Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- you will use the copy only for the purposes of research or private study
- you will recognise the author's right to be identified as the author of the thesis and due acknowledgement will be made to the author where appropriate
- you will obtain the author's permission before publishing any material from the thesis.
EFFECTS OF GRASSLAND AFFORESTATION ON SOIL NUTRIENT DYNAMICS AND AVAILABILITY IN NEW ZEALAND

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Doctor of Philosophy
at
Lincoln University

By
Chengrong Chen

Lincoln University
Abstract of a thesis submitted in partial fulfilment of the requirement for the Degree of Doctor of Philosophy

EFFECTS OF GRASSLAND AFFORESTATION ON SOIL NUTRIENT DYNAMICS AND AVAILABILITY IN NEW ZEALAND

By Chengrong Chen

Continued expansion of plantation forestry in New Zealand is expected to alter soil chemical, biochemical and biological properties which will have important implications for long-term sustainable land management. The major objectives of this study were to investigate the impacts of grassland afforestation on the dynamics of soil organic matter and associated phosphorus and sulfur with respect to chemical, biochemical and biological processes. This study involved a combination of field and glasshouse experiments.

A paired-site study of a 19-year old mixed conifer forest stand (*Pinus ponderosa*, *Pinus nigra*) and adjacent unimproved grassland was conducted at Craigieburn, Waimakiriri catchment, Canterbury. It was found that concentrations of organic carbon, phosphorus and sulfur were significantly lower, but levels of labile inorganic phosphorus and sulfur were higher in topsoil under forest compared with grassland. These findings supported earlier research indicating that afforestation caused enhanced mineralization of organic matter and associated nutrients. On the other hand, concentrations of microbial biomass carbon, phosphorus and sulfur and microbial and enzyme activities were lower in soils under forest compared with grassland. It is possible that the apparent enhanced mineralization of organic matter and associated nutrients in soil under forest may be due to elevated levels of microbial and enzyme activity during the earlier stages of forest development.

A seasonal study at the Craigieburn site further confirmed that afforestation enhanced mineralization of organic matter and organic phosphorus. Moreover, seasonal changes in soil moisture and temperature influenced the biological and biochemical processes involved in phosphorus recycling. Results showed that phosphorus recycling was primarily driven by plant demand and sustained mainly by inputs of root litter under grassland and needle litter under forest. Microbial biomass played a pivotal role in
phosphorus recycling, and phosphorus turnover through the microbial biomass was higher under forest than under grassland.

Results from a short-term glasshouse experiment using 15 different grassland soils showed that perennial ryegrass (*Lolium perenne*) and radiata pine (*Pinus radiata*) caused redistribution of inorganic phosphorus from slowly exchangeable to rapidly exchangeable pools. Once again, soil organic phosphorus mineralization was consistently greater under radiata pine compared with ryegrass. Higher mineralization was accompanied by higher concentrations of water soluble organic carbon, microbial biomass carbon and carbon dioxide respiration under pine. This supported the previous contention that enhanced mineralization of organic phosphorus occurred during the early stages of forest development. Enhanced mineralization of organic phosphorus under radiata pine may also be partly attributed to higher levels of root phosphatase activity. The importance of microbial and enzyme activities in soil organic phosphorus mineralization was confirmed by results from a detailed study of rhizosphere processes in two soils under ryegrass and radiata pine. The precise role of root exudates (particularly organic acids) in the mineralization of soil organic phosphorus under conifer warrants further investigation.

**Keywords:** Soil, grassland, afforestation, *Lolium perenne, Pinus radiata*, carbon, phosphorus, sulfur, microbial biomass, extracellular enzyme activity, mineralization, fractionation, isotopic exchange kinetics.
ACKNOWLEDGEMENTS

I would like to express my most sincere thanks to my supervisor Dr. Leo Condron for his great expertise, encouragement and enthusiasm throughout this project, particularly in the completion process of this thesis. I would also like to thank my co-supervisor Mr. Murray Davis and associate supervisor Dr. Rob Sherlock for their advice, guidance and encouragement.

I acknowledge the New Zealand Forest Research Institute for granting me a Ph.D. Scholarship and Lincoln University for providing the necessary research funds (including funding for manufacturing a microtome adapter for use in the rhizosphere study). I would also like to thank the New Zealand Soil Science Society for awarding me the 2000 Summit-Quinphos Postgraduate Scholarship.

I would like to thank Dr. Sokrat Sinaj of Institute of Plant Science, Swiss Federal Institute of Technology Zürich (ETHZ) for teaching me isotopic exchange kinetics technique.

Special thanks to Roger Cresswell for his technical assistance and proofreading of this thesis and to Zheng for his help with computing.

Many thanks to Neil Smith for his assistance with field soil sampling, measurement of bulk density and setting up the glasshouse experiments.

I would also like to thank staff and postgraduate students in the Soil and Physical Group for their helpful discussion, assistance and tolerance throughout the period of my study at Lincoln.

My thanks also to:

Dr. Chris Frampton for his advice in the statistical analysis.

Dr. Mike Beare of New Zealand Institute for Crop and Food Research Limited for his comments on the original research proposal,
Dr. Benjamin Turner and Dr. Hongjie Di for their comments on some chapters of this thesis,

John Milne for his help with manufacture of the specific microtome adapter,

Gay Bruce for her assistance with field soil sampling,

Merv Spurway for his help with setting up of the glasshouse experiments,

Jason Breitmeyer, Colin Gray and Neil Smith for their proofreading of parts of this thesis.

Dr. Colin Gray, Dr. Ed Gregorich, Frank O'Meara, Roger McLenaughen, Sjef Lamers and Wim Rijkse for their assistance with collection of the soil samples used in the glasshouse experiments.

Finally, I am most grateful to my wife Xiujuan for her love, understanding and patience. I would also like to thank my lovely daughter, Hong, and my son, Jeff, for bringing me the joyful and happy time.
LIST OF CONTENTS

Abstract  
i
Acknowledgements iii
List of contents v
List of Tables ix
List of Figures xii

Chapter 1  INTRODUCTION 1
1.1 Plantation Forestry in New Zealand 1
1.2 Impacts of Afforestation on Soil Properties 3
  1.2.1 Soil nutrient availability 3
  1.2.2 Soil microbial properties 4
  1.2.3 Other soil properties 4
  1.2.4 Possible mechanisms for the elevated levels of available inorganic nutrients in soil under forest 5
1.3 Soil Organic Nutrient Dynamics in Terrestrial Ecosystems 5
1.4 Research Program 8
  1.4.1 Hypothesis 8
  1.4.2 Objectives 11

Chapter 2  MATERIALS AND METHODS 14
2.1 Materials 14
  2.1.1 Field study site 14
  2.1.2 Glasshouse experiment soils 14
2.2 Methods 15
  2.2.1 Treatment of soil samples 17
  2.2.2 Chemical and physical analyses 17
  2.2.3 Biochemical and biological analyses 22

Chapter 3  LONG-TERM EFFECTS OF AFFORESTATION ON SOIL PHOSPHORUS DYNAMICS AND AVAILABILITY 25
3.1 Introduction 25
3.2 Materials and Methods 26
  3.2.1 Research site 26
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.2</td>
<td>Sampling</td>
<td>26</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Soil analyses</td>
<td>27</td>
</tr>
<tr>
<td>3.2.4</td>
<td>Statistical analysis</td>
<td>28</td>
</tr>
<tr>
<td>3.3</td>
<td>Results</td>
<td>28</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Root biomass</td>
<td>28</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Soil chemical properties</td>
<td>29</td>
</tr>
<tr>
<td>3.3.3</td>
<td>Soil microbial biomass C and P</td>
<td>33</td>
</tr>
<tr>
<td>3.3.4</td>
<td>Soil respiration and metabolic quotients</td>
<td>33</td>
</tr>
<tr>
<td>3.3.5</td>
<td>Soil phosphatase activity</td>
<td>36</td>
</tr>
<tr>
<td>3.4</td>
<td>Discussion</td>
<td>38</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Soil microbial parameters and biological fertility</td>
<td>39</td>
</tr>
<tr>
<td>3.4.2</td>
<td>Soil P fractions</td>
<td>41</td>
</tr>
<tr>
<td>3.4.3</td>
<td>Soil phosphatase activity</td>
<td>42</td>
</tr>
<tr>
<td>3.4.4</td>
<td>Soil organic P mineralization</td>
<td>43</td>
</tr>
<tr>
<td>3.4.5</td>
<td>Role of the forest floor in P cycling</td>
<td>44</td>
</tr>
<tr>
<td>3.5</td>
<td>Conclusions</td>
<td>45</td>
</tr>
</tbody>
</table>

