difference between ECM inoculations)

Seedling parameters	Treatment	Autoclaved soil	Fungicided soil	Natural soil	*S. luteus* Inoculated soil	*R. rubescens* inoculated soil	Diff. at P <0.05
Height (cm)	P unfertilised	12.8 ± 0.3 aB	13.2 ± 0.2 aB	14 5 ± 0 7 aB	13.5 ± 0.6 aB	13.7 ± 0.4 aB	$P < 0.0001$
	P fertilised	18.9 ± 2 0 bA	26.1 ± 0.8 aA	26.8 ± 1.1 aA	25.8 ± 0.4 aA	26.6 ± 1.0 aA	$LSD_{(soil)} = 1.1$
			$P < 0.0001$; $LSD_{(inoc)} = 1.8$				
Shoots DM (g)	P unfertilised	1 9 ± 0 02 aB	1.9 ± 0.02 aB	2.3± 0 04 aB	2.1 ± 0.05 aB	2 2 ± 0.04 aA	$P < 0.0001$
	P fertilised	4.9 ± 0.18 cA	8.2 ± 0.07 bA	9.5 ± 0.08 aA	8.9 ± 0.11 bA	9.5 ± 0.04 aB	$LSD_{(soil)} = 0.5$
			$P < 0$ 0001; $LSD_{(inoc)} = 0$ 7				
Roots DM (g)	P unfertilised	1.3 ± 0.03 B	1 0 ± 0.02 B	1.0± 0 01 B	1.1 ± 0.02 B	1 2 ± 0.04 B	$P < 0.0001$
	P fertilised	2 8 ± 0.11 A	3.6 ± 0 01 A	3.6 ± 0.04 A	3 7 ± 0.04 A	3 8 ± 0.05 A	$LSD_{(soil)} = 0.3$
			$NS_{(inoc)}$				
Root/shoot	P unfertilised	0 7 ± 0.01 aA	0 5 ± 0 00 cA	0.5± 0.01 cA	0.5 ± 0.01 cA	0.6 ± 0.02 bA	$P < 0.001$
	P fertilised	0.6 ± 0 01 aB	0 4 ± 0.00 bB	0.4 ± 0 01 bB	0 4 ± 0 01 bB	0.4 ± 0.01 bB	$LSD_{(soil)} = 0.06$
			$P < 0.001$, $LSD_{(inoc.)} = 0.09$				
ECM Tip density (tip m^{-1} root)	P unfertilised	15 ± 1.7 bB	57 ± 6 bB	141 ± 20 aB	109 ± 20 aB	126 ± 20 aB	$P < 0.0001$
	P fertilised	99 ± 29 dA	256 ± 20 cA	350 ± 36 bA	349 ± 35 bA	422 ± 16 aA	$LSD_{(soil)} = 29$
			$P < 0.0001$, $LSD_{(inoc)} = 46$				
P conc in shoots (mg g^{-1} DM)	P unfertilised	0.24 ± 0 01 bB	0 34 ± 0 06 aB	0.36 ± 0.04 aB	0.29 ± 0.03 bB	0.29 ± 0.04 bB	$P < 0.0001$
	P fertilised	0.45 ± 0.09 bA	0.70 ± 0 03 aA	0.70 ± 0.07 aA	0.78 ± 0.04 aA	0.71 ± 0.04 aA	$LSD_{(soil)} = 0.06$
			$P < 0.001$; $LSD_{(inoc)} = 0$ 1				
P conc in roots (mg g^{-1} DM)	P unfertilised	0.36 ± 0.03 bB	0.50 ± 0.03 aB	0.51 ± 0.06 aB	0.41 ± 0 03 aB	0.43 ± 0.05 aB	$P < 0.0001$
	P fertilised	0.61 ± 0.05 bA	0.91 ± 0.04 aA	0.86 ± 0.04 aA	0 90 ± 0 10 aA	0 80 ± 0.05 aA	$LSD_{(soil)} = 0.07$
			$P < 0.001$, $LSD_{(inoc)} = 0$ 1				
N conc in shoots (mg g^{-1} DM)	P unfertilised	15.4 ± 0.9 A	17.3 ± 1.0 A	19.7± 1.3 A	18.4 ± 0 8 A	16.1 ± 0.9 A	$P < 0.0001$
	P fertilised	12.2 ± 1.9 B	11.0 ± 0.4 B	11.1 ± 0.7 B	12 0 ± 0 4 B	11.0 ± 0.6 B	$LSD_{(soil)} = 1.2$
			$NS_{(inoc)}$				
N conc in roots (mg g^{-1} DM)	P unfertilised	15.2 ± 0.5 A	16.3 ± 0.8 A	15.1 ± 0.9 A	15 0 ± 0.2 A	14.9 ± 1.1 A	$P < 0.0001$
	P fertilised	11.9 ± 1.0 B	11.3 ± 0.8 B	10.8 ± 0.4 B	11.6 ± 0.6 B	10.4 ± 0.7 B	$LSD_{(soil)} = 0.95$
			$NS_{(inoc)}$				
P uptake in shoots (mg pot^{-1})	P unfertilised	0.5 ± 0.04 aB	0.6 ± 0.10 aB	0.8 ± 0 14 aB	0.6 ± 0.13 aB	0.6 ± 0.06 aB	$P < 0.0001$
	P fertilised	2.4 ± 0 89 cA	5.8 ± 0.43 bA	6.5 ± 0.82 aA	6.9 ± 0.37 aA	6.8 ± 0.38 aA	$LSD_{(soil)} = 0.55$
			$P < 0.0001$, $LSD_{(inoc)} = 0.88$				
P uptake in roots (mg pot^{-1})	P unfertilised	0.5 ± 0.04 aB	0.5 ± 0.07 aB	0.5 ± 0.07 aB	0.5 ± 0.06 aB	0.5 ± 0.02 aB	$P < 0.01$
	P fertilised	1.8 ± 0.40 cA	3.3 ± 0.19 aA	3.0 ± 0.33 bA	3.4 ± 0.52 aA	3.0 ± 0 13 bA	$LSD_{(soil)} = 0.5$
			$P < 0.0001$; $LSD_{(inoc)} = 0.31$				

Amongst the non-autoclaved treatments in the P-fertilised soils, the seedling height and P concentration in shoots and roots did not show any difference, but significantly higher shoot DM was found in the natural and the *R. rubescens* inoculated treatments. This higher shoot DM in the above treatments was associated with significantly higher ECM tip density, indicating that *R. rubescens* may be more effective than the other species on the growth of young *P. radiata* seedlings as reported by others (e.g. Chu-Chou and Grace, 1985). In P-fertilised soils the order of ECM tip density was: *R. rubescens* inoculated > *S. luteus* inoculated = natural > fungicide > autoclaved treatments (Table 6.1). Phosphorus concentration and P uptake did not show any significant difference between natural soil and *S. luteus* and *R. rubescens* inoculated soil treatments (Table 6.1). The lack of these differences might have been partly due to the difficulty in maintaining a pure introduced ECM species in soil growth medium. This difficulty in introducing and maintaining specific vegetative mycelium in soils without prior sterilisation has also been reported by others (such as Lamb and Richards, 1974). The fungicide treatment resulted in lower ECM tip density compared to the natural soil and the two ECM inoculated treatments, but its effect on seedling growth was not significant.

The P concentrations in shoots and roots of the seedlings were significantly correlated with ECM tip density in P-fertilised soil (Figure 6.1), but no such relationship was observed in unfertilised soils, probably due to the relatively lower ECM infection in P-unfertilised soils.

6.3.1.2 Effects of P fertilisation

Application of BGPR to the soils significantly ($P < 0.05$) increased seedling height (1.2 – 1.9-fold) and ECM tip density (2 – 7 fold) (Plate 6.2-Bottom; Table 6.1). The dry matter (DM), P concentration and P uptake in both seedling shoots and roots were also significantly higher in P-fertilised soils (Table 6.1) than in P-unfertilised soils as observed in another glasshouse study on the same soil (Chapter 3). The growth and P uptake response of the seedlings to BGPR-P application in this study is consistent with the low plant-available P concentration in the native soil causing P-limited, seedling growth. The Bray-2 P concentration in the P-unfertilised soil was 1.8 μg g^{-1} soil, which is lower than the critical Bray-2 P concentration of 12 μg g^{-1} soil suggested for mature

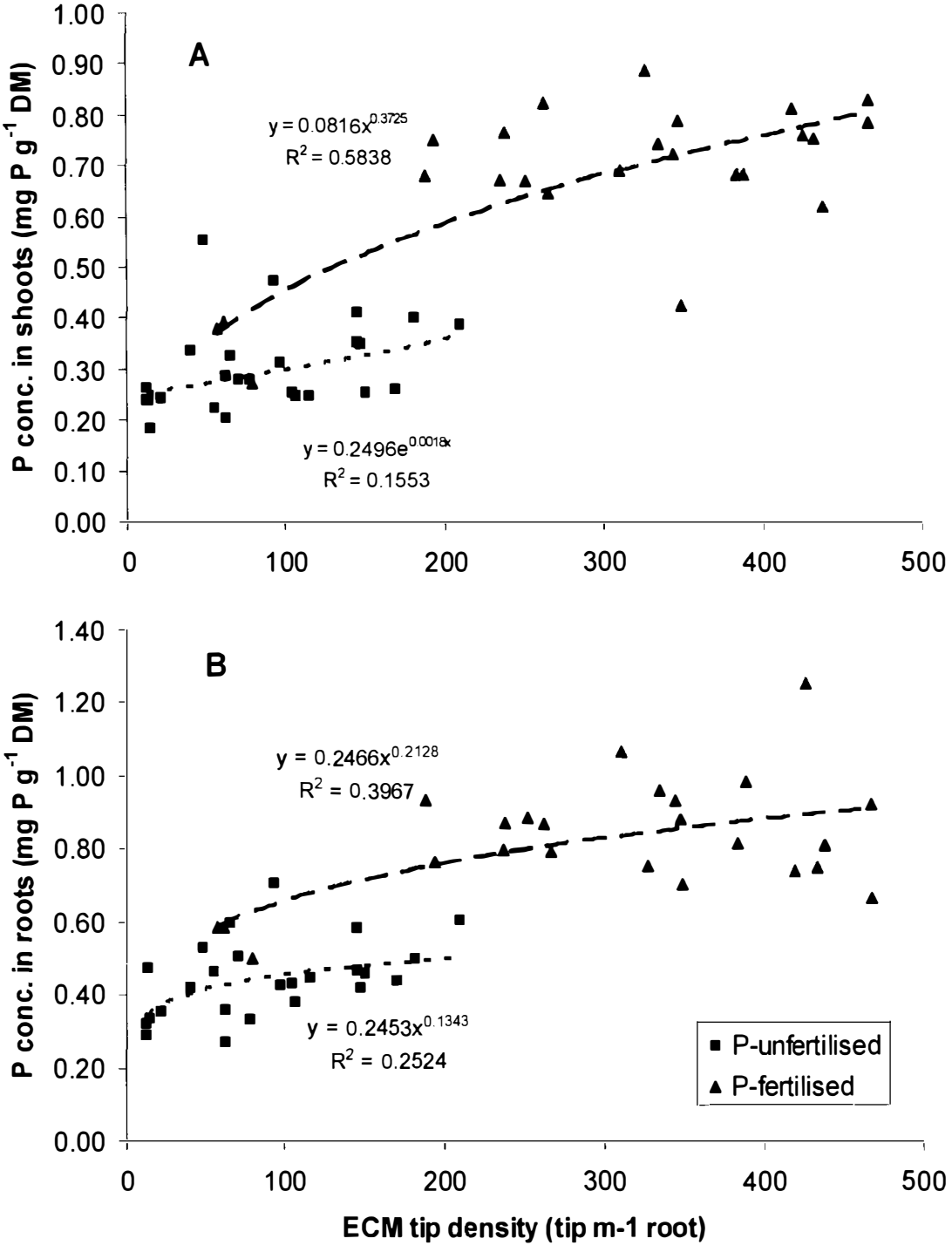

Figure 6.1 Relationship between ECM root tip density and P concentration in shoots (A) and roots (B) of *P. radiata* seedlings.

P. radiata trees (Ballard, 1973). At harvest, the seedlings in unfertilised soils appeared unhealthy compared to those in P-fertilised treatment (Plate 6.2-Bottom).