**Chapter 4**

LONG-TERM EFFECTS OF AFFORESTATION ON SOIL SULFUR DYNAMICS AND AVAILABILITY 46

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Introduction</td>
<td>46</td>
</tr>
<tr>
<td>4.2</td>
<td>Materials and Methods</td>
<td>47</td>
</tr>
<tr>
<td>4.2.1</td>
<td>Site</td>
<td>47</td>
</tr>
<tr>
<td>4.2.2</td>
<td>Soil analyses</td>
<td>47</td>
</tr>
<tr>
<td>4.2.3</td>
<td>Statistical analysis</td>
<td>47</td>
</tr>
<tr>
<td>4.3</td>
<td>Results</td>
<td>48</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Sulfur fractions</td>
<td>48</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Microbial biomass S</td>
<td>48</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Soil arylsulfatase activity</td>
<td>49</td>
</tr>
<tr>
<td>4.4</td>
<td>Discussion</td>
<td>51</td>
</tr>
<tr>
<td>4.5</td>
<td>Conclusions</td>
<td>54</td>
</tr>
</tbody>
</table>

**Chapter 5**

SEASONAL CHANGES IN SOIL PHOSPHORUS AND ASSOCIATED BIOLOGICAL PROPERTIES UNDER ADJACENT GRASSLAND AND FOREST 55

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Introduction</td>
<td>55</td>
</tr>
</tbody>
</table>
5.2 Materials and Methods
5.2.1 Research site
5.2.2 Sampling
5.2.3 Soil analysis
5.2.4 Rainfall and soil temperature
5.2.5 Statistical analysis

5.3 Results
5.3.1 Rainfall, soil moisture and temperature
5.3.2 Soil pH, C, N and total P
5.3.3 Labile P, inorganic and organic P
5.3.4 Soil microbial biomass and activity
5.3.5 Phosphatase enzyme activities
5.3.6 Relationships between soil properties and environmental factors

5.4 Discussion
5.4.1 Seasonal comparisons of soil characteristics
5.4.2 Seasonal fluctuation in soil P fractions and soil P recycling
5.4.3 Seasonal fluctuation in microbial biomass and activity and contribution to P recycling
5.4.4 Role of the forest floor in P recycling in the forest ecosystem

5.5 Conclusions

Chapter 6 EFFECTS OF PLANT SPECIES ON SOIL PHOSPHORUS DYNAMICS AND ASSOCIATED BIOLOGICAL PROPERTIES

6.1 Introduction
6.2 Materials and Methods
6.2.1 Soils
6.2.2 Glasshouse experiment
6.2.3 Soil and plant analyses
6.2.4 Statistical analysis

6.3 Results
6.3.1 Plant growth and P uptake
6.3.2 Soil pH and total organic C and total N
6.3.3 Chemically extractable P pools
6.3.4 Isotopically exchangeable soil P
6.3.5 Microbial biomass and activity
6.3.6 Soil and root phosphatase activity

6.4 Discussion
6.4.1 Plant P uptake in different soils
6.4.2 Effects of plant species on soil P availability and redistribution
6.4.3 Effects of plant species on soil biochemical and biological properties
6.4.4 Chemical, biochemical and biological processes involved in soil P transformations under different plant species

6.5 Conclusions

Chapter 7 PHOSPHORUS DYNAMICS IN THE RHIZOSPHERE OF RYEGRASS AND RADIATA PINE

7.1 Introduction
7.1.1 The rhizosphere
7.1.2 Impacts of root-induced changes on rhizosphere P dynamics
7.1.3 Rhizosphere study technique

7.2 Materials and Methods
7.2.1 Soil sample and plant species
7.2.2 Glasshouse experiment
7.2.3 Soil and plant analysis
7.2.4 Statistical analysis

7.3 Results
7.4 Discussion
7.5 Conclusions

Chapter 8 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

8.1 Summary
8.2 Conclusions
8.3 Future work

REFERENCES
LIST OF TABLES

2.1 Selected New Zealand grassland soils: location, classification, parent material and texture. 16

3.1 Root biomass determined in adjacent soils under grassland and forest. 29
3.2 Chemical and physical properties determined for adjacent soils under grassland and forest. 30
3.3 Resin (Lewatit MP500A) extractable P (µg g\(^{-1}\)) determined for adjacent soils under grassland and forest. 31
3.4 Phosphorus fractions (µg g\(^{-1}\)) determined for adjacent soils under grassland and forest. 32
3.5 Microbial biomass C and P contents, respiration and metabolic quotient determined for adjacent soils under grassland and forest. 34
4.1 Organic C, total S and water soluble organic C determined for adjacent soils under grassland and forest. 49
4.2 Sulfur fractions and microbial biomass S and C concentrations determined for adjacent soils under grassland and forest. 50
5.1 F ratios for two-way analyses of variance of seasonal data on chemical, biochemical and biological properties determined for adjacent soils under grassland and forest over the period July 1998 to July 1999 at the Craigieburn site. 65
5.2 F ratios for two-way analyses of variance of seasonal data on chemical, biochemical and biological properties determined for forest floor materials (F, L layers) over the period July 1998 to July 1999 at the Craigieburn site. 67
5.3 Range and mean values for chemical properties determined for adjacent soils under grassland and forest and forest floor materials (F, L layers) over the period July 1998 to July 1999 at the Craigieburn site. 68
5.4 Range and mean values for biochemical and biological properties determined in adjacent soils under grassland and forest and forest floor materials (F, L layers) over the period July 1998 to July 1999 at the Craigieburn site. 73
5.5 Correlation coefficients (r) of monthly average soil temperature, rainfall and soil moisture content and selected other soil properties determined for grassland and forest soils and forest floor materials (F, L layers) over the period July 1998 to July 1999 at the Craigieburn site. 79
5.6 Calculated seasonal turnover of soil P through the microbial biomass (0-5 cm) under grassland and forest for the period July 1998 to July 1999 at the Craigieburn site.

6.1 Chemical and physical properties determined for the selected New Zealand grassland soils before planting.

6.2 Isotopic exchange kinetics parameters and Pi pools (µg g⁻¹) determined for the selected New Zealand soils before planting.

6.3 Root, shoot and total biomass (dry weight, g pot⁻¹), and shoot: root ratios (S:R) of ryegrass and radiata pine determined after a 40-week period of growth.

6.4 Phosphorus uptake and P use efficiency by ryegrass and radiata pine determined after a 40-week period of growth.

6.5 Selected soil chemical properties determined after a 40-week period of growth under ryegrass and radiata pine.

6.6 Bicarbonate extractable P (BPi and BPo) (µg g⁻¹) and resin extractable P (Resin Pi, Po) (µg g⁻¹) determined in soils after a 40-week period of growth under ryegrass and radiata pine.

6.7 Concentrations of soil inorganic, organic and total P (µg g⁻¹) determined after a 40-week period of growth under ryegrass and radiata pine.

6.8 Isotopic exchange kinetics parameters (Cp, R/r₁, n) determined in soils after a 40-week period of growth under ryegrass and radiata pine.

6.9 Isotopically exchangeable Pi pools (µg g⁻¹) determined in soils after a 40-week period of growth under ryegrass and radiata pine.

6.10 Concentrations of E₁₅min-3m Pi (sum of E₁₅min Pi, E₁₅min-24h Pi and E₂₄h-3m Pi) determined in original soils and soils after a 40-week period of growth under ryegrass and radiata pine.

6.11 F ratios for two-way analyses of variance of data for various microbial biomass and phosphatase enzyme activities determined in soils after a 40-week period of growth under ryegrass and radiata pine (values are the means for all 15 soils).

6.12 Microbial biomass and activity determined in soils after a 40-week period of growth under ryegrass and radiata pine.

6.13 Phosphatase activities determined in soils after a 40-week period of growth under ryegrass and radiata pine.

7.1 Dry matter yield, root surface area and P contents determined for ryegrass and radiata pine after 60 days.
7.2 F ratios for two-way analyses of variance of data for pH and P fractions of rhizosphere Fork and Hurunui soils under ryegrass and radiata pine determined after 60 days.

7.3 F ratios for two-way analyses of variance of data for biochemical and biological properties of rhizosphere Fork and Hurunui soils under ryegrass and radiata pine determined after 60 days.
LIST OF FIGURES

1.1 Annual plantation of exotic forests (A) and total planted forest area in New Zealand. 2
1.2 Soil phosphorus cycle. 6
1.3 Hypothetical model of processes involved in nutrient dynamics following land-use changes from grassland to forest. 9
1.4 Flow diagram of research programme and thesis structure. 13
2.1 Field research site at the Craigieburn. 15
2.2 Soil P fractionation scheme. 20
2.3 Effects of buffer pH on phosphomonoesterase activity in soil and forest floor material samples from the field trial at the Craigieburn site. 24
3.1 Evolution of CO$_2$-C from adjacent grassland and forest soils. 35
3.2 Evolution of CO$_2$-C from forest floor materials. 36
3.3 Phosphomonoesterase activity determined for soils and forest floor materials. 37
3.4 Phosphodiesterase activity determined for soil and forest floor materials. 37
4.1 Arylsulfatase activity determined for soils and forest floor material under adjacent grassland and forest. 52
5.1 Distribution of daily rainfall (A) and soil (0-5 cm) and forest floor material moisture content (B and C) over the period July 1998 to July 1999 at the Craigieburn site. 61
5.2 Seasonal changes in soil (A) and forest floor (B) temperature over the period July 1998 to July 1999 at the Craigieburn site. 62
5.3 Changes in the pH of soils (A) and forest floor materials (B) determined over the period July 1998 to July 1999 at the Craigieburn site. 63
5.4 Changes in water soluble organic C (WSOC) determined for soils (A) and forest floor materials (B) over the period July 1998 to July 1999 at the Craigieburn site. 63
5.5 Changes in total organic C (TOC), total N, total P, C:N and C:Po ratios (C:P for the forest floor material) determined for soils (left) and forest floor materials (right) over the period July 1998 to July 1999 at the Craigieburn site. 64
5.6 Changes in water soluble Pi (WSPi) determined for soils (A) and forest floor materials (B) over the period July 1998 to July 1999 at the Craigieburn site. 69
5.7 Changes in bicarbonate extractable P (Pi, Po), resin extractable P (Pi, Po) determined for soils (left) and forest floor materials (right) over the period July 1998 to July 1999 at the Craigieburn site.

5.8 Changes in total Pi (A) and Po (B) determined for soils under grassland and forest over the period July 1998 to July 1999 at the Craigieburn site.

5.9 Changes in microbial biomass C and P determined for soils (left) and forest floor materials (right) over the period July 1998 to July 1999 at the Craigieburn site.

5.10 Changes in microbial C:P, microbial biomass C: total organic C (MBC:TOC) and microbial biomass P: total P (MBP:total P) ratios determined for soils (left) and forest floor materials (right) over the period July 1998 to July 1999 at the Craigieburn site.

5.11 Seasonal dynamics of respiration determined for soils (A) and forest floor materials (B) over the period July 1998 to July 1999 at the Craigieburn site.

5.12 Seasonal dynamics of in situ carbon dioxide (CO2) efflux determined for soils and forest floor materials over the period July 1998 to July 1999 at the Craigieburn site.

5.13 Seasonal changes in phosphomonoesterase (PME) and phosphodiesterase (PDE) activities determined for soils (left) and forest floor materials (right) over the period July 1998 to July 1999 at the Craigieburn site.

5.14 Relationships between CO2 respiration and moisture content determined for soils (A) and forest floor materials (B, C) over the period July 1998 to July 1999 at the Craigieburn site.

5.15 Relationships determined between in situ CO2 efflux and soil moisture over the period July 1998 to July 1999 at the Craigieburn site.

5.16 Relationships determined between CO2 respiration and the temperature of soils (A) and forest floor materials (B, C) over the period July 1998 to July 1999 at the Craigieburn site.

5.17 Relationships determined between in situ CO2 efflux and soil temperature over the period July 1998 to July 1999 at the Craigieburn site.

5.18 Relationships determined between bicarbonate extractable Po (BPo) in forest floor materials (F, L layers) and the underlying soil for samples collected from the Craigieburn site between July 1998 and July 1999.

5.19 Seasonal P dynamics in soils under grassland and forest at the Craigieburn site.
6.1 Effects of buffer pH on root phosphatase activities determined under ryegrass and radiata pine. 105
6.2 Specific mineralization rate (SMR) determined in soils after a 40-week period of growth under ryegrass and radiata pine. 115
6.3 Root phosphatase activities of ryegrass and radiata pine after a 40-week period of growth. 124
6.4 Relationship between the levels of $E_{1\min Pi}$ in the original soils and ratios of P uptake by radiata pine to ryegrass determined after a 40-week period of growth. 126
6.5 Relationships determined between specific mineralization rate (SMR) and P uptake by ryegrass (left) and radiata pine (right) after a 40-week period of growth. 128
6.6 Relationships determined between root phosphatase activity and P uptake of ryegrass (left) and radiata pine (right) after a 40-week period of growth. 129
6.7 Relationships determined between root weight of ryegrass and radiata pine and water soluble organic C (WSOC). 132
6.8 Relationships determined between the specific mineralization rate of organic P (SMR) and the turnover rate of organic C in soils under ryegrass and radiata pine after a 40-week period of growth. 138
7.1 Schematic representation of the plant growth container (PGC) and irrigation system. 144
7.2 Rotary microtome and custom adaptor. 145
7.3 Effects of plant species on pH determined in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days. 148
7.4 Effects of plant species on inorganic P fractions determined in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days. 151
7.5 Effects of plant species in organic P and residual P fractions determined in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days. 153
7.6 Effects of plants species on total extractable Pi (TEPi), Po (TEPo) and total P determined in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days. 154
7.7 Effects of plant species on microbial biomass C (MBC) and water soluble organic C (WSOC) determined in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days. 156
7.8 Effects of plant species on acid phosphatase (AcPME), alkaline phosphatase (AlPME) and phosphodiesterase (PDE) activities determined in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days.

7.9 Relationships determined between microbial biomass C (MBC) and water soluble organic C (WSOC), and between MBC and acid phosphatase (AcPME), alkaline phosphatase (AlPME) and phosphodiesterase (PDE) activities in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days.

7.10 Relationships determined between phosphatase activities and concentrations of sodium hydroxide extractable organic P (NPo) in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days.

7.11 Relationships determined between water soluble organic C (WSOC) and microbial biomass C (MBC) and concentrations of NPo in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days.
Chapter 1

Introduction

1.1 Plantation Forestry in New Zealand

It is estimated that around 75% of New Zealand was covered by native forests until about 1200 years ago (Wards, 1973). Loss of these native forests started following Polynesian colonization (Davidson, 1984) and was accelerated by organized European settlement of New Zealand, especially during the period of 1840 to 1900 (Molloy, 1980). The area of native forests is currently 6.4 million ha, covering approximately 23% of New Zealand (Statistics New Zealand, 1996).

Large-scale development of exotic forests was brought about by government realization that indigenous forest timber resources were decreasing and that there was a need to provide for future domestic timber markets. The New Zealand government started forest plantation efforts in 1898, but it was not until the State Forest Act was passed in 1921-1922 that significant areas of land were planted with exotic trees (Poole, 1969). The State Forest Service formulated plans for an expansion in planting on land which was difficult or uneconomic to develop for agriculture. The planting boom of 1925-1936 resulted in 172,000 ha of predominantly radiata pine (*Pinus radiata*) being planted in the central North Island. However, between 1936 and 1960 only 30,500 ha of new plantation forest was established. The annual new planting rate increased from 6,000 ha in 1960 to 43,000 ha in 1973 (Figure 1-1A). The impetus for this increase in new plantings was the result of government policy to provide enough timber for the domestic market and develop export markets. For 1973 to 1985, the rate of new annual planting fluctuated between 38,000 and 56,000 ha, divided equally between the state and private sectors. Total planted forest area reached 1,131,000 ha in 1985 (Figure 1-1B). The sharp decrease in annual new forest plantation which occurred between 1985 and 1991 (Figure 1-1A) was brought about by a combination of government reorganization of state forestry and the fact that forest companies delayed new planting while preparing bids for cutting rights to state-owned plantations (Levack, 1990). However, new forest establishment increased again over the
Figure 1-1 Annual plantation of exotic forests (A) and total planted forest area (B) in New Zealand (Ministry of Agriculture and Forestry of New Zealand, 1999).
period 1992 – 1998 at 50,000 - 98,000 ha per annum (New Zealand Ministry of Agriculture and Forestry, 1999), stimulated by a combination of factors including expected high returns from forestry and declining returns from hill and high country pastoral farming (Maclaren, 1996). The current expansion in plantation forestry is expected to continue for the next 10 years (Glass, 1997). At present, plantation forestry covers 1.679 million ha (Ministry of Agriculture and Forestry, 1999) (Figure 1-1B), which represents 7% of New Zealand total land area of 27 million ha. The area under plantation forestry represent 11% of land useable for agriculture and forestry (15.5 million ha). Assuming an annual rate of new forest establishment of 50,000 ha, the area under plantation forest in New Zealand in 2010 will be 2.23 million ha (14% of useable land area). Radiata pine represents over 90% of the total exotic forest plantation while the remainder includes Douglas fir (*Pseudotsuga menzieii*), eucalypts (*Eucalyptus spp.*) and cypresses (e.g. *Cupressus macrocarpa*). The conversion of land use from pasture to forest (i.e. afforestation) is likely to significantly alter soil chemical, physical and biological properties and may have important implications for long-term sustainable land management. This will influence aspects of soil and environmental quality, which in turn may affect access and price for timber products in export markets (especially in North America and Europe).