The BGPR-P application resulted in significant ($P < 0.05$) increase in ECM tip density as well (Table 6.1). The ECM tip densities in P-fertilised soils were 2 to 7-folds higher than these in P-unfertilised soils. This indicates that in extremely P deficient soils, such as the one used in this study, an addition of BGPR at the P rate of 100 μg g^{-1} soil stimulated the infection of ECM fungi in newly germinated *P. radiata* seedlings. Many studies conducted under nursery and laboratory conditions have shown that ECM fungi infection is higher under P-deficient condition and P fertiliser application decreases the degree of infection (Bougher et al., 1990; Jones et al., 1990; Newton and Browning, 1991 and Wallander et al., 1997). However, in these studies the soil P status were generally higher than that in unfertilised soil used in this study (Bray- 2 P < 4 μg P $soil^{-1}$). The extreme P deficiency in the P-unfertilised soil in this study might have caused competition for P between seedlings and ECM fungi, thereby hindering the ECM infection on the roots. The results are consistent with the findings of some others. For example, Lamb and Richards (1974) reported that in two soils with low P status (total P concentrations were 7.9 and 38.3 μg g^{-1} soil) the addition of single superphosphate at the rate of 40 kg P ha^{-1} greatly increased ECM formation in *P. radiata*. Ballard (1972) also reported that on severely P deficient soils of the first rotation of *P. radiata*, application of superphosphate appeared to stimulate mycorrhizal infection of roots. In another study reported in Chapter 3 on the same soil as used in this Chapter, ECM root tip density in 1-year-old seedling increased with the application of Sechura PR at the rate of 50 μg P g^{-1} soil. The tip density however decreased at higher rate of P application (>100 μg P g^{-1} soil) as reported by others (Newton and Pigott, 1991; Browning and Whitney, 1992). The reason for tip density not decreasing at 100 μg P g^{-1} soil in this study as in the study in Chapter 3 might be due to the difference in the way the two experiments were conducted (e.g. seedling age, number of seedling per pot and PR type and % dissolution etc.) and the difference in the dissolution rates of used RPR.

The N concentration in root tissues may also be important in determining the success of ECM infection in the newly geminated seedlings. Gagnon et al. (1988) have shown a large decline in formation of ECM by *Laccaria bicolor* on black spruce (*Picea mariana*) when N application rates were increased from 8.5 to 15 mg per seedling per pot at a

constant P level of 1.5 mg per seedling. In this study, the N concentrations in the tissues of P-unfertilised seedlings were significantly ($P < 0.05$) higher than that in P-fertilised seedlings (Table 6.1). This is probably due to a dilution effect caused by the higher growth of seedlings in P-fertilised soils. The relatively higher (1.2 - 1.5 folds) concentration of N in the P-unfertilised seedlings might have reduced the ratio of carbohydrate to N in the root system of the seedlings, thereby decreasing roots susceptibility to ectomycorrhizal development (Marx et al., 1977).

Seedlings in P-unfertilised and in autoclaved treatments had significantly ($P < 0.05$) larger root:shoot ratio, compared with ECM infected seedlings in P-fertilised soils (Table 6.1). This indicates that the benefits of P fertilisation and ECM infection resulting in extra shoot growth outweighed the additional carbon demands of ECM fungi on the seedlings. The results obtained are consistent with increased P supply decreasing exploratory root growth and the observations made by Ekblad et al. (1995) in *P. sylvestris* and alder (*Alnus incana*) where N and P fertiliser addition had a strong negative effect on the root:shoot ratio in the plants.

6.3.2 Effects of P fertilisation and ECM inoculation on soil properties

6.3.2.1 Effects on hyphal length density

Compared to P-unfertilised soils (mean hyphal length density of 26 m g^{-1} soil), application of BGPR significantly ($P < 0.05$) increased hyphal length density in soils (mean 42 m g^{-1} soil). The larger hyphal length density (y) in P-fertilised soils was associated with higher ECM tip density (x) (Table 6.1) ($y = 8.452Ln(x) - 0.9555$, $r = 0.44$). In both P-unfertilised and P-fertilised soils, there was no significant difference in hyphal length density among soil inoculation treatments, mainly due to large variations amongst replicates. In P-fertilised soils significantly ($P < 0.05$) higher hyphal length density was observed in rhizosphere soil than in the bulk soils, mainly in the fungicide and the two ECM inoculated treatments (Table 6.2).

Table 6.2 Effects of P fertilisation and soil treatments on selected soil properties. Numbers associated with same letters are not significantly different at $P < 0.05$; upper case letters after numbers in columns – difference between bulk and rhizosphere soils; lower case letters after numbers in rows – differences between soil treatments

A. P unfertilised treatments							
Soil properties	**Treatment**	**Autoclaved**	**Fungicide treated**	**Natural untreated**	***S.luteus* inoculated**	***R. rubescens* inoculated**	**Diff. at *P* <0.05**
Soil pH (1:2.5 H_2O)	Bulk soil	5.27±0.01aA	4.85±0.03 cB	4.99±0.02 bB	4.93±0.05 bB	4.95±0.03 bB	$P < 0.0001$ $LSD_{(soil)}$ =0.07
	Rhizosphere	5.31±0.08 aA	5.07±0.04 bA	5.09±0.05 bA	5.17±0.01 bA	5.12±0.07 bA	
			$P < 0.0001$; $LSD_{(inoc.)} = 0.11$				
Acid phosphatase ($\mu g\ g^{-1}\ h^{-1}$)	Bulk soil	498±39 d	701±46 c	887±38 a	788±33 b	862±19 ab	NS $_{(soil)}$
	Rhizosphere	561±51 c	766±33 b	855±66 a	850±43 a	931±15 a	
			$P < 0.0001$; $LSD_{(inoc.)}$ = 82				
Alkaline phosphatase ($\mu g\ g^{-1}\ h^{-1}$)	Bulk soil	115±26 b	253±20 a	284±38 a	292±44 a	241±20 a	NS $_{(soil)}$
	Rhizosphere	147±15 b	226±27 a	273±46 a	277±42 a	278±34 a	
			$P < 0.001$; $LSD_{(inoc.)}$ = 68				
Oxalate conc. ($mg\ L^{-1}$)	Bulk soil	0.40±0.00 c	0.85±0.25 b	0.30±0.00 c	0.51±0.00 c	1.35±0.35 a	NS $_{(soil)}$
	Rhizosphere	0.75±0.05 b	0.45±0.05 c	0.35±0.05 c	0.45±0.05 c	1.65±0.25a	
			$P < 0.001$; $LSD_{(inoc.)}$ =0.36				
B. P fertilised treatments							
Soil properties	**Treatment**	**Autoclaved**	**Fungicide treated**	**Natural untreated**	***S.luteus* inoculated**	***R. rubescens* inoculated**	**Diff. at *P* <0.05**
Soil pH (1:2.5 H_2O)	Bulk soil	5.57±0.02 aA	5.56±0.03 aA	5.50±0.02 bA	5.52±0.02 bA	5.54±0.01 aA	$P < 0.0001$ $LSD_{(soil)}$ =0.03
	Rhizosphere	5.45±0.00 aB	5.36+0.03 bB	5.33±0.02 bB	5.41±0.02 aB	5.36±0.02 bB	
			$P < 0.01$; $LSD_{(inoc.)}$ = 0.05				
Acid phosphatase ($\mu g\ g^{-1}\ h^{-1}$)	Bulk soil	245±68cA	551±57bA	698±80aA	618±40baB	587±61bB	$P < 0.05$
	Rhizosphere	321±97cA	629±65bA	763±52aA	748±78aA	724±51aA	$LSD_{(soil)}$ =83
			$P < 0.0001$; $LSD_{(inoc.)}$ = 132				
Alkaline phosphatase ($\mu g\ g^{-1}\ h^{-1}$)	Bulk soil	95±12bA	166±15abB	189±29aA	185±20aA	135±18bB	$P < 0.05$
	Rhizosphere	100±12bA	234±12aA	206±33aA	207±25aA	216±33aA	$LSD_{(soil)}$ =30
			$P < 0.001$; $LSD_{(inoc.)}$ = 48				
Hyphae density (m g^{-1} soil)	Bulk soil	25±8 A	25±8 B	36±8 A	37±20 B	21±2 B	$P < 0.05$
	Rhizosphere	34±3 A	70±14 A	36±14 A	64±14 A	42±14 A	$LSD_{(soil)}$ =16
			NS $_{(inoc.)}$				
Oxalate conc. ($mg\ L^{-1}$)	Bulk soil	0.40±0.10 A	0.25±0.05 B	0.45±0.15 A	0.15±0.05 B	0.35±0.25 B	$P < 0.05$
	Rhizosphere	0.40±0.00 A	0.80±0.50 A	0.25±0.05 A	1.00±0.20 A	1.10±0.10 A	$LSD_{(soil)}$ =0.28
			NS $_{(inoc.)}$				

6.3.2.2 Effects on soil pH

Application of BGPR significantly ($P < 0.05$) increased soil pH for overall data (mean 5.1 for P-unfertilised soils and 5.5 for P-fertilised soils, Table 6.2). Higher soil pH in P-fertilised soils might have been due to the liming action of the BGPR (Loganathan et al., 1995).

In P-unfertilised soils, soil pH in non-autoclaved soil treatments were significantly ($P < 0.05$) lower than in autoclaved soils for both the rhizosphere and bulk soils (Table 6.2), but in P-fertilised treatments significant differences were observed mostly in rhizosphere soils with an exception in the rhizosphere soil of *S. luteus* inoculated treatment. The decreased soil pH in non-autoclaved treatments was probably due to excretion of protons by the mycorrhizal roots of *P. radiata* as observed in the studies reported in Chapters 3 and 4.

Soil pH in P-fertilised rhizosphere soils were significantly ($P < 0.05$) lower than that in the corresponding bulk soils for all ECM infected treatments (Table 6.2). In P-unfertilised soils which had significantly lower ECM infection, however, soil pH was significantly ($P < 0.05$) higher in rhizosphere soils than in bulk soils for all but the autoclaved treatments (Table 6.2). This contrasting observation in pH between P-fertilised and P-unfertilised soils may be associated with the N uptake form by *P. radiata* seedlings under varying degrees of ECM infection. Olykan and Adams (1995) reported that ECM infected *P. radiata* seedlings take up N predominantly in the cationic NH_4^+ form than in the anionic NO_3^- form from soil. Other studies with mycorrhizal pines in solution culture (Taber and McFee, 1972) and in soil culture (McFee and Stone, 1969) also strongly indicate that NH_4^+-N is the preferred form of nitrogen. Skinner (1978) found that non-mycorrhizal infected *P. radiata* seedlings grown in solution culture prefer to take up NO_3^--N than NH_4^+-N. Similar results were reported by Krajina et al. (1973) and Driesshe and Dangerfield (1975) in non-mycorrhizal Douglas-fir seedlings grown in solution culture. This difference in N uptake form between mycorrhizal and non-mycorrhizal seedlings suggests that the degree of mycorrhizal fungi infection on pine roots has a strong influence on changes in rhizosphere pHs (Figure 6.2).

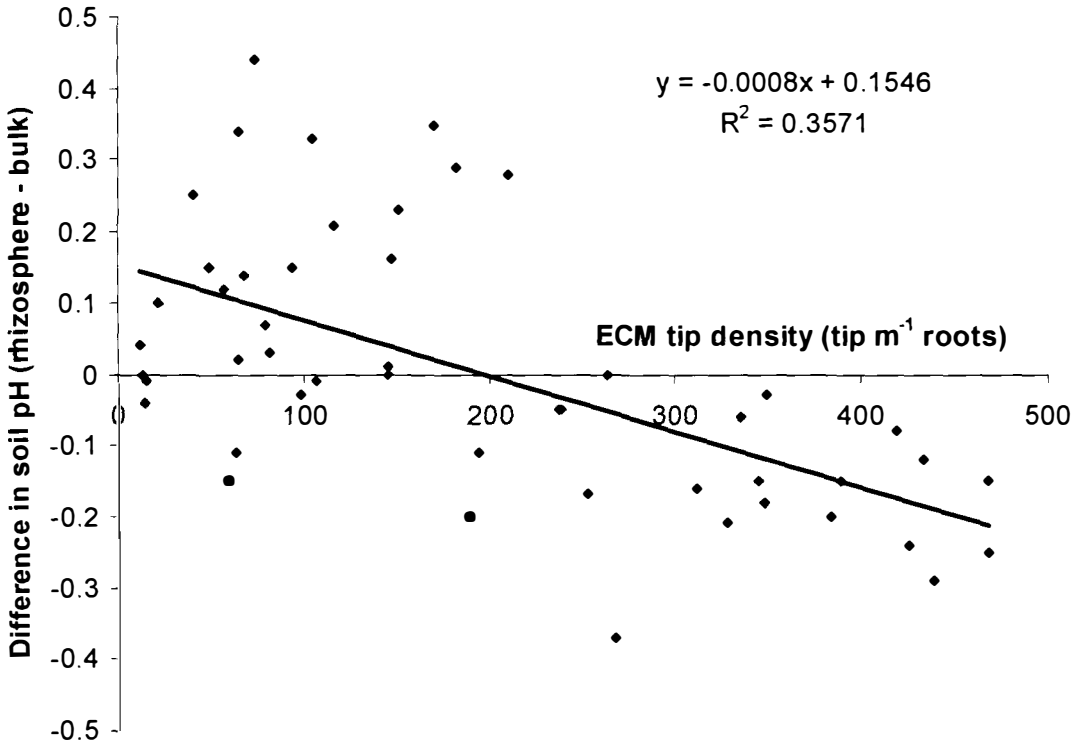

Figure 6.2 Relationships between ECM root tip density and the difference in soil pH between the rhizosphere and bulk soils

6.3.2.3. Effects on acid and alkaline phosphatase activities

In P-unfertilised soils root processes did not result in any significant difference in acid and alkaline phosphatase activities between the bulk and rhizosphere soils (Table 6.2). This is probably due to low ECM infection of the roots. However, in the P-fertilised soils significantly higher rhizosphere acid phosphatase activities, compared to the bulk soils, were observed in the two ECM inoculated soil treatments (Table 6.2). In the *R. rubescens* and the fungicide treatments, significantly higher alkaline phosphatase activities were also observed in the rhizosphere than in the bulk soils.

Increased phosphatase activities in the rhizosphere soil were associated with higher hyphal length density in soils, particularly for *S. luteus* and *R. rubescens* inoculated soil treatments in P-fertilised soils (Table 6.2). This indicates that ECM fungi may play an important role in increasing phosphatase production. This suggestion is also supported by significantly higher acid and alkaline phosphatase activities in non-autoclaved soils

than in autoclaved soils at both P-unfertilised and P-fertilised soils (Table 6.2). However, it should be noted that the increases in ECM tip density and hyphal length density in P-fertilised treatments did not result in higher phosphatase activities compared to P-unfertilised treatments. Conversely, BGPR application resulted in significant ($P < 0.05$) decreases in both acid and alkaline phosphatase activities in P-fertilised soils for overall data (Table 6.2). The decreases in phosphatase activities with BGPR application are in consistent with the suggestion that synthesis of phosphatase enzymes can be induced when plants and mycorrhizal fungi are deficient in P (Hedley et al., 1982b; Häussling and Marschner, 1989). Similar results were observed in the experiment reported in Chapter 3.

6.3.2.4. Effects on oxalate exudation

As observed in Chapter 3, the concentrations of oxalate in soil solution were highly variable in the different treatments (Table 6.2 and Figure 6.3). The reasons for this high variability were discussed in Chapter 3. Amongst the ECM inoculation treatments, *R. rubescens* inoculated treatment produced higher oxalate concentration, particularly in P-unfertilised treatments (Figure 6.3). As discussed in previous studies reported in Chapters 3 and 4, in P deficient soils oxalate production could have helped increase plant P uptake by releasing P from P-fixing soil minerals, though the shoot and root P concentrations were lower in P-unfertilised treatment.

Compared to the bulk soils, significantly higher oxalate concentrations were measured in rhizosphere soil solutions in P-fertilised soils with fungicide, *S. luteus* and *R. rubescens* treatments (Table 6.2), but there was no difference in oxalate concentration between rhizosphere and bulk soil solutions in P-unfertilised soils (Table 6.2), probably due to the relatively large variation within treatments.

6.3.2.5 Effects on BGPR dissolution and soil P fractions

Application of BGPR significantly ($P < 0.05$) increased soluble P concentration in soil solution (1.3 fold) and all P fractions (except for residual-P) in the soils (Table 6.3). As

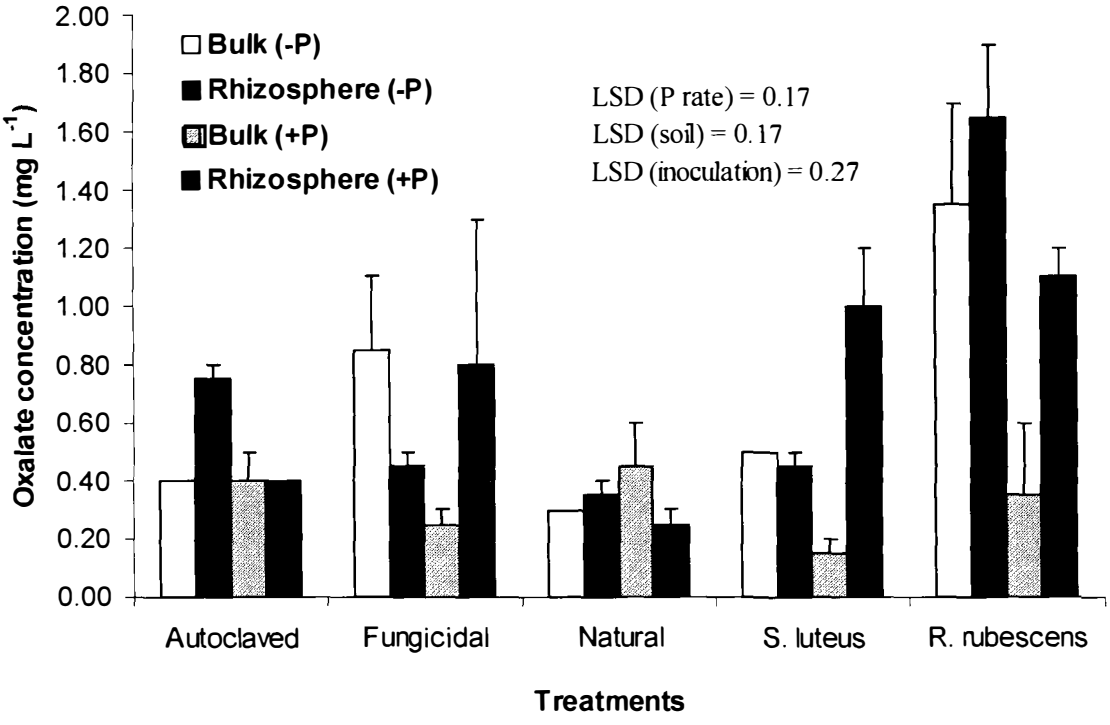

Figure 6.3 Concentration of oxalate in soil solution

expected, the increased P concentrations in all the above fractions except the residual-P fraction were mainly caused by the high BGPR dissolution (dissolution of 57 – 69% determined using TEA/NaCl (pH 7) as the initial step) (Tambunan et al., 1993) (Table 6.3). The % recovery of BGPR added ranged from 85 to 104%. As discussed in Chapter 3, the very high % dissolution of BGPR was encouraged by the mildly acidic soil pH, the long period of experimental time (10 months) and fine particle size of BGPR used (150 to 250 μm) (Kanabo and Gilkes 1988). The high P retention (92%) and low Ca concentration in the Allophanic Soil will have promoted the dissolution of BGPR in soil (Kanabo and Gilkes 1987; Bolan et al. 1997). Similar to the previous pot trial (see Chapter 3), little dissolved BGPR-P remained in the easily plant-available resin-P pool (mean 1.3 $\mu g\ g^{-1}$ soil in P-fertilised soils), while most of the dissolved BGPR-P was redistributed to 0.1 *M* NaOH-P_i fraction (around 44%). This shows that the dissolved BGPR-P was sorbed mainly onto allophane and hydrous oxide surfaces of Al and Fe.

ECM inoculation did not cause any change in the concentration of soil P fractions except for significant ($P < 0.05$) decreases in H_2SO_4-P concentration in non-autoclaved treatments compared to the autoclaved treatment in P-fertilised soils (Table 6.3). The

Table 6.3 Effects of P fertilisation and soil treatments on soil phosphorus fractions. Numbers associated with same letters are not significantly different at $P < 0.05$; upper case letters after numbers in columns – difference between bulk and rhizosphere soils; lower case letters after numbers in rows – differences between soil treatments

A. P unfertilised treatments							
Soil properties	**Treatment**	**Autoclaved**	**Fungicide treated**	**Natural untreated**	***S.luteus* inoculated**	***R. rubescens* inoculated**	**Diff. at P <0.05**
Solution P (mg L^{-1})	Bulk soil	0.01	0.02	0.02	0.01	0.01	$NS_{(soil)}$
	Rhizosphere	0.02	0.01	0.01	0.01	0.01	
				$NS_{(inoc.)}$			
Resin P (μg g^{-1})	Bulk soil	0.44±0.1	0.32±0.1	0.76±0.1	0.59±0.2	0.60±0.1	$NS_{(soil)}$
	Rhizosphere	0.44±0.1	0.58±0.1	0.62±0.1	0.55±0.1	0.68±0.3	
				$NS_{(inoc.)}$			
0.1 *M* NaOH P_i (μg g^{-1})	Bulk soil	35.1±2.0	33.1±1.3	35.3±0.9	36.8±1.9	33.5±2.0	$NS_{(soil)}$
	Rhizosphere	37.7±2.7	33.9±0.9	33.3±1.3	34.2±2.5	36.5±1.8	
				$NS_{(inoc.)}$			
***0.1 M* NaOH P_o (μg g^{-1})**	Bulk soil	182±3	175±4	169±7	180±2	174±14	$NS_{(soil)}$
	Rhizosphere	185±4	180±6	167±14	179±9	165±18	
				$NS_{(inoc.)}$			
0.5 *M* H_2SO_4 P (μg g^{-1})	Bulk soil	20.8±0.8	19.8±0.5	19.2±0.9	19.6±0.6	20.3±1.7	$NS_{(soil)}$
	Rhizosphere	19.0±0.7	19.0±0.6	18.8±0.5	20.5±1.3	19.9±1.0	
				$NS_{(inoc.)}$			
Residual P (μg g^{-1})	Bulk soil	39.9±3.0	38.9±3.2	40.0±2.3	38.4±1.8	39.5±2.5	$NS_{(soil)}$
	Rhizosphere	38.8±2.5	39.7±1.8	39.6±3.0	39.3±1.7	39.8±2.8	
				$NS_{(inoc.)}$			
B. P fertilised treatments							
Soil properties	**Treatment**	**Autoclaved**	**Fungicide treated**	**Natural untreated**	***S.luteus* inoculated**	***R. rubescens* inoculated**	**Diff. at P <0.05**
Solution P (mg L^{-1})	Bulk soil	0.02 A	0.01 B	0.02 A	0.02 B	0.02 B	$P < 0.01$
	Rhizosphere	0.02 A	0.02 A	0.02 A	0.04 A	0.03 A	$LSD_{(soil)}$ =0.005
				$NS_{(inoc.)}$			
Resin P (μg g^{-1})	Bulk soil	0.60±0.1 A	1.20±0.3 B	1.03±0.3 B	0.79±0.1 B	1.15±0.1 A	$P < 0.05$
	Rhizosphere	1.09±0.4 A	2.08±0.7 A	1.59±0.3 A	1.69±0.4 A	1.24±0.3 A	$LSD_{(soil)}$ =0.5
				$NS_{(inoc.)}$			
0.1 *M* NaOH P_i (μg g^{-1})	Bulk soil	64.3±4.3	66.9±3.0	69.7±2.0	67.1±2.7	61.0±7.5	$NS_{(soil)}$
	Rhizosphere	66.2±2.7	70.7±1.5	71.2±1.7	67.9±1.5	69.3±2.2	
				$NS_{(inoc.)}$			
***0.1 M* NaOH P_o (μg g^{-1})**	Bulk soil	198±3	185±9	191±6	188±7	185±5	$NS_{(soil)}$
	Rhizosphere	183±10	181±7	192±3	199±6	195±3	
				$NS_{(inoc.)}$			
0.5 *M* H_2SO_4 P (μg g^{-1})	Bulk soil	49.6±3.6 a	44.3±0.9 b	41.8±2.6 b	43.0±2.2 b	45.1±2.5 b	$NS_{(soil)}$
	Rhizosphere	53.3±3.6 a	42.1±2.6 bc	41.0±1.4 c	46.1±1.8 b	46.1±1.4 b	
			$P < 0.01$; $LSD_{(inoc.)} = 4.9$				
Residual P (μg g^{-1})	Bulk soil	42.3±4.4	36.9±2.0	37.6±2.3	39.4±2.9	37.8±2.8	$NS_{(soil)}$
	Rhizosphere	39.4±4.8	36.1±3.1	38.3±2.3	39.0±3.1	39.1±2.5	
				$NS_{(inoc.)}$			
PR dissolution (%)	Bulk soil	61±3	66±2	67±1	65±2	68±2	$NS_{(soil)}$
	Rhizosphere	57±3	69±2	68±1	67±1	67±2	
				$NS_{(inoc.)}$			

significantly lower H_2SO_4-P concentrations in non-autoclaved treatments were partly associated with lower soil pHs.