### 1.2 Impacts of Afforestation on Soil Properties

#### 1.2.1 Soil nutrient availability

It has been reported that conifers increase mineralization of soil organic matter and thus the bioavailability of associated nutrients. For example, Fisher and Stone (1969) revealed higher availability of nitrogen (N) and phosphorus (P) in the root zone of conifers in New York State, USA. In Canada, conversion from grassland to forest also caused the decrease in organic matter and soil moisture (Anderson, 1987). In New Zealand, Davis and Lang (1991) and Davis (1994) investigated soils under conifers and adjacent tussock grassland and found that levels of mineralizable N, Olsen and Bray-2 extractable P and sulfate-sulfur (S) in surface soils were higher under conifers than under grassland, but levels of organic carbon (C) and P were lower. Belton et al. (1995) reported that levels of labile P (bicarbonate extractable) in topsoils under conifers in the Canterbury high country across a wide precipitation range were 2 to 4 fold greater compared with adjacent unimproved grassland soils. Davis (1995) conducted a pot trial which involved growing radiata pine and cocksfoot (*Dactylis*...
glomerata) for one year in grassland soils collected from eight sites in the South Island montane zone and found that concentrations of plant available N, P and S levels were substantially higher under pine compared with cocksfoot despite greater nutrient uptake by pine. Similar results were also reported from other studies carried out in New Zealand by Hawke and O'Connor (1993), Condron et al. (1996) and Alfredsson et al. (1998).

1.2.2 Soil microbial properties
Different types of land-use systems can also significantly affect soil microbial properties. Sparling et al. (1994) compared the 0-20 cm soils under long-term indigenous native forest (Nothofagus truncata), exotic forest (Pinus radiata) and grass/clover pasture and found concentrations of microbial biomass C and N were highest in soil under pasture. Yeates et al. (1997) also reported the concentrations of total and microbial biomass C and N, the microbial C:N ratio, total nematode numbers and most nematode functional groups and diversity in topsoils were lower under radiata pine compared with adjacent grassland at 11 sites. Similar results were also reported by Yeates and Saggar (1998) and Ross et al. (1999). Reduction in total C, N and P pools and microbial biomass C and N in soil under radiata pine compared with adjacent grassland soil have been regarded as indicating a decline in biological fertility and soil quality (Yeates et al, 1997).

1.2.3 Other soil properties
A number of studies have shown that conifers may increase the acidity of soils (Davis and Lang, 1991; Davis, 1994; 1995; Binkley, 1995; Giddens et al., 1997; Alfredsson et al., 1998), reduce the number of earthworms and other fauna and increase the risk of compaction of soil during site preparation and harvesting (Maclaren, 1996). The reduction in soil pH under forest may be associated with uptake of excess cations over anions by coniferous trees (Giddens et al., 1997).

1.2.4 Possible mechanisms for the elevated levels of available inorganic nutrients in soil under forest
The studies cited above indicated that the mineralization of soil organic matter was a major mechanism contributing to increased availability of nutrients (N, P, S) in topsoils under forest, since this was usually accompanied by a decrease in organic N, P and S (Davis and Lang, 1991; Davis, 1995; Condron et al., 1996). However, the lower
microbial biomass and activity found in soils under conifers is inconsistent with the enhanced mineralization of organic matter compared with adjacent grassland (Yeates et al., 1997).

Some other mechanisms have also been proposed. For example, nutrient pumping from deeper horizons to the topsoil via nutrient uptake by roots and subsequent litterfall may also be important in some cases (Fisher, 1990; Davis and Lang, 1991). Other possible mechanisms include transfer of nutrients from surrounding areas by roosting birds, domestic stock and/or feral animals, aeolian deposition, and release of nutrients from understory vegetation after suppression at the time of canopy closure (Davis, 1995; Davis, 1998). In addition, organic acids such as citric, oxalic, maleic, and acetic acids secreted by tree roots and associated microorganisms (including mycorrhizas) may increase the availability of sparingly soluble P minerals by a combination of lower pH and/or metal chelation (Comerford and Skinner, 1989; Fox et al., 1990a; Grierson, 1992; Jones and Darrah, 1994).

1.3 Soil Organic Nutrient Dynamics in Terrestrial Ecosystems

Organic forms of N, P and S make up >95%, 5-95% (usually >50%) and 80-99% (usually >90%) of total N, P and S in surface soils, respectively (Stevenson, 1985; Mitchell et al., 1992). Significant amounts of N, P and S are contained in plant, animal and microbial residues returned to soil, and the turnover and availability of organic forms of N, P and S are important factors in the long-term maintenance of soil fertility and productivity (Magid et al., 1996; Zhao et al., 1996).

Soil P dynamics in terrestrial ecosystems has been comprehensively reviewed by Dalal (1977), Stewart and McKercher (1982), Stewart and Tiessen (1987), Sanyal and De Datta (1991), Richardson (1994), Magid et al. (1996) and Frossard et al. (2000). Figure 1-2 shows the major chemical, physical and biological pools and processes in soil P dynamics. Physiochemical processes such as adsorption – desorption and dissolution – precipitation control the abiotic transfer between the solid phase and soil solution. Biological mineralization (decomposition) and immobilization govern the transformation of P between inorganic and organic forms (predominantly ester P). The major processes involved in the dynamics of soil organic S are similar to those for organic P (Zhao et al., 1996).
Figure 1-2 Soil phosphorus cycle.
The mineralization of organic nutrients in soil is primarily mediated by microbial processes. The microbial biomass makes up 2% - 3% of the total organic C in the soil, and represents the labile fraction in soil organic matter and associated nutrients (Sanyal and De Datta, 1991). Microbial biomass P and S comprise approximately 3 - 20% and < 0.1% - 1.0% of total soil organic P and S, respectively (Stevenson, 1985; Zhao et al., 1996). The mineralization of organic P and S are believed to involve both 'biochemical' and 'biological' processes. Organic ester bound P and S (C-O-P, C-O-S) which are stabilized independently of the main moiety of organic matter may be biochemically mineralized by extracellular enzymes (phosphatases and sulphatases), whereas the organic S (and P) stabilized through direct association with C is mineralized as a result of biological C mineralization which in turn is related to energy demand (McGill and Cole, 1981). It has been argued that these two pathways are closely related and cannot be separated completely (Magid et al., 1996; Zhao et al., 1996).

Many studies on the mineralization of organic nutrients associated with extracellular enzymes have been reported. These processes mainly occur in the rhizosphere which is the area 0-1500 µm from the root surface (Foster et al., 1983), and regarded as the most chemically, biochemically and biologically active region in soils (Martin, 1983; Rovira, 1991). Extracellular phosphatases produced by mycorrhizal fungi, bacteria and plant roots are considered to play a role in the mineralization of organic P by hydrolyzing organic P (Eivazi and Tabatabai, 1977). Depletion of organic P by rape (Brassica oleracea), onion (Allium cepa), wheat (Triticum aestivum) and clover (Trifolium alexandrinum) have been positively correlated with soil phosphatase activity (Tarafdar and Jungk, 1987). Häussling and Marschner (1989) also revealed that in 80-year-old Norway spruce (Picea abies) phosphatase activity was correlated with the length of mycelial hyphae of ectomycorrhizae, and reduced levels of organic P in rhizoplane soil were associated with an increase in phosphatase activity compared with the bulk soil. It has been reported that barley (Hordeum vulgare) genotypes differ in the activity of soluble extracellular phosphatase and the depletion of organic P in the rhizosphere soil and a negative correlation was found between the activity of soluble extracellular phosphatase and the quantity of labile organic P (bicarbonate extractable) (Asmar et al., 1995). However, a number of studies showed no direct correlation
between soil phosphatase activity and the depletion of organic P (e.g. Speir and Ross, 1978; Joner et al., 1995; McDowell et al., 1996).