In the P-unfertilised treatments, no statistically significant differences in P fractions between the bulk and rhizosphere soils were found. This may be due to the limited root growth. In P-fertilised treatments, higher concentrations of solution and resin-P were found in the rhizosphere soils of 3 of the 4 non-autoclaved treatments than in the corresponding bulk soils (Table 6.3). The increased plant-available P concentrations in the rhizosphere soils were generally associated with the higher hyphal length density, acid and alkaline phosphatase activities and oxalate concentration in the rhizosphere soils than in the bulk soils (Table 6.2). The results obtained in this study are consistent with the observations made by others (e.g. Häussling and Marschner, 1989) and a field study reported in Chapter 4. As discussed in Chapter 4, these root-induced changes in the rhizosphere soils may have produced favourable conditions for P_o conversion to P_i (Gobran and Clegg, 1996; Chen et al., 2002; Sanchez and Bursey, 2002) and also remediated P fixation by allophane and hydrous oxides of Fe and Al compounds in soil (Fox and Comerford, 1992a). Nevertheless, it should be noted that the techniques employed for sampling rhizosphere soil (collecting rhizosphere soil by shaking the roots) may have had the possibility of overestimating the concentration of plant-available P, because the P in broken ECM tips and the extrametrical mycelium is likely to be released into rhizosphere soil/soil solution. The air-drying effect of the soil samples may have also influenced the resin-P concentration. This is discussed in Chapter 4.

There were no significant differences in NaOH-P_i, NaOH-P_o and residual-P fractions as well as the % dissolution of BGPR between the rhizosphere and bulk soils (Table 6.3). The lack of these differences is probably due to poor demarcation of bulk soils from rhizosphere soils. Unlike in the trial reported in Chapter 3, where one seedling per pot containing 1 kg soil was grown, in this trial 5 seedlings per pot containing 0.5 kg soil was used. This resulted in higher root density in the soil in this trial, particularly in P-fertilised treatments (23 - 58 m roots kg^{-1} soil in this trial compared to 28 - 45 m roots kg^{-1} soil in previous trial) which made it difficult to separate the bulk soils from the rhizosphere soils.

6.4 Conclusions

The results obtained from this pot study further supported our previous hypothesis that RPRs are an effective P fertiliser for *P. radiata* growing in marginally acidic Allophanic Soil. In this study, application of Ben Guire PR (BGPR; 150 – 250 μm particle size) at a rate of 100 μg P g^{-1} soil to newly geminated *P. radiata* seedlings grown in a P deficient Allophanic Soil significantly increased *P. radiata* seedling height (1.5 – 2.0-times), ECM root tip density (2 – 7-times) and P concentration in shoots (1.9 – 2.7-times) 10-months after application, compared to P unfertilised soil. The fate of the BGPR added to the soil was similar to that of Sechura PR discussed in Chapter 3. More than 55% BGPR added to the soil had been dissolved in 10 months after application.

The hypothesis on the influence of the type of fungal species on radiata seedling growth was not different in this study. Compared to the autoclaved treatment, however, the ECM infected non-autoclaved treatments significantly increased seedling growth (1.4-times in height; 1.7 – 1.9-times in shoot DM) and P-use efficiency (1.6 -1.7-times in shoot P concentration; 2.4 – 2.9-times in shoot P uptake) in P-fertilised soils. Positive relationship between ECM tip density and P concentration in seedlings was also observed in P-fertilised soils. The results demonstrated that ECM association with *P. radiata* plays an important role in seedling growth and P uptake by trees. In P-unfertilised soils, ECM inoculation had little effect on seedling growth, indicating that the P deficiency in the Allophanic Soil has limited ECM development and its influence on seedling growth.

Root-induced changes in the rhizosphere biochemical properties were mainly observed in P-fertilised soils. In these soils, both *S. luteus* and *R. rubescens* inoculated treatments, as well as the fungicidal treatment, produced higher concentrations of resin-P in the rhizosphere than in the bulk soils. In these treatments, higher phosphatase activities and oxalate concentration, and lower pH were mostly observed in the rhizosphere soils than in the bulk soils. These changes were associated with higher soil ECM hyphae density in these treatments. This further supports the hypothesis tested in Chapter-5 that *P. radiata* rhizosphere processes are associated with ECM hyphae.

Compared with *S. luteus* species, *R. rubescens* inoculation produced greater ECM tip density in seedling roots in P-fertilised soils and higher oxalate concentration in rhizosphere solution, particularly in P-unfertilised soils. The results indicate that *R. rubescens* are more susceptible to infection with radiata seedling and may be a more effective fungal species in improving P uptake by young *P. radiata* seedlings in P deficient Allophanic Soils.

CHAPTER 7

Attempts to develop a $^{14}CO_2$ pulse labelling technique to quantify the active external mycorrhizal hyphae in soil

7.1 Introduction

The external mycelia of ectomycorrhizal (ECM) fungi comprise a dominant component of soil microbial biomass in most coniferous and deciduous forest ecosystems (Finlay and Soderstrom, 1992). Rousseau et al. (1994) reported 500 m of external hyphae per meter root of pine seedlings. At this density the external mycelium would contribute 75% of the total nutrient absorbing surface. This large absorptive area is of importance in nutrient (such as N, P and C) cycles and uptake by trees (Smith and Read, 1997; Leake et al., 2001). Also, this dense ECM mycelium influences rhizosphere environments and P bio-availability as reported at Chapter 5.

Despite an increasing awareness of the importance of ECM hyphae in forest ecosystem, there still remain some gaps in our understanding of the quantity of active ECM hyphae and the distribution of C allocated to the hyphal network by the host plants. **A** major constraint for researchers are the lack of methods to determine the amount and the spatial distribution of the active mycorrhizal hyphae in natural soils and distinguish this active biomass from those of other soil fungi. The current available methods and their limitation for determining external hyphae density have been reviewed in Chapter 2.

In the experiment presented in this Chapter, we tested an indirect technique using ^{14}C labelling of hyphae via a host plant to assess the amount of active ECM hyphae in the soils. The novel rhizosphere study container (RSC) technique (Hedley et al., 1982a, b;

Zoysa et al., 1997) was used to create soil volumes that could be explored only by mycorrhizal hyphae.

7.2 Materials and methods

7.2.1 Soil and seedlings

The rhizosphere study container (RSC) described in Chapter 5 was employed in this experiment. The RSC had two compartments – upper and lower and separated by a nylon mesh having one of three different opening sizes (10, 90 and 210 μm) to constrain roots to the upper compartment of the RSC. The use of three mesh sizes was expected to produce varying ECM hyphal density at the root interface in the lower compartment. In the experiment described in Chapter 5, meshes with openings of 10 and 24 μm were used and it was observed that the hyphae penetrated through both these meshes. The RSC was packed by a non-fertilised amended Allophanic Soil (air-dried, passed through a 2 mm sieve and detailed in Chapter 3) to a bulk density of 0.8 cm g^{-1} for both compartments. Non-shoot-trimmed *P. radiata* seedlings (10 months old, inoculated by *Hebeloma crustuliniforme*; produced at the same nursery as described in Chapter 3) were transplanted into upper compartments (one seedling per RSC) and grown for 100 days inside a soil bath containing non-fertiliser amended soil (passed through 2 mm sieve) same as the one packed inside the RSC. The soil bath was kept moist by a water reservoir with water table at 180 mm below the base of the RSCs (Figure 7.1). This enables the RSCs to be kept at a constant water potential of approximately – 1.6 kPa, and the water content in the soil of inter-compartment boundary at mean of 53% (w/w). The treatments were duplicated and the RSCs were arranged in a completely randomise block design in a glasshouse maintained at 28°C maximum and 15°C minimum. A additional set of treatments without seedling was also included in this experiment as the control treatments.

7.2.2 ^{14}C pulse labeling

At the end of 100-days growth, the seedlings grown in RSC pots were removed from the soil bath to a solid surface inside the glasshouse for $^{14}CO_2$ pulse labelling. In order to avoid the $^{14}CO_2$ diffusing from RSC surface into the soil, each RSC pot (root-soil-

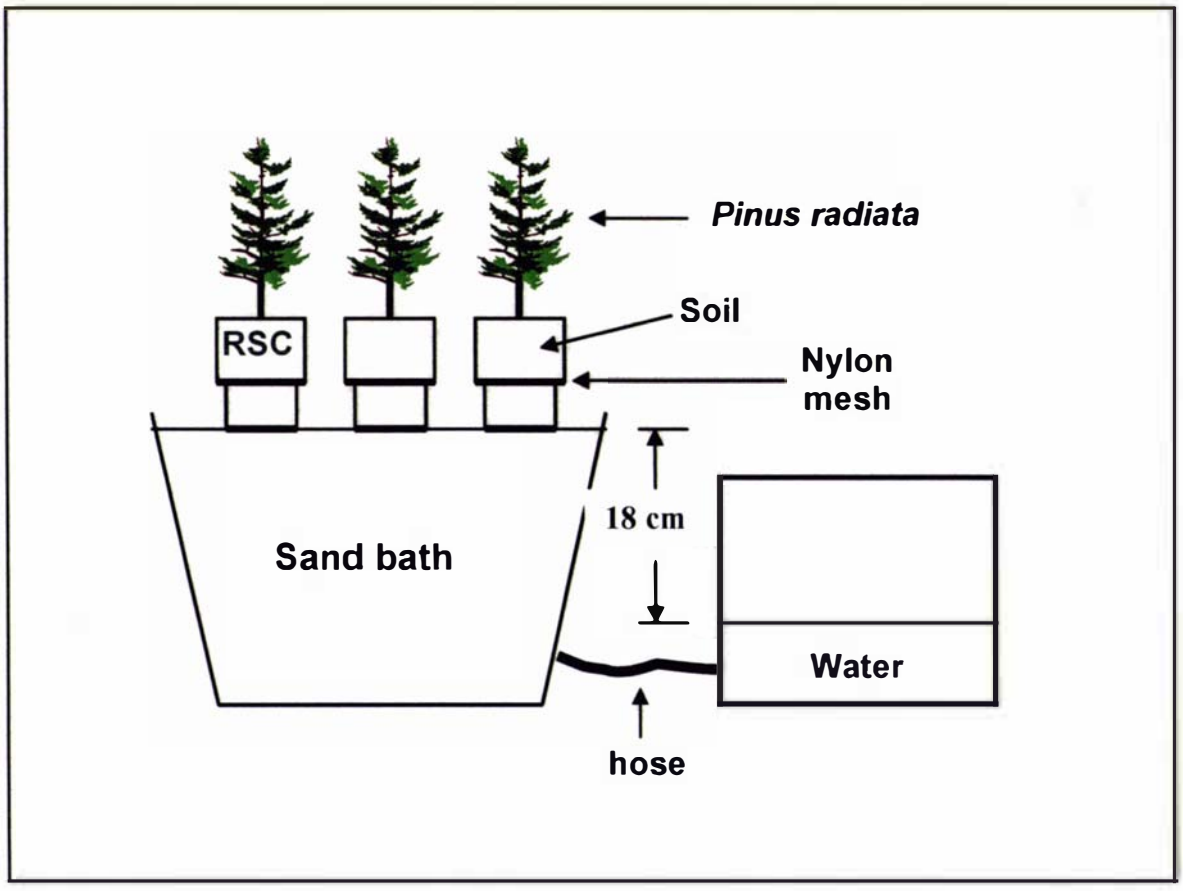

Figure 7.1 Schematic representation of the root study container (RSC) technique

Plate 7.1 $^{14}CO_2$ pulse-labelled onto the shoots of seedlings grown in two-compartmental rhizosphere study containers (RSCs) in a glasshouse

system) was enclosed in a polyethylene bag (Plate 7.1). The bag was sealed (using silicon glue) at the base of the seedling stem. The seedling shoots were then enclosed in another thick polyethylene bag (0.07 mm wall thickness) with the neck taped firmly and sealed at the base of the stem (leaving approximately 5 mm gap between the bags). The approximate volume of the air enclosed inside the thick polyethylene bag was measured as 2 L to the point where the bag mouth was taped to the stem.