Sulphatases are involved in the mineralization of soil organic S and their activity also depends on their origin (plant root and microorganisms) and the nature of organic S (Ganeshamurthy and Nielsen, 1990; Zhao et al., 1996). However, many authors have reported no significant correlation between the mineralization of organic S and arylsulphatase activity (e.g. Tabatabai and Bremner, 1970: Zhao et al., 1996).

The lack of correlation between enzyme activities (phosphatase and sulphatase) and the mineralization of organic P (and S) has been attributed to a combination of factors including the stability and availability of substrates in soils (Ganeshamurthy and Nielsen, 1990), the artificial substrates used in enzyme assays (Fitzgerald et al., 1985; Leake, 2001), and the possible involvement of other enzymes (Zhao et al., 1996).

1.4 Research Program

1.4.1 Hypothesis

It is hypothesized that on conversion of grassland to forest differences in vegetation, plant nutrient uptake, root distribution and specific interactions between plant species and soil can significantly change the quantity and quality (composition) of soil organic matter and associated nutrients. Figure 1-3 outlines hypothetical changes in major variables of the ecosystem after conversion from grassland to forest. These changes are expected to have strong impacts on the physical, chemical, biochemical and biological processes that govern the transformation of nutrients in soil.

Carbon input can differ significantly between the grassland ecosystem and the forest ecosystem. It has been suggested that residual organic C in the mineral soil decreases at the early stage of forest stand development due to little production of litterfall and root debris (Parfitt et al., 1997) and continuous decomposition of the dense fibrous roots associated with the grass/clover (Davis, 1995). Condron and Newman (1998) found more recalcitrant forms of organic C in soil under forest compared with grassland by using solid state $^{13}$C NMR spectroscopy, reflecting the reduced inputs of fresh organic matter to the soil under forest. Differences in the quantity and quality of
Figure 1-3 Hypothetical model of processes involved in nutrient dynamics following land-use change from grassland to forest.
organic C will change the community of microorganisms and their activities, which in turn will affect the biochemical and biological transformation of organic nutrients in soil.

Root distribution, characteristics and dynamics play an important role in the mineralization of organic nutrients in soil (Schubert and Mengel, 1989; Vogt et al., 1991b). Helal and Sauerbeck (1989) showed that 19% of the total photosynthetic production of maize (*Zea mays*) plants was released into the rhizosphere as organic material through roots. Root exudation stimulated the growth of microorganisms in the rhizosphere, which in turn affects the availability of organic nutrients (Richards, 1987; Cheng and Coleman, 1991; Marschner, 1991). Organic acids secreted by roots acidify the rhizosphere and promote the solubilization of insoluble phosphates (Hoffland et al., 1989) or compete with phosphate esters for adsorption sites (Lopez-Hernandez et al., 1986; Bar-Yosef, 1996; Hinsinger, 2000) and thus enhance P availability. These effects of root exudation have yet to be quantified adequately. In addition, Perrott et al. (1999) reported that fine roots in soil under grassland can maintain the higher proportion of soil aggregates >0.5 mm and thus provided more physical protection of organic matter compared with forest.

Moreover, conversion from grassland to forest may alter the soil environment (temperature, moisture etc.) through reduction in radiation and evaporation, particularly after closure of the tree canopy and accumulation of a litter layer.

Ectomycorrhizas may play a key role in the utilization of organic P as described earlier. It has been postulated that the increased nutrient availability in soil under forest compared with grassland may be attributed to differences in microorganisms associated with the rhizosphere of conifers and pasture (Häussling and Marschner, 1989; Pasqualini et al., 1992; Condron et al., 1996). However, plant root-ectomycorrhizas-rhizosphere associations are very complex and may be system specific. Further, detailed investigation of interrelationships between these components are required to determine and interpret the rhizosphere processes which control the availability of soil organic nutrients.
1.4.2 Objectives

A number of studies have been carried out to investigate the impacts of afforestation on soil properties in the past decade, but there are some limitations to these studies and a number of questions remain. First of all, most ‘paired-site’ studies to date have focused on topsoil (≤ 10 cm depth) (Davis and Lang, 1991; Davis, 1994; Giddens et al., 1997; Yeates and Saggar, 1998; Perrott et al., 1999) and comparisons between soils under grassland and forest from these studies were made at only one point in time (Davis and Lang, 1991; Davis, 1994; Giddens et al., 1997; Yeates et al., 1997; Alfredsson et al., 1998). Secondly, in many of these studies, chemical fertilizer (e.g. superphosphate) was applied to the grassland soils which were grazed. This will influence the distribution and cycling of organic matter and associated nutrients in soil and consequently make it difficult to differentiate the effect of the fertilizer P from P released from the mineralization of organic P on the levels of soil available inorganic P. Moreover, although some work has been done on the effects of afforestation on biological properties, determination of chemical properties has been the major focus of most of these studies (Yeates et al., 1997; Yeates and Saggar, 1998). The biochemical and biological processes involved in the dynamics of organic matter and associated nutrients following plantation of conifers in soils developed under improved and unimproved grassland are inadequately understood.

There is a need to conduct further detailed studies on the long-term and seasonal effects of afforestation on the availability and dynamics of organic matter and associated nutrients, and to examine specific biochemical and biological processes involved in the mineralization of organic nutrients in soils under grassland and conifers.

The major focus of this study was on the dynamics of soil P although the dynamics of soil S was also considered. The structure of this project in the thesis is outlined in Figure 1-4 and the main objectives of the proposed research are:

i) to quantify the effects of conifers on the long-term (one rotation) and seasonal dynamics of P and S in soils developed under unimproved pasture (field studies – Chapters 3, 4 and 5);

ii) to examine the detailed effects of ryegrass and radiata pine on P dynamics in grassland soils (glasshouse experiment – Chapter 6);
iii) to investigate P dynamics in the rhizosphere of ryegrass and radiata pine (glasshouse experiment – Chapter 7).
Figure 1-4. Flow diagram of research programme and thesis structure.
Chapter 2

Materials and Methods

2.1 Materials

2.1.1 Field study site

The field study site was located on a flat terrace at Cave Stream within the Craigieburn Research Area, central South Island, New Zealand (43° 10' S, 171° 20' E) (Figure 2-1).

The soil is a Craigieburn silt loam, an Allophanic Brown Soil (Dystrochrept: USDA Soil Taxonomy) formed from greywacke sandstone loess overlying gravelly alluvium. Altitude at the site is 800 m, annual rainfall is 1400 mm, and the mean annual temperature is 8 °C (July 2 °C; January 13.9 °C). The original vegetation was mountain beech (*Nothofagus solandri var cliffortioides*) which was cleared and burnt over 100 years ago. Grassland consisted of a mixture of native and exotic grasses (e.g. short tussock (*Festuca novae-zealandiae*) and browntop (*Agrostis capillaris*)) which were lightly grazed. A mixed stand of Ponderosa pine (*Pinus ponderosa*) and Corsican pine (*Pinus nigra*) was established in 1979 at a density of 2200 stems ha⁻¹ (total stand area: 400 m²). The stand was 19 years old and the canopy was closed at the time of sampling. The forest stand had not been thinned, the forest and adjacent grassland area had never received fertilizer, and the grassland had not been grazed since forest establishment and has been invaded by native shrubs (*Cassinia, Hebe, Leptospermum* and *Dracophyllum* spp) since closure. The selected area under forest (20 m x 20 m) was divided into 4 subplots; corresponding subplots of the same size were also located within the adjacent grassland area, approximately 20 m from the edge of the forest.