Volumes of $^{14}CO_2$ gas were produced in a large air tight plastic syringe fitted with a 3-way stopcock. Twelve ml of 0.2 *M* $Na_2{}^{14}CO_3$ (cc.1.85 MBq ml^{-1}) were drawn into the syringe and reacted in the body of the syringe with 8 ml of 1 *M* H_2SO_4 by manipulation of the stopcock. This produced 52 ml $^{14}CO_2$ gas and 19 ml liquid solution according to the equation below.

$$Na_2{}^{14}CO_3 + H_2SO_4 \rightarrow Na_2SO_4 + {}^{14}CO_2 \uparrow + H_2O$$

Once evolution of CO_2 had ceased, the solution was decanted and stored to determine residual ^{14}C activity, and 7 ml of $^{14}CO_2$ per pot was injected through the shoot-enclosed bag into the atmosphere above each seedling. The hole in the plastic bag was sealed with the masking tape immediately after the injection of $^{14}CO_2$. To check for a leakage of $^{14}CO_2$ from the bag, 7 ml of $^{14}CO_2$ was also injected into a plastic bag without seedling (a stick replaced the seedling) as a control treatment. The remaining 3 ml $^{14}CO_2$ gas was injected into a 10 ml Vacutainer tube as a standard sample.

The temperature inside the glasshouse during the pulse labelling period varied between 19°C at the start of pulse labelling (1000 h) to 22 °C at the1200 h, then decreased to 19°C at the end of the pulse (1600 h). After injection, the $^{14}CO_2$ activity in the enclosed plastic atmosphere was monitored at 20 min, 1 h, 2 h, 4 h and 6 h, using a Geiger counter and 10 ml air samples were taken, using a pre-vacuumed 10 ml Vacutainer tube through the 3-way stopcock. After sampling, 2 ml of 2 *M* NaOH + 0.02 *M* Na_2CO_3 was injected into the Vacutainer tubes as a CO_2 trap solution.

Six hours after pulse labelling (1000 h to 1600 h), the plastic bags enclosing the seedlings were removed, and the seedlings were grown in ambient glasshouse

conditions for another 42 h, before harvesting. During the whole experimental period of 48 h (6 + 42), an additional light resource of 250 W incandescence lamp was fitted approximately 0.5 m above the seedlings (Plate 7.1) to stimulate seedling CO_2 assimilation.

7.2.3 Plant and soil sampling

At the end of the experimental period (48 hours after $^{14}CO_2$ pulse), the upper and lower compartments of the RSCs were physically separated from the mesh position, and the seedlings in the upper compartments were harvested as four fractions – needles, stems, excised ECM root tips and residual roots. Soil from the upper compartment was collected, mixed and dried for later analysis of ^{14}C activity. Soils from the lower compartment were sliced into thin sections using a piston microtome starting at the inter-compartment boundary (see Plate 5.2 in Chapter 5). The first section was sliced at 0.5 mm thickness and the next 7 sections were sliced at 1 mm, and the final set of 3 sections was taken at 2 mm thickness.

7.2.4 Measurement of hyphae length

A simplified agar film technique (Jones and Mollison, 1948) described in Chapter 4 was used to measure the external soil hyphal density. The hyphal length density per g soil was estimated from 36 observation fields, and the inactive fungal hyphae in soil were excluded by subtracting the hyphal density measured from the control treatment (the pots without seedlings).

7.2.5 Analysis of ^{14}C activities

7.2.5.1 ^{14}C in air samples

Duplicate 0.2 ml aliquots of the trapping solution (2 *M* NaOH + 0.02 *M* Na_2CO_3) were transferred to polypropylene scintillation vials and diluted to 2 ml with deionised water. Scintillation cocktail solution (10 ml) was added to these vials and shaken vigorously to produce a clear emulsion and stored overnight in the dark prior to counting ^{14}C activity using a Wallac 1414 WinSpectral™ LSC (standard parameters for ^{14}C counting and

automatic quench correction applied). The scintillation cocktail used toluene base was prepared according to the recipe described by Singh (2000).

7.2.5.2 Total C and ^{14}C in shoot, root and soil samples

Roots, tips and other herbage were dried at 60°C inside an oven and finely chopped using an electric coffee grinder inside a fume cupboard. Soil samples were air-dried and milled < 0.5 mm. Total C in herbage and soil samples was determined by the dichromate digestion procedure (Amato, 1983; Sparling et al., 1991). In this procedure, plant (15 – 20 mg), or soil material (0.25 – 0.35 g) was added to the bottom of a screw top Kimax glass digestion tube (50 ml volume) and digested using 7.5 ml of digestion mixture at 135 ± 5 °C for 1.5 h. A 5 ml graduated tube containing 3 ml of 2 *M* NaOH + 0.2 *M* Na_2CO_3 was placed on the twisted glass support in the digestion tube for trapping CO_2 evolved. After digestion, an aliquot (1 ml) of the trapping solution was titrated against 0.1 *M* HCl using phenolphthalein as indicator to determine the residual NaOH, after first precipitating out the carbonates by adding 25 ml of 10% $BaCl_2$ (Singh, 2000).

For the measurement of ^{14}C activity, another aliquot (0.2 ml) of the trapping solution was taken in the polypropylene scintillation vial and diluted to 2 ml with deionised water. The scintillation cocktail solution (10 ml) was added and the vial was shaken thoroughly to produce a clear solution and counted for ^{14}C activity.

The % recovery of the ^{14}C in different components of the shoot, root, tips and soils was expressed as a percentage of the total ^{14}C injected into the plastic bag at the start of the pulse labeling. The injected total ^{14}C was calculated as 2.47 MBq seedling^{-1}, based on the measurement of the standard sample.

7.3 Results and discussion

7.3.1 Dry weight of seedling fractions

The dry weights of the seedling fractions are shown in Figure 7.2. There was no significant (at $P < 0.05$) difference in seedling dry weights between mesh pore-size treatments. The highest proportion of the dry weight amongst the fractions was recorded

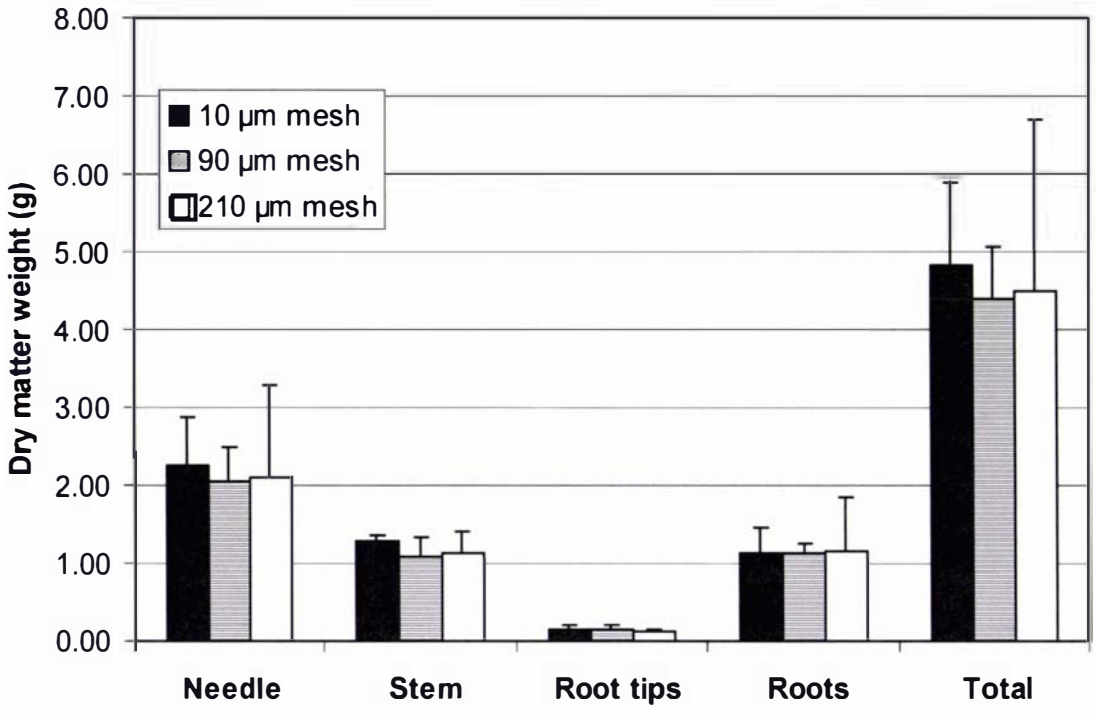

Figure 7.2 Dry weight of seedling fractions in three mesh pore-size treatments (Line on the top of bars show 1 SE of mean)

in needles, while the lowest in root tips. The mean weights of the fractions as a percentage of total seedling dry weight were 46%, 25%, 3% and 25%, respectively for needle, stem, root tips and roots. The ratio of the aboveground/belowground plant dry weight was 2.5 – 2.8.

7.3.2 ^{14}C changes in air samples

The data obtained from both air samples analysis (Figure 7.3A) and Geiger counter (Figure 7.3B) of the atmosphere enclosed in plastic bags showed that the $^{14}CO_2$ activity in the control (stick) treatment remained essentially constant, whereas the $^{14}CO_2$ activity in the bags covering seedling dramatically ($P < 0.001$) decreased with increased pulse time after the injection of $^{14}CO_2$, particularly in the first hour after $^{14}CO_2$ application. This decrease in $^{14}CO_2$ activity indicated that the radiata seedlings used in this study grew vigorously and therefore assimilated $^{14}CO_2$ rapidly.

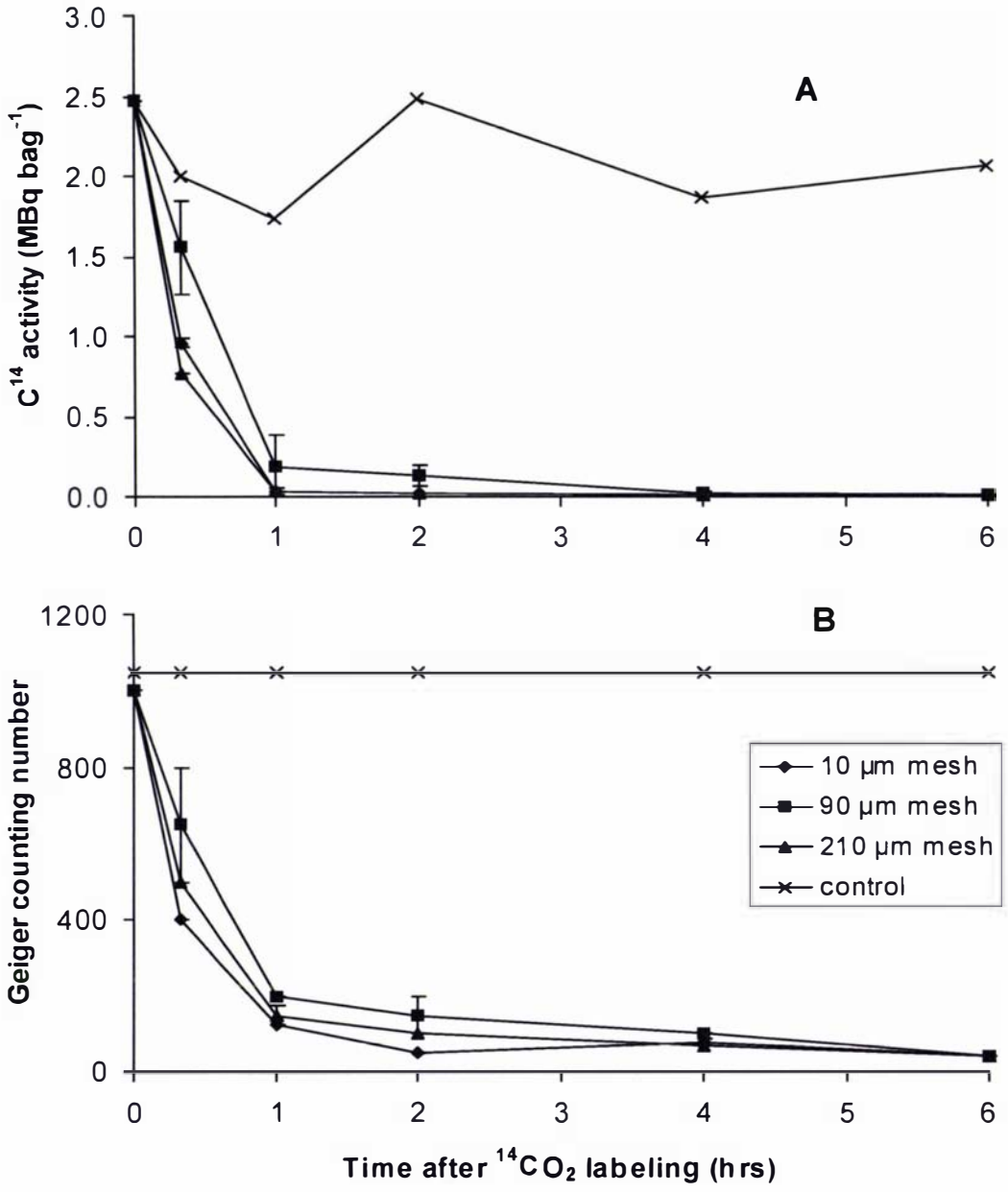

Figure 7.3 Changes in $^{14}CO_2$ activity in bags enclosing pine seedlings with increased time after $^{14}CO_2$ pulse labeling (A – measured by air samples; B – counted by a Geiger counter; line bars - ± 1 SE).