The litter (L) and fermentation (F) layers under forest were 2-5 cm and 2-3 cm thick, respectively, but the humic layer (H) was not clearly distinguished. Studies on the long-term effect of afforestation on the dynamics of soil phosphorus (P) (Chapter 3) and sulfur (S) (Chapter 4) and on seasonal changes in soil P under grassland and forest (Chapter 5) were carried out at this field site.
2.1.2 Glasshouse experiment soils

Fifteen topsoils (0 – 7.5 cm: depth used for assessing the fertility status of soil under grassland in New Zealand) that were originally developed under native vegetation (mainly forest) prior to European settlement of New Zealand and were subsequently redeveloped under improved and unimproved grassland were collected from around New Zealand. These included soils from the North Island (Egmont, Stratford, Mangamahu, Himatangi, Taupo, Oruanui, Patoka and Te Kauwhata) and South Island (Pukaki, Fork, Hurunui, Richmond, Temuka, Mapua and Okarito) and covered six major soil groups (New Zealand Soil Classification) (Hewitt, 1998). The origin, classification, parent material and texture of the soils are shown in Table 2-1.

All fifteen soils were used in the glasshouse experiment to determine the effects of plant species on soil P dynamics and biological properties (Chapter 6), while the Fork and Hurunui soils were used in the study on rhizosphere P dynamics (Chapter 7). All soil samples were air-dried and sieved < 4 mm prior to use in glasshouse experiments (Chapter 6), while < 1 mm sieved soils were used in the rhizosphere P dynamics experiment (Chapter 7).
Table 2-1  Selected New Zealand grassland soils: location, classification, parent material and texture.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Location</th>
<th>NZ Genetic Soil Classification</th>
<th>US Soil Taxonomy</th>
<th>Parent material</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(district)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Egmont</td>
<td>Wanganui</td>
<td>Orthic Allophanic</td>
<td>Udand</td>
<td>andesitic tephra</td>
<td>loam</td>
</tr>
<tr>
<td>2 Stratford</td>
<td>Taranaki</td>
<td>Orthic Allophanic</td>
<td>Udand</td>
<td>andesitic tephra</td>
<td>sandy loam</td>
</tr>
<tr>
<td>3 Mangamahu</td>
<td>Wairarapa</td>
<td>Firm Brown</td>
<td>Dystrochrept</td>
<td>siltstone</td>
<td>loam</td>
</tr>
<tr>
<td>4 Himatangi</td>
<td>Manawatu</td>
<td>Orthic Brown</td>
<td>Ustochrept</td>
<td>coastal sand</td>
<td>loamy sand</td>
</tr>
<tr>
<td>5 Taupo</td>
<td>Taupo</td>
<td>Orthic Pumice</td>
<td>Vitrand</td>
<td>rhyolitic tephra</td>
<td>loamy sand</td>
</tr>
<tr>
<td>6 Oruanui</td>
<td>Rotorua</td>
<td>Orthic Pumice</td>
<td>Vitrand</td>
<td>rhyolitic tephra</td>
<td>sandy loam</td>
</tr>
<tr>
<td>7 Pukaki</td>
<td>Mackenzie Basin</td>
<td>Allophanic Brown</td>
<td>Dystrochrept</td>
<td>greywacke alluvium</td>
<td>sandy loam</td>
</tr>
<tr>
<td>8 Fork</td>
<td>Mackenzie Basin</td>
<td>Allophanic Brown</td>
<td>Dystrochrept</td>
<td>greywacke alluvium</td>
<td>sandy loam</td>
</tr>
<tr>
<td>9 Hurunui</td>
<td>Canterbury</td>
<td>Orthic Brown</td>
<td>Dystrochrept</td>
<td>greywacke alluvium</td>
<td>loam</td>
</tr>
<tr>
<td>10 Richmond</td>
<td>Nelson</td>
<td>Firm Brown</td>
<td>Dystrochrept</td>
<td>greywacke/schist alluvium</td>
<td>clay loam</td>
</tr>
<tr>
<td>11 Temuka</td>
<td>Canterbury</td>
<td>Acid Gley</td>
<td>Aquept</td>
<td>greywacke alluvium</td>
<td>loam</td>
</tr>
<tr>
<td>12 Patoka</td>
<td>Hawkes Bay</td>
<td>Orthic Allophanic</td>
<td>Udand</td>
<td>andesitic tephra</td>
<td>sandy loam</td>
</tr>
<tr>
<td>13 Te Kauwhata</td>
<td>Waikato</td>
<td>Oxidic Granular</td>
<td>Humult</td>
<td>greywacke/holocene tephra</td>
<td>loam</td>
</tr>
<tr>
<td>14 Mapua</td>
<td>Nelson</td>
<td>Orthic Brown</td>
<td>Dystrochrept</td>
<td>Pliocene- Pleistocene</td>
<td>loam</td>
</tr>
<tr>
<td>15 Okarito</td>
<td>Westland</td>
<td>Orthic Podzol</td>
<td>Orthod</td>
<td>glacial outwash</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>greywacke alluvium/loess</td>
<td>sandy loam</td>
</tr>
</tbody>
</table>

† Hewitt (1998).
2.2 Methods

2.2.1 Treatment of soil samples

*Air-dried soil samples*

A subsample of each soil (from both field and glasshouse experiments) was ground < 2 mm and a subsample of each soil was finely ground (< 150 µm) and stored at room temperature prior to analysis. Soil samples (< 2 mm) were used for all chemical and physical analyses, except for total C, N and S and P fractionation (< 150 µm).

*Moist soil samples*

Moist soil samples (from both field and glasshouse experiments) were sieved (< 2 mm) and stored at 4 °C prior to chemical, biochemical and biological analyses.

*Forest floor materials*

A subsample of forest floor materials from field experiments was oven-dried at 70 °C and finely ground for the analysis of total C, N, P and S. Another subsample of forest floor materials was cut into 2-4 mm pieces, and stored at 4 °C prior to biochemical and biological analyses.

2.2.2 Chemical and physical analyses

*Soil texture*

Soil particle size distribution was determined by a Malvern Mastersizer-S particle sizer after soil organic matter was oxidized by treatment with hydrogen peroxide and samples were dispersed using Calgon (mixture of sodium hexametaphosphate \(((\text{NaP}_3\text{O}_6)\)) and sodium carbonate \((\text{NaCO}_3)\)).

*Soil pH*<sub>(water)</sub>*

A 10 g sample of air-dried soil or moist soil was dispersed in 25 ml of deionized water, left overnight at 22 °C, and pH was read on a pH meter (PHM84, Radiometer Copenhagen) (Blakemore et al., 1987). The pH of forest floor material was measured using a solid: water ratio of 1:5.

*Total organic C, N and S*

Soil total organic C, N and S were determined using a LECO 2000 CNS analyzer at 1330 °C.
Water soluble organic C

Water soluble organic C was extracted at a solid: water ratio of 1:2.5 (soil) and 1:5 (forest floor material) in an end-to-end shaker for 1 hour, and filtered (Whatman No.42 filter paper followed by 0.45-μm membrane) (Huang and Schoenau, 1998). The concentration of soluble C in the extracts was determined using a Shimadzu TOC-5000A analyzer.

Extractable soil Fe and Al

Soil iron (Fe) and aluminum (Al) oxides were extracted with dithionite-citrate reagent and acid ammonium oxalate (Ross and Wang, 1993). For the dithionite-citrate method, 0.500 g of soil was extracted with 25 ml sodium citrate (Na3C6H5O7·2H2O)(200 g L⁻¹) and 0.4 g dithionite (Na2S2O4) overnight and then centrifuged for 20 minutes at 10,000 rpm and filtered through a No.42 Whatman filter paper. For the ammonium oxalate method, 0.25 g of soil was equilibrated with 10 ml of acid oxalate reagent (0.2 M ammonium oxalate ((NH4)2C2O4·H2O), 0.2 M oxalic acid (H2C2O4·2H2O), pH 3) in the dark on an end-to-end shaker for 4 hours and then centrifuged and filtered as described above. Concentrations of Fe and Al in the extracts were determined using a Shimadzu flame atomic absorption spectrophotometer (Shimadzu AA-6200).