No significant ($P < 0.05$) difference in $^{14}CO_2$ activities in the bags was found between the mesh pore-size treatments. Slower rates of $^{14}CO_2$ decline were associated with seedlings that had lower needle weights (Figure 7.2), but this was not significant.

7.3.3 ^{14}C distribution in plant and soil

7.3.3.1 ^{14}C allocation in shoot-root-soil system

At harvest (48 h after ^{14}C pulse labelling), the total of 70 – 82% injected ^{14}C was recovered (Table 7.1) in the plant-soil system. The high % total recovery of the injected ^{14}C indicates that in the present study there was a relatively small proportion of the ^{14}C lost from the respiration of seedling shoots and roots. The distribution of ^{14}C amongst the seedling parts and soils were highly variable between the mesh pore-size treatments (Table 7.1). In general, the injected $^{14}CO_2$ was mainly recovered in the seedling shoots (particularly in the needles) which ranged from 40% to 77%, and 2 – 37 % of injected ^{14}C were recovered in the roots (tips + roots) and the root-contacted soil in the RSC upper compartment (Table 7.1). The recovery percentage of ^{14}C in the lower compartment soils (0 - 13.5 mm depth from mesh) was only 0.04 - 0.16% of the total injected ^{14}C (Table 7.1).

The ^{14}C determined in the root-contacting soils (i.e. the soil in upper compartment) can be attributed to rhizodeposition (Tinker et al., 1991) that might originate three ways: (i) the debris of the root tips and the residues of the external ectomycorrhizal hyphae that formed a part of the roots system; (2) the root exudation; and (3) the diffusion of root and microbial respiration. The residues of external hyphae may contribute a high proportion to the ^{14}C recovered in the soil in upper compartment, because high hyphal density had been found in this compartment, particularly in the interface layer of the mesh (will be discussed in later section).

In the present study, as the roots had been visibly stopped by the mesh, the ^{14}C recorded in the soil of the lower compartment might have mostly originated in two ways: (i) assimilated ^{14}C transferred by active hyphae, which penetrated through the mesh into

Table 7.1 Distribution of total injected ^{14}C (as MBq and %) in different components of *P. radiata* shoot-root-soil system (mean ± 1 SE) at 48 hrs after $^{14}CO_2$ pulse labelling.

Mesh	Shoot		Root		Soil		Total
pore-size	needle	stem	tips	other roots	above mesh	below mesh	
			Measured ^{14}C activity (MBq pot^{-1})				
10 μm	0.771±0.058	0.218±0.054	0.026±0.011	0.321±0.144	0.558±0.155	0.002±0.001	1.897±0.198
90 μm	1.361±0.016	0.325±0.004	0.004±0.003	0.018±0.014	0.029±0.019	0.001±0.000	1.738±0.055
210 μm	1.470±0.305	0.437±0.071	0.008±0.001	0.044±0.000	0.076±0.053	0.004±0.001	2.038±0.326
			Percentage of recovered ^{14}C activity (%)*				
10 μm	31.18±2.36	8.83±2.20	1.04±0.44	12.97±5.81	22.56±6.28	0.08±0.02	76.66±8.00
90 μm	55.02±0.63	13.14±0.15	0.15±0.11	0.73±0.56	1.16±0.77	0.04±0.01	70.25± 2.23
210 μm	59.41±12.33	17.65±2.89	0.29±0.04	1.79±0.01	3.09±2.15	0.16±0.05	82.39±13.18

*100 × measured ^{14}C / total injected ^{14}C

the lower part compartment; (2) ^{14}C diffusion from upper compartment through root exudates and root respiration.

The relatively small ^{14}C recovered in roots in 90μm and 210μm of mesh pore-size treatments indicates that only small amount of ^{14}C assimilated through photosynthesis was transported into the root system (Table 7.1). This small translocation ratio of the pulsed ^{14}C from shoots into root system might be involved in the shortage of ^{14}C chase time of 2 days. It was unclear what caused the large variation in the allocation of assimilated ^{14}C between mesh pore-size treatments (Table 7.1). A possible explanation of this may be associated with the seedling status. Because the seedlings used in the present study were produced in a commercial nursery, the seedlings would have differences in the physiological conditions.

7.3.3.2 ^{14}C distribution in the soil in the lower compartment

The ^{14}C activity recorded in the soil of the hyphae-compartment (i.e. the lower RSC compartment) contributed a very small proportion (only ranging from 0.04% to 0.16%) of the injected ^{14}C, compared to the other fractions (Table 7.1). All ^{14}C was recovered in the 0 - 13.5 mm depth because the ^{14}C activity measure at 13.5 mm depth was essentially a background value (Figure 7.4).

Both ^{14}C activity and specific activity decreased significantly ($P < 0.001$) with increasing distance from the mesh. Notably the largest activities were observed in 0 – 2.5 mm distance from the mesh. Mesh size had a significant ($P < 0.05$) effect on ^{14}C activity (Figure 7.4). The reason for the higher ^{14}C activity recorded in the 210 μm mesh treatment was possibly due to the large mesh pore-size because very fine root tips may have penetrated the mesh pore-size.

7.3.4 Hyphae activity in the soil of the lower compartment

The changes in the hyphal length density in the soil of the lower compartment are shown in Figure 7.5. There was no significant difference in the mesh pore-size treatments, but the hyphal length density significantly ($P < 0.001$) decreased with the increasing distance from the mesh (Figure 7.5). The decrease in hyphal length density

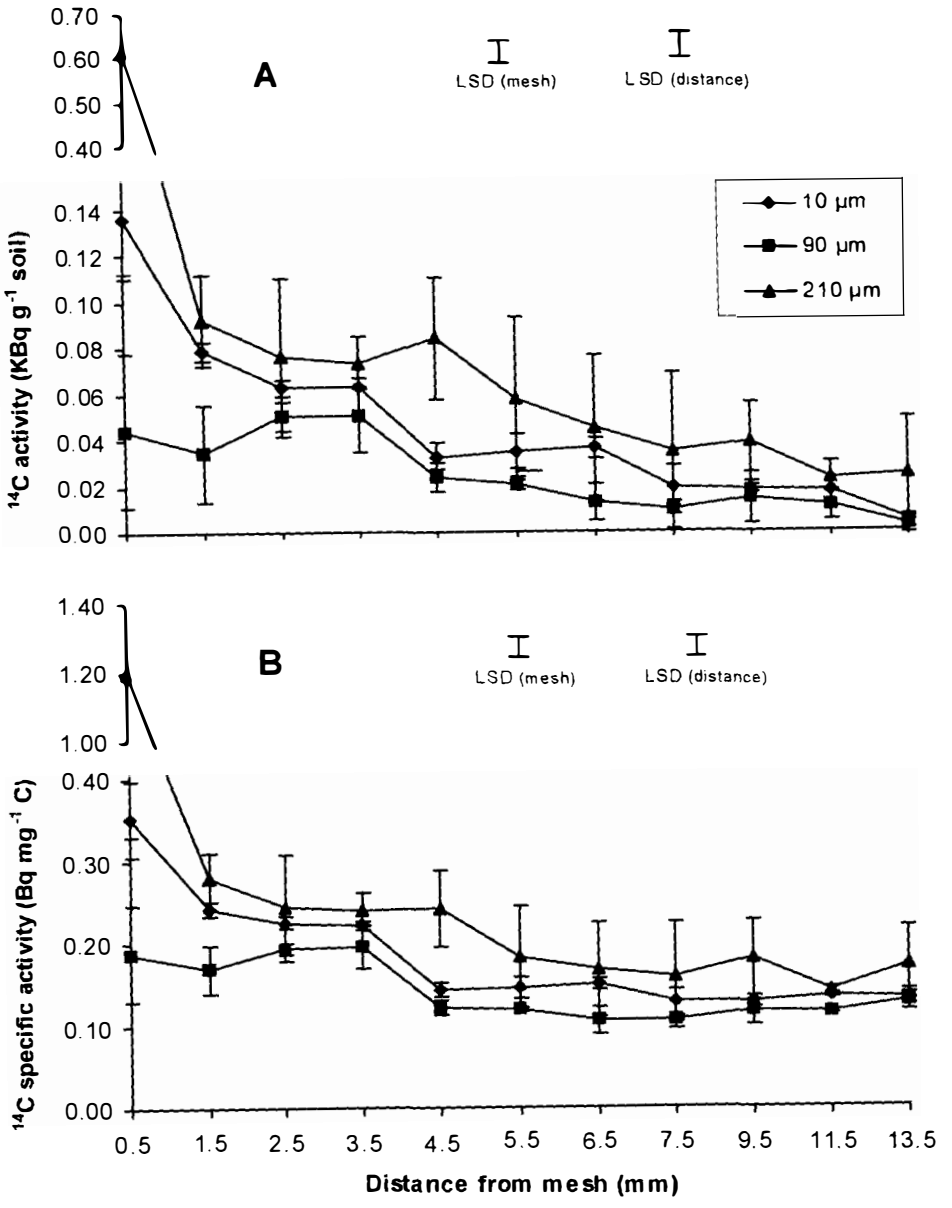

Figure 7.4 Changes in ^{14}C activity (A) and specific activity (B) in the soil at the lower compartments (Mean ± 1 SE)

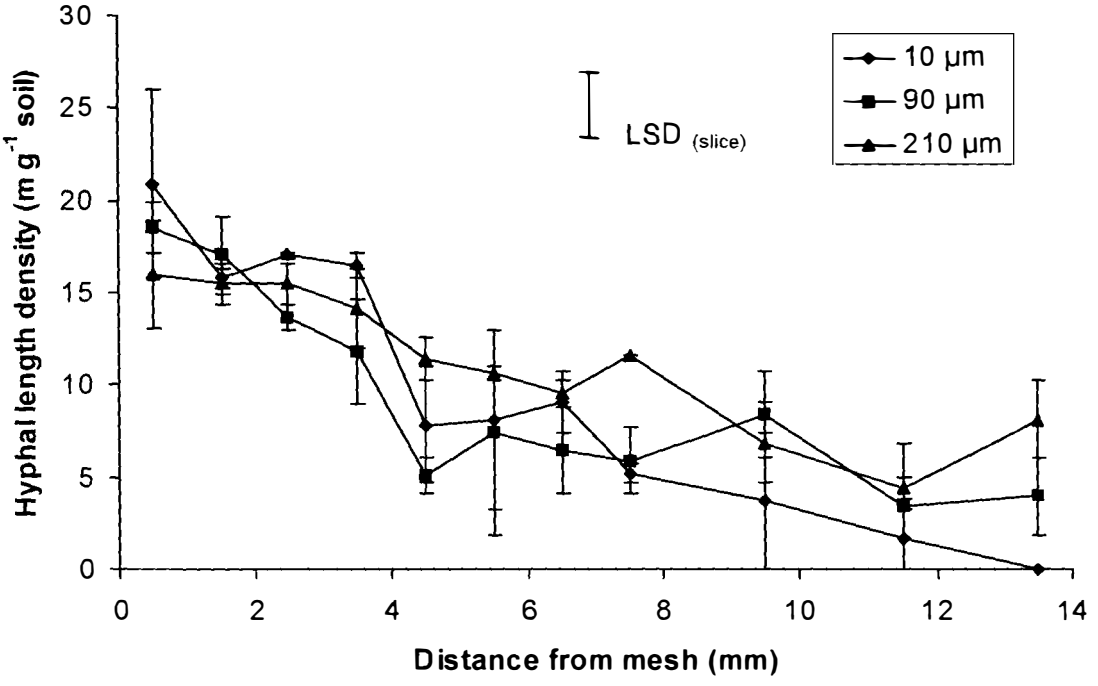

Figure 7.5 Changes of hyphal length density with distance from mesh in the lower compartments soils (lines show ± 1 SE)

with distance from the mesh (Figure 7.5) and the differences between mesh treatments (210 µm > 10 µm > 90 µm) appear closely associated with the pattern of ^{14}C activity away from the mesh (Figure 7.4).