Phosphorus sorption index (PSI)

Phosphorus sorption index was determined using the method described by Bache and Williams (1971). Duplicate 5 g air-dried soil samples (< 2 mm) were weighed into 250 ml plastic bottles, one receiving an addition of 100 ml of 0.02 M KCl and the other 100 ml of 75 mg P L⁻¹ (as KH2PO4) solution (in 0.02 M KCl), giving an addition of 150 mg P per 100 g soil. Two drops of chloroform were added to inhibit microbial activity and the bottles were shaken in an end-to-end shaker at 20 °C for 18 hours. The suspensions were filtered through a Whatman no.41 filter paper and the filtrates centrifuged at 5,000 rpm for 30 min. Phosphate remaining in the solution was determined by the method of Murphy and Riley (1962), and P sorbed was calculated by difference. Phosphate sorption index was expressed as X/log C, where X is the phosphate adsorbed (mg (100 g)⁻¹) and log C is the logarithm of the concentration of the remaining phosphate in equilibrium solution (μmol L⁻¹).
Soil P fractionation

Soil P fractionation schemes described by Hedley et al. (1982b, c) and Condron et al. (1996) were modified by including an analysis of the residual P (i.e. non-extracted P) using nitric acid (HNO₃)- perchloric acid (HClO₄) digestion (Olsen and Sommers, 1982). This scheme is shown in Figure 2-2 and involved the determination of various forms of inorganic (Pi) and organic (Po) soil P by sequential extraction of finely ground (< 150 µm) soil with 1 M ammonium chloride (NH₄Cl) (APi), 0.5 M sodium bicarbonate (NaHCO₃) (pH 8.5) (BPi, BPo), 0.1 M sodium hydroxide (NaOH) (N(I)Pi, N(I)Po), 1 M hydrochloric acid (HCl) (HPi) and 0.1 M NaOH (N(II)Pi, N(II)Po). The concentration of inorganic P in the extracts was determined after the precipitation of organic matter by acidification, and the concentration of total P in the extracts was determined after persulfate oxidation, while the concentration of organic P in the extracts was calculated as the difference between total P and inorganic P (Ties sen and Moir, 1993). The fractions separated by the above procedure correspond to the following soil P pools:

- **NH₄Cl extractable Pi (APi)** – solution Pi;
- **NaHCO₃ extractable Pi (BPi)** – labile Pi, adsorbed on the surface of more crystalline P compounds, sesquioxides or carbonate;
- **First NaOH extractable Pi ((N(I)Pi)** – moderately labile Pi, associated with amorphous and some crystalline Al, Fe hydrous oxides;
- **HCl extractable Pi (HPi)** – less labile Pi, associated with primary calcium minerals (e.g.apatite).
- **Second NaOH extractable Pi ((N(II)Pi)** – moderately stable Pi, which adsorbed into the mineral structure of soil components, or occluded by Fe and Al coatings.
- **NaHCO₃ extractable Po (BPo)** – labile Po, easily mineralizable.
- **First NaOH extractable Po ((N(I)Po)** – moderately labile Po, associated with Fe and Al hydrous oxides;
- **Second NaOH extractable Po ((N(II)Po)** – stable Po, adsorbed into the mineral structure of soil components, or occluded by Fe and Al coatings.
- **Residual P** – non-extracted/ recalcitrant Pi and Po.
1,000 g air-dried soil → Add 30 ml 1 M NH₄Cl, shake for 16 h, then centrifuge for 10 min at 10,000 RPM → Solution-P (AP)

Residue → Add 30 ml 0.5 M NaHCO₃, shake for 16 h, then centrifuge for 10 min at 10,000 RPM → BPi & BPo

Residue → Add 30 ml 0.1 M NaOH, shake for 16 h, then centrifuge for 10 min at 10,000 RPM → N(I)Pi & N(I)Po

Water wash (add 30 ml H₂O, shake for 4 h and centrifuge for 10 min at 10,000 RPM, supernatant discarded).

Residue → Add 30 ml 1 M HCl, shake for 16 h, then centrifuge for 10 min at 10,000 RPM → HPI

Water wash as above

Residue → Add 30 ml 0.1 M NaOH, shake for 16 h, then centrifuge for 10 min at 10,000 RPM → N(II)Pi & N(II)Po

Water wash as above

Residue → Transferred to digestion tube with deionised H₂O, dried at 60°C, digested using 8 ml concentrated HNO₃ and 4 ml HClO₄ → Residual-P

**Figure 2-2** Soil P fractionation scheme (modified from Hedley et al. (1982b) and Condron et al. (1996)).
Soil total P
Soil total P concentration (Chapters 3 and 7) was determined by adding all P fractions from the P fractionation scheme. In the experiments where soil P fractionation was not carried out (Chapters 5 and 6), total P was determined by the ignition method of Saunders and Williams (1955) (see below).

Soil organic P
Soil organic P was determined by the ignition method of Saunders and Williams (1955). One gram of soil sample was ignited at 550 °C for 1 hour. This ignited soil and an unignited soil sample (1 g) are then extracted with 50 ml 0.5 M H₂SO₄ for 16 hours. The P in the extracts was determined by the method of Murphy and Riley (1962). The concentration of organic P was estimated by the difference in extractable P between ignited (total P) and unignited samples (inorganic P).

Bicarbonate extractable P
In this study, sodium bicarbonate extractable Pi and Po were determined using the extracts of non-fumigated soils during microbial biomass P measurements. The concentration of inorganic P in the bicarbonate extracts was determined after the precipitation of organic matter by acidification and the concentration of total P in bicarbonate extracts was determined after persulfate oxidation, while the concentration of organic P in the bicarbonate extracts was calculated as the difference between total P and Pi.

Resin extractable P
Labile soil P (total, inorganic and organic) extracted by macroporous anion exchange resin (Lewatit MP500A in HCO₃⁻ form) was determined according to the method described by Rubæk and Sibbesen (1993). Five ml of macroporous resin was enclosed in bags made of polyester netting (0.4 mm mesh) and fitted into a 75 ml centrifuge tube. Moist soil equivalent to 2 g of dry soil and 40 ml water were added and shaken for 17 hours at 25 °C. The resin bags were then removed, washed to remove soil particles, and drained for 15 minutes before they were eluted batch-wise with two portions of 20 ml of 1 M sodium chloride (NaCl) each for 30 minutes. Inorganic P (Pi) in the eluents was determined by the molybdic blue method (Murphy and Riley, 1962). Total P in the eluents was determined as follows: 4 ml of each eluent was transferred to a 50 ml digestion tube, followed by 1 ml of concentrated HClO₄ and 0.5 ml of
concentrated sulfuric acid (H$_2$SO$_4$). The samples were then placed on a digestion block. Temperature was brought to 130 °C, and when water had evaporated, the temperature was raised to 250 °C for 5 min to allow HClO$_4$ to evaporate. After cooling, the samples were diluted, and total P was determined as Pi. Organic P (Po) was calculated as the difference.

**Plant P analysis**
Phosphorus concentrations in plant samples were determined following HNO$_3$- HClO$_4$ digestion (Olsen and Sommers, 1982). Plant root and shoot samples from the glasshouse experiments and forest floor materials (F and L layers) from the field experiments were oven-dried and finely ground (< 150 μm) prior to analysis.

2.2.3 Biochemical and biological analyses

**Microbial biomass C**
Microbial biomass C (MBC) was measured by fumigation-extraction according to the methodology described by Vance et al. (1987) using a conversion factor (Kc) of 0.45 (Wu et al., 1990). Moist soil was sieved (< 2 mm) and visible roots were removed. The fumigated and non-fumigated soils (10 g dry wt equivalent moist soil) were extracted with 50 ml 0.5 M potassium sulfate (K$_2$SO$_4$) (soil: extractant ratio 1:5) for 30 min. For forest floor materials, the ratio 1:20 (forest floor material: extractant, 4 g dry wt equivalent fresh materials were extracted by 80 ml 0.5 M K$_2$SO$_4$) was used. Soluble organic C in extracts was determined using a Shimadzu TOC-5000A analyzer.

**Microbial biomass P**
Microbial biomass P (MBP) in soil and forest floor materials was measured by fumigation-extraction (1:20 soil and L/F layers) according to the methodology described by Brookes et al. (1982) using a conversion factor (Kp) factor of 0.4.

**Phosphomonoesterase activity**
Phosphomonoesterase (PME) (EC 3.1.3) activity was measured by the method of Tabatabai and Bremner (1969) and modified by Adams (1992). Moist soil or forest floor material (1g dry wt equivalent) was amended with 0.25 ml toluene and then incubated with 1 ml 115 mM p-nitrophenyl phosphate solution (p-nitrophenyl phosphate, disodium hexahydrate, Sigma 104) in modified universal buffer (MUB) at
37 °C for 1 hour. The amount of p-nitrophenol released was measured on a UV/VIS spectrophotometer (Philips) at 400 nm. Controls (blanks) were also performed for each soil. The buffer pH and incubation temperature used varied with different experiments depending on the specific research objectives. In the field experiment (Chapter 3), a series of preliminary experiments were carried out to determine the optimum buffer pH for forest and grassland soils and forest floor materials. The results are shown in Figure 2-3. The optimum buffer pH for PME activity was 6.5 for grassland soil, 6.0 for forest soil, 5.1 for L layer material and 5.2 for F layer material. In the seasonal study (Chapter 5), buffer pH was adjusted to fresh soil pH values and soil samples were incubated at the field temperature. In the glasshouse experiments (Chapters 6 and 7), acid phosphatase (EC 3.1.3.2) (AcPME) and alkaline phosphatase (EC 3.1.3.1) (AIPME) activities were measured at 37 °C at buffer pH 6.5 and 11, respectively (Tabatabai and Bremner, 1969).

**Phosphodiesterase activity**
Phosphodiesterase (EC 3.1.4) (PDE) activity was measured by using the method of Browman and Tabatabai (1978). The procedures were the same as above, except bis(p-nitrophenyl) phosphate (Sigma, N1256) was used as the substrate in the assay.
Figure 2-3 Effects of buffer pH on phosphomonoesterase activity in soil and forest floor material samples from the field trial at the Craigieburn site (standard errors are shown by vertical bars).
Chapter 3

Long-Term Effects of Afforestation on Soil Phosphorus Dynamics and Availability

3.1 Introduction

Land-use change from grassland to plantation forest, predominantly radiata pine (Pinus radiata), has been occurring widely in hill country areas of New Zealand in recent years due to a combination of factors including low economic returns from pastoral farming, the desire to reduce soil erosion, and expected high economic returns from forestry in the future (Maclaren, 1996). Several studies have examined the influence of this land-use change on soil chemical properties and demonstrated that despite increased soil acidity, levels of plant available nutrients such as phosphorus (P) and sulfur (S) were higher under forest compared with adjacent grassland (Davis and Lang, 1991; Belton et al., 1995; Condron et al., 1996; Parfitt et al., 1997; Alfredsson et al., 1998). These chemical changes were mainly attributed to enhanced mineralization of soil organic matter and associated nutrients under forest. The processes responsible for increased soil organic nutrient mineralization under trees are not yet understood, while the potential long-term impacts of changes in the nature and distribution of organic nutrients under short rotation plantation forestry in New Zealand warrant continued investigation.

Soil P is a major nutrient that controls plant growth and development in agricultural and forest ecosystems. Organic P is the predominant form of P in many soils and its mineralization is mediated by both microbial activity and extracellular phosphatase enzymes produced by plant roots and soil microorganisms (Magid et al., 1996; Tarafdar and Claassen, 1988). The microbial biomass is the most active fraction of soil organic matter and represents a dynamic nutrient pool which responds rapidly to changes in the soil environment (Richardson, 1994). Microbial biomass and enzyme activities, together with microbial respiration and metabolic quotient, have been increasingly recognized as sensitive indicators of soil quality and function in relation to land use and management (Elliott et al., 1996; Oberson et al., 1996; Bergstrom et al., 1998).
Most studies to date on the effects of grassland afforestation have been limited to soil chemical properties and processes (Davis and Lang, 1991; Belton et al., 1995; Giddens et al., 1997; Parfitt et al., 1997), although some aspects of soil biological properties in improved pasture soils have also been examined (Yeates et al., 1997; Ross et al., 1999).

The principle objective of this study was to conduct a detailed investigation of the immediate effects of afforestation on the nature of soil P by comparing relevant chemical, biochemical and biological properties of soils taken from adjacent areas under unimproved grassland and 19 year old pine forest at a site in the South Island high country.

3.2 Materials and Methods

3.2.1 Research site

The study site was located on a flat terrace at Cave Stream within the Craigieburn Research Area, central South Island. Details of this site were described in Chapter 2. In brief, the soil is a Craigieburn silt loam (Allophanic Brown Soil). Grassland consisted of a mixture of native and exotic grasses (e.g. short tussock (*Festuca novae-zealandiae*) and browntop (*Agrostis capillaris*) and native shrub (*Cassinia, Hebe, Leptospermum* and *Dracophyllum* spp). A mixed stand of Ponderosa pine (*Pinus ponderosa*) and Corsican pine (*Pinus nigra*) was established in 1979 at a density of 2200 stems ha\(^{-1}\) (total stand area: 400 m\(^2\)). The stand was 19 years old and the canopy was closed at the time of sampling. The selected area under forest (20 m x 20 m) was divided into 4 subplots; corresponding subplots of the same size were also located within the adjacent grassland area, approximately 20 m from the edge of the forest. The litter (L) and fermentation (F) layers under forest were 2-5 cm and 2-3 cm thick, respectively, but the humic layer (H) was not clearly distinguished.

3.2.2 Sampling

Mineral soil samples (0-5, 5-10, 10-20, 20-30 cm) were randomly taken from the subplots by soil corer (6 cm diameter) in grassland, and along tree rows, 50-70 cm from tree boles in the forest stand in late autumn (May 1998). A total of 15 soil cores were collected from each subplot and bulked. Field moist soil samples were sieved (<
2 mm) and stored at 4 °C during determination of pH, microbial properties, enzyme activities and resin extractable P. A subsample of each soil was air dried and ground (< 150 μm) prior to determination of organic C, total N, total P and soil P fractions.

Forest floor material (L and F layers, < 6 mm) was randomly sampled from 5 areas (10 x 10 cm²) within each subplot. A subsample of forest floor material was oven-dried at 70 °C for 72 h ) prior to determination of organic C, total N and total P. A subsample of the forest floor material was cut into 2-4 mm pieces and stored at 4 °C during determination of pH, microbial properties, enzyme activities and resin extractable P.

Enzyme activities were measured within 72 h, while pH, microbial biomass C and P, and resin extractable P were determined within 7 days. The soil moisture content was measured gravimetrically and all results are expressed on an oven-dry basis.

An additional 8 soil cores from each subplot of the grassland and forest soil were collected for root biomass measurement. Roots retained on a 0.6 mm screen were divided into the following classes: < 2 mm, 2-5 mm and > 5 mm, and dried at 70 °C for 72 h and then weighed. Fine roots (<2 mm) were bulked and finely ground for total C, N and P measurements.

3.2.3 Soil analyses
Total organic C (TOC) and total N, resin extractable P and pH in soil and forest floor materials and total P in forest floor materials and root samples were determined using the methods described in Chapter 2. Soil P fractionation was carried out according to the scheme described in Chapter 2. In brief, this involved sequential extraction of soil with 1 M ammonium chloride (NH₄Cl) (APᵢ), 0.5 M sodium bicarbonate (NaHCO₃) (pH 8.5) (BP; Bₚ), 0.1 M sodium hydroxide (NaOH) (N(I)Pᵢ; N(I)Pₒ), 1 M (hydrochloric acid) HCl (HPᵢ), and 0.1 M NaOH (N(II)Pᵢ; N(II)Pₒ). Total soil P was determined as the sum of extractable P fractions and residual P (non-extracted). Soil bulk density was measured in situ by a combined neutron γ-probe (Strata Gauge, MC-S-36, CPN Corporation, California, USA).

Microbial biomass C (MBC) and P (MBP), phosphomonoesterase (PME) and phosphodiesterase (PDE) activities were also determined using the methods described
in Chapter 2. Soil respiration was measured using the method described by Bartha and Pramer (1965). Soil was aerobically incubated in a 1 L sealed glass jar and carbon dioxide (CO₂) evolved from soil was trapped in 0.1 M NaOH and measured by titration after 1 h, 5 h, 24 h, 3 days, 7 days, 21 days and 28 days. Metabolic quotient (qCO₂) was calculated as the ratio of microbial respired C (µg g⁻¹ over the 28 days) to MBC and used as an indicator of microbial metabolism in soil (Alvarez et al., 1995).

3.2.4 Statistical analysis
Descriptive statistics and one-way ANOVA were carried out using Genstat 4.2 (Lawes Agricultural Trust, Rothamsted, UK) to calculate means and standard errors, and test for significant differences between means.

3.3 Results
3.3.1 Root biomass
Total root biomass to 30 cm was similar under grassland (24.2 kg m⁻³) and forest (24.9 kg m⁻³). Under grassland, most roots (84%) were present in the 0-10 cm soil layer, and root biomass decreased with depth (Table 3-1). Fine roots (< 2 mm) made up 88% - 94% of the total root biomass through the profile. Under the forest, total root biomass in the 0-5 cm soil layer was markedly lower compared with the grassland. Fine roots made up 85% and 60% of total root biomass in 0-5 cm