In five of the six RSCs the hyphal length density in the soils of lower compartment was significantly ($P < 0.01$) correlated with ^{14}C activity (Table 7.2). Significant correlation was also found in the overall data (Figure 7.6). In these correlation analyses, two highest ^{14}C values recorded at 0 - 0.5 mm slice of the 210 µm mesh treatment have been excluded from calculations, because they were considered to be affected by relatively larger mesh pore-size of 210 µm which was too large to stop the penetration of fine roots.

Table 7.2 Regression equations describing the relationships between hyphal length density (y) (m g^{-1} soil) and ^{14}C activities (x) (KBq g^{-1} soil)

Mesh opening (μm)	Replicate	Regression equation	R^2	Significant level (P at $_{0.05}$)
10	1	y = 157.0x + 1.67	0.799	<0.001
	2	y = 179.6x + 2.42	0.632	0.003
90	1	y = 186.3x + 4.27	0.216	0.159
	2	y = 186.2x + 4.96	0.713	0.002
210	1	y = 118.1x + 7.5	0.442*	0.032
	2	y = 95.0x + 2.88	0.753*	0.002

*The data at 0.5 mm depth have been removed as they were considered to be affected by the root tips penetration.

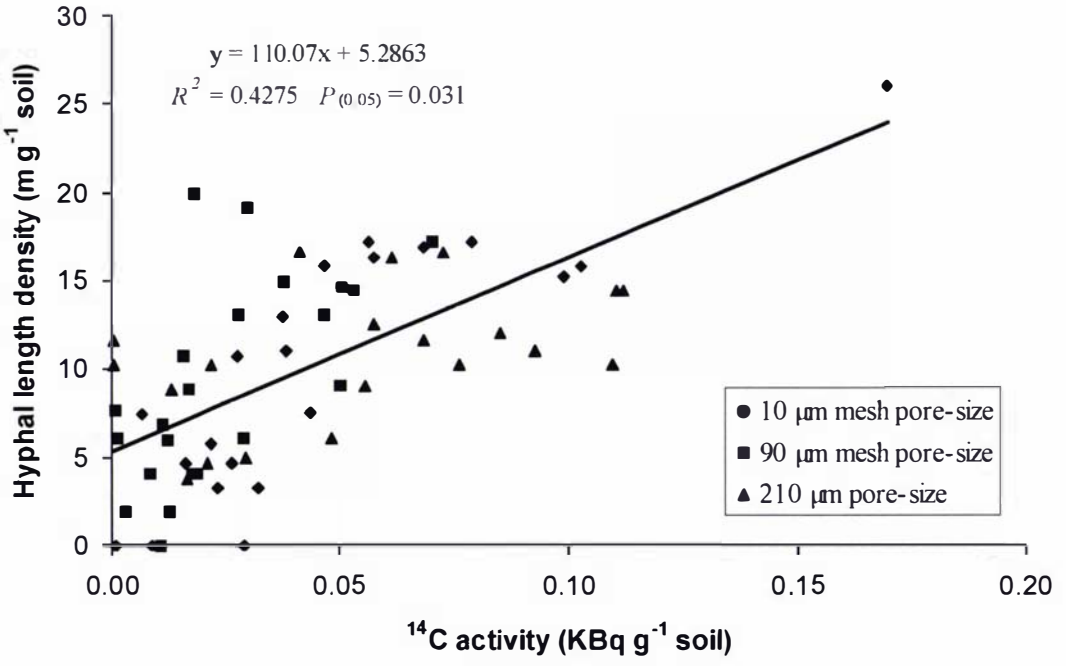

Figure 7.6 Relationship between ^{14}C activity and hyphal length density in the lower compartment soil for all mesh pore-size treatments

The significant relationship between ^{14}C activities and hyphal length density indicated that the $^{14}CO_2$ pulse labelling method may have the potential to indirectly estimate the density and distribution of active ECM hyphae of young plants grown in soil in small pots. This result is despite the fact that the measured ECM hyphal length density in the present study is 10 times lower than reported values in the natural forests (such as 400 – 1500 m ml^{-1} soil in 80-year-old Norway spruce, Haüssling and Marschner, 1989). This relatively lower hyphal length density might have limited the amount of the ^{14}C transferring into the lower compartment. Thus, attempts to improve the development of mycorrhizal hyphae in the lower compartment may improve the sensitivity of $^{14}CO_2$ pulse labelling for identifying areas of hyphal density. In the present study, the extremely low plant-available P (Bray-2 P < 2 μg g^{-1} soil) and the fine particles (< 2 mm) in the test soil may have restricted the development of the ectomycorrhizae (Slankis, 1974; Schack-Kirchner et al., 2000). Therefore, a modification of plant-available P and particle size in the lower compartment soil would stimulate the penetration of the ECM hyphae through the mesh.

7.4 Conclusions

The results obtained in this study showed that the ^{14}C activities obtained in the hyphae compartment were significantly correlated with hyphal length density measured by a simplified agar film technique. This suggests that the $^{14}CO_2$ pulse labelling method has the potential to indirectly estimate the density and distribution of active ECM hyphae of young plants grown in soils in pots. If this approach is further developed, it would provide a relatively rapid and simple way to evaluate the effects of cultivar types and applied treatment on mycelial activity in the soil. Further research on the improvement of this technique needs to consider the following:

1) The density and distribution of active fungi mycelia in soil estimated by $^{14}CO_2$ labelling method is likely to be interfered by ^{14}C metabolisation and subsequent root release into the soil. Isotopic materials other than C could be tested.
2) The 2-days chase time used in the current study might not be long enough for the ^{14}C transferring from shoot to root system of older than 1-year *P. radiata* seedlings. Optimal chase time needs to be researched.

3) The relatively large variation of ^{14}C allocation in the seedling parts may be due to the heterogenic seedling status used in this study. Further studies need to be conducted on more uniform seedlings.

4) Meshes of pore-size openings of 10 – 90 μm diameters are recommended for future studies. This is because the mesh with 210 μm pore-size openings was not able to stop root penetration into the lower compartment of the rhizosphere study containers in this study and this gave a poor correlation between hyphal density and ^{14}C activity in the lower compartment soils.

CHAPTER 8

Summary, conclusions and recommendations for future work

8.1 Need for the study

Inherent low phosphorus (P) status and high P fixing capacity coupled with low or no fertiliser input compared with agricultural soils, have resulted in low levels of plant-available P in volcanic ash soils supporting *Pinus radiata* plantations in New Zealand. However, *P. radiata* generally grows well on these low P status soils with additions of low rates of and in some cases no P inputs. The mechanisms by which *P. radiata* obtains sufficient P for growth on these soils are not well understood. A review of literature in Chapter-2 highlighted the importance of ectomycorrhizal (ECM) fungi associated with *P. radiata* roots in the acquisition of P from soils containing low levels of plant-available P. The interactions between soil, ECM fungi and *P. radiata* roots create a mutually supportive system in the *P. radiata* rhizosphere (Gobran and Clegg, 1996). Ectomycorrhizal root-induced soil acidification and production of oxalate and hydrolytic phosphatase enzymes in the rhizosphere of conifers appear to mobilise insoluble soil P. The turnover of ECM fungi and fine roots of the conifers may also influence soil P bioavailability in the rhizosphere of conifers. Compared to the agricultural plants, relatively little research has been conducted to understand the effect of rhizosphere processes on soil P dynamics under *P. radiata.* Hence, there is a need to study the effect of rhizosphere processes on the soil and fertiliser-P dynamics under *P. radiata* so that the nutritional requirements of large tracts of managed *P. radiata* in New Zealand can be better understood. This study has concentrated on using rhizosphere study techniques combined with soil P fractionation, enzyme and microbial assays to examine evidence for solid phase P mobilisation in the *P. radiata* rhizosphere.

8.2 Main findings of this study

This study produced direct evidence of accelerated mobilisation of solid phase soil P associated with the activities of the mycorrhizal *P. radiata* rhizosphere in volcanic ash soils. A visual concept model of P uptake and cycling under *P. radiata* is proposed to better describe the soil-plant-fungi processes governing P supply (Figure 8.1).

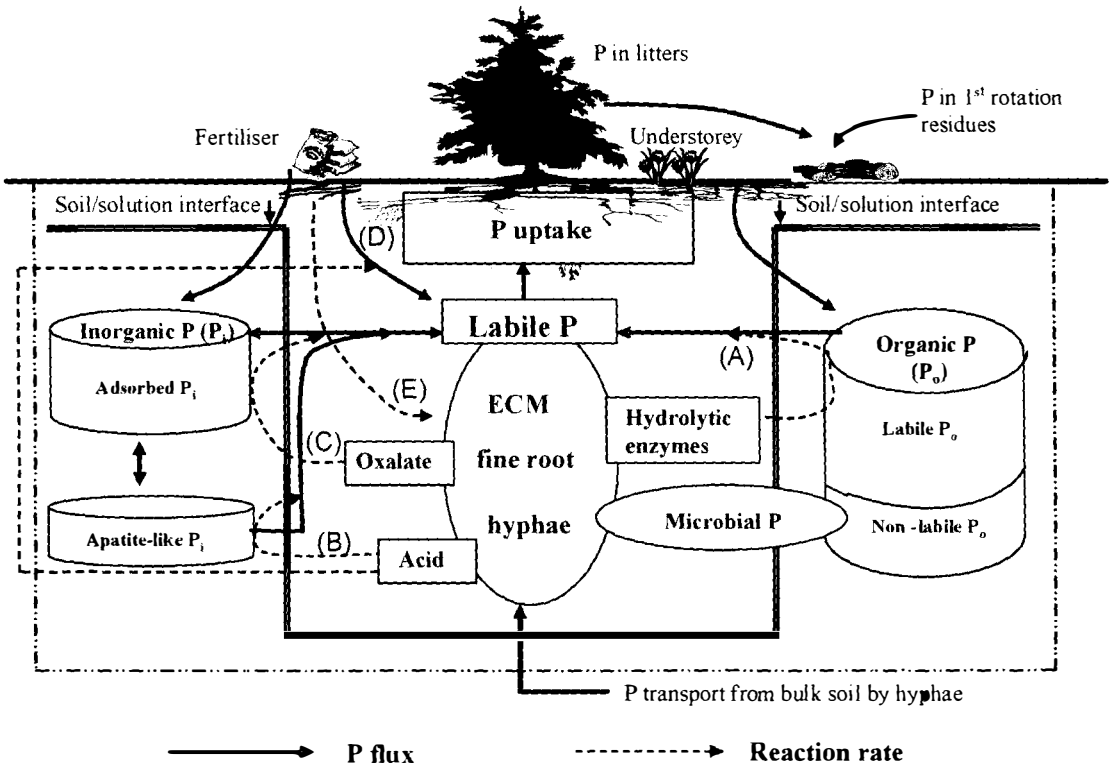

Figure 8.1 A conceptual model of the rhizosphere processes governing P supply and uptake by *P. radiata*. Rhizosphere processes discussed in text are denoted by capital letters.

8.2.1 *P. radiata* root processes increase soil phosphatase activity and thereby increase soil organic P mineralisation rate

The soil organic P constituted more than 60% of the total soil P in volcanic ash top soils (0 – 10 cm depth). The efficient use of this soil organic P by *P. radiata* has been considered to be a major process of supplying P to *P. radiata* in forest soils but very little quantitative data is available in the literature to support this suggestion. When 1-

year-old *P. radiata* seedlings were grown for 10-months in a RPR treated Allophanic Soil, it was found that the labile organic P (0.1 *M* NaOH-P_o) fraction was depleted by 8 – 17% in the rhizosphere soil (Chapter-3), showing that this process (A in Figure 8.1) is a significant pathway of generating labile P for uptake by the radiata pine.

This mobilisation of organic P to inorganic P in the rhizosphere was associated with root-induced increases in soil phosphatase enzyme activities. Both acid and alkaline phosphatase activities were found to be > 50% higher in the rhizosphere than in the bulk soils. The results indicate that significant quantities of soil organic P reserves can be mobilised in the *P. radiata* rhizosphere.

The root-induced increases in phosphatase activities in the rhizosphere soils were also observed in other studies in this thesis (Chapters 4, 5 and 6). But there was no direct relationship found between P_o mineralisation rate and phosphatase activities. This may partly be due to the fact that a suite of other hydrolytic enzymes would be required for the mineralisation of soil P_o and simple esterases such as those that can use the phosphatase assay substrate used in this study (sodium *p*-nitrophenyl phosphate) may not be the limiting step.

8.2.2 *P. radiata* rhizosphere processes are able to induce soil acidification thereby solubilise poorly soluble PR-P

RPR-P bioavailability relies on its dissolution in the soil. Soil acidification is an important factor controlling RPR dissolution. In Chapter-3, a short-term (10-months) pot trial was conducted on 1-year-old *P. radiata* seedlings to examine the fate of a finely divided (75 – 150 µm size) Sechura phosphate rock (SPR) applied to an Allophanic Soil at rates equivalent to 0, 50, 100 and 150 kg P ha^{-1}, demonstrating that the rhizosphere processes of *P. radiata* significantly increased the rate of dissolution of SPR (by 5 – 7%) and this was associated with a decrease in rhizosphere soil pH (decrease of 0.5 – 0.6 pH unit from pH 5.9 - 6.1 in the bulk soil) compared to the bulk soil. These results indicate that *P. radiata* root-induced acidification (B in Figure 8.1) is an important process controlling the dissolution of RPR-P (D in Figure 8.1) added to soils.

The root-induced acidification in rhizosphere soil appears to be associated with ECM association. In Chapter-6, *P. radiata* rhizosphere processes were compared in RPR-P fertilised and unfertilised soils with different types of ECM fungal inoculations. The experimental results demonstrated that after 10-months of *P. radiata* seedling growth the highly ECM infected seedlings treated with P fertilisers significantly decreased pH in the rhizosphere soil (by 0.1 – 0.2 pH unit) compared to that in the bulk soil (pH = 5.6). However, there was no such decrease in rhizosphere pH in the seedlings with poor ECM infection in P-unfertilised and autoclaved soils, indicating that ECM association with *P. radiata* induces rhizosphere acidification.

8.2.3 *P. radiata* root-induced changes in rhizosphere oxalate concentration are highly variable

Oxalate exudation by coniferous roots (C in Figure 8.1) has been considered to play an important role in mobilising insoluble soil inorganic P. The efforts to confirm this in the soils under *P. radiata* in this thesis were unsuccessful, probably due to variable rates of oxalate adsorption onto soil and decomposition by soil microorganisms. However, the mean oxalate concentration in the *P. radiata* rhizosphere was found to be higher than that in the bulk soil in both the pot and field trials reported in Chapters 3 and 4, respectively.

8.2.4 *P. radiata* rhizosphere processes have a greater potential to mobilise solid phase soil P than the understorey grass rhizosphere processes

In Chapter-4, rhizosphere processes of *P. radiata* were compared with those of understorey grass in two 4- to 5-year plantations, one in an Allophanic Soil and the other in a Pumice Soil. The results demonstrated that *P. radiata* rhizosphere processes have a greater potential to mobilise soil P than the understorey grass rhizosphere processes.

Compared to the understorey grass roots, ECM roots of *P. radiata* markedly increased rhizosphere organic matter content (15 - 17% in radiata rhizosphere soil and 12 – 13% in radiata bulk soil; 15 – 17% in grass rhizosphere soil and 14 – 15% in grass bulk soil), acid phosphatase enzyme activities (> 50% increase in radiata rhizosphere soil, < 25%

increase in grass rhizosphere soil) and concentrations of dissolved organic carbon and oxalate compared to the corresponding bulk soils in both the Allophanic and Pumice Soils. These rhizosphere processes raised resin-P concentration in the radiata rhizosphere (9 μg g^{-1} soil) in the Pumice Soil, compared to the corresponding bulk soil (3.5 μg g^{-1} soil), but there was no significant difference in resin P concentration between understorey grass rhizosphere and bulk soils. The greater potential to mobilise soil P in *P. radiata* rhizosphere than in the grass rhizosphere appeared to be as a result of ECM association on *P. radiata* roots and labile P_o cycling in the rhizosphere through ECM fine roots turnover.

8.2.5 *P. radiata* rhizosphere processes are greatly associated with ECM hyphae

In Chapter-5, by creating high and low soil hyphae activities using a novel technique employing rhizosphere study containers with different pore-sized nylon meshes to allow (mesh opening diameter 26 μm) or reduce (< 10 μm mesh plus 3 μm filter) hyphae penetration into the rhizosphere soil, it was shown that the increase in soil acid phosphatase activity was associated with increase in ECM hyphal length density in soil. The increase in soil acid phosphatase activity was also associated with a decrease in soil organic P (particularly the P_o in residual form). The ECM hyphae induced further dissolution (1.5 - 9.4% higher in the high hyphae activity treatment than in the low hyphae activity treatments) of a finely-divided (155 – 250 μm pore-size) Ben Guire phosphate rock in soil 10-months after this RPR application. These results demonstrated that the ECM hyphal strand has a significant role in mobilising low solubility organic and inorganic soil P forms for utilisation by the tree.

Further evidence of the role of ECM hyphae strands in mobilising low solubility inorganic and organic P forms was obtained in an ECM inoculation trial. In Chapter-6, a glasshouse study on ectomycorrhizal fungal inoculation of newly germinated *P. radiata* seedlings grown in P-fertilised and unfertilised soils demonstrated that in P-fertilised soils, inoculation of seedlings with *S. luteus* and *R. rubescens* produced higher concentrations of resin-P, phosphatase activities and oxalates, and lower pH in the rhizosphere soils than in the bulk soils. These changes were associated with higher soil ECM hyphae density in the rhizosphere soil. These results further support the hypothesis that *P. radiata* rhizosphere processes are associated with ECM hyphae.

In the study reported in Chapter-6, *R. rubescens* inoculation, compared with *S. luteus* inoculation, produced greater ECM tip density in seedling roots in P-fertilised soils and higher oxalate concentration in rhizosphere solution, particularly in P-unfertilised soils. However, there was no difference between the two inoculants in their effects on P uptake or seedling growth, though both inoculation treatments in P-fertilised soils produced higher P uptake and seedling growth than when seedlings were grown in autoclaved soil.

8.2.6 Finally-divided reactive phosphate rocks (RPRs) are effective P fertilisers for *P. radiata* in Allophanic Soils

RPR is promoted as an effective P fertiliser for improving *P. radiata* growth in marginal acidic (pH 5.8) Allophanic Soils. The results obtained in Chapter-3 and Chapter-6 demonstrated that the application of finally-divided RPRs to a P deficient Allophanic Soil improved seedling growth and P uptake. This was due to increased RPR dissolution in the soils.

The results of the glasshouse trial reported in Chapter-3 showed that SPR (75 – 150 μm particle) application at a rate equivalent to 150 kg P ha^{-1} significantly increased height (1.2-times) and P uptake (1.7-times) of 1-year-old *P. radiata* 10-months after SPR application. In this trial, more than 87% of the added SPR was found to be dissolved in the rhizosphere soil

The application of another RPR, Ben Guire PR (BGPR; 150 – 250 μm particle size) at a rate equivalent to 100 kg P ha^{-1} to newly geminated *P. radiata* seedlings also significantly increased *P. radiata* seedling height (1.5 – 2.0-times) and P concentration in shoots (1.9 – 2.7-times) 10-months after application. In this trial, more than 57% of the added BGPR was found to be dissolved in the rhizosphere soil

8.2.7 Soil P fertility influences ECM formation on *P. radiata* roots

Though the ECM hyphae have a strong influence on the radiata rhizosphere processes and their effects on solid phase P mobilization in rhizosphere soils, the development and

formation of ectomycorrhizas in radiata seedlings appeared to be hindered by high or extremely low soil P status (Chapter 3 and 6) (E in Figure 8.1). High soil P concentration was reported to decrease carbohydrate content of pine roots and therefore decreases their susceptibility to ECM fungi infection. Conversely evidence was produced to show that extremely low soil P concentrations may also limit the growth of mycorrhizal fungi thereby hindering the ECM infection on the roots of *P. radiata.*

8.2.8 $^{14}CO_2$ pulse-labelling technique may have the potential to indirectly estimate the density and distribution of active ectomycorrhizal hyphae in soil

Despite an increasing awareness of the importance of ECM hyphae in rhizosphere P bioavailability, there is currently no suitable method for the determination of the density and distribution of active hyphae in field soils. The methods currently available cannot also clearly distinguish active ECM hyphae from other fungal hyphae in soil.

In Chapter-7, a preliminary study on determining active mycorrhizal hyphae distribution in the soil by using a $^{14}CO_2$ pulse-labelling technique demonstrated close correlation between hyphal length density and ^{14}C activities in the soils. This suggests that the $^{14}CO_2$ pulse-labelling technique may have the potential to indirectly estimate the amount and distribution of active ectomycorrhizal hyphae in soils. However, the ^{14}C measured in the soil may not be entirely derived from the hyphae. ^{14}C diffusion from the roots into the soil also contributes to the measured ^{14}C activity. Further research perhaps using other isotopes needs to be conducted to improve this technique.

8.3 Recommendations for future work

More than 60% of the P in the topsoil (0 – 15 cm depth) of forests exists in organic form. The study reported in this thesis showed that *P. radiata* rhizosphere processes influence the bioavailability of this organic P fraction. The current soil P test (Bray-2 P) used in forest plantations considers P availability from only the inorganic P pool in the soil. Attempts need to be made to develop soil P tests to include P release from both inorganic and organic pools. We also need to revisit the whole area of organic P dynamics, particularly P_o cycling through ECM roots, in the rhizosphere to assist our

understanding of P availability and transport processes to roots and reasons for the observed organic P depletion in the rhizosphere soils in some studies but not in others.

The main limitation of this and similar studies with other plant species is the current lack of techniques suited to measuring the small biochemical changes that take place in the rhizosphere of plants under field conditions but changes which are extremely important in the uptake of P and other nutrients. For example, oxalate in the *P. radiata* rhizosphere has been implicated to have a significant role in P mobilisation but this was not able to be tested in this thesis due to large variations in soil solution oxalate concentration observed, probably as a result of the interactions in the rhizosphere among roots, soil, mycorrhizal fungi and other soil micro-organisms and soil adsorption and microbial decomposition of the oxalate released. Improved techniques are needed to quantify the role of ECM root-released oxalates (and other organic acids) in effecting rhizosphere P mobilisation. It would be worth examining other areas of science (e.g. nano-technologies) for new techniques measuring low concentrations of organic acids that can be employed for studying the effect of soil colloid surfaces and soil microbes on the released organic acids. Improved techniques are also required to measure ECM hyphae lengths and distribution in soils; so that the root-induced changes in rhizosphere soil properties can be better understood.

The root-induced changes in rhizosphere properties appear to be associated with varying mycorrhizal fungal symbionts. If this is true, isolating P-efficient ECM fungi and inoculating them to radiata seedlings may have significance in terms of sustainable management of forestry and this aspect warrant further investigation.

The study reported in this thesis demonstrated that there are interactive influences of the application of phosphate fertiliser on radiata seedling growth and ECM fungi development. In order to establish whether fertiliser application rates and their interaction with fungal infection have an agronomic impact on rate and quality of pine production, variable rates of P fertiliser application and seedlings inoculated with different ECM fungi are worth investigating.

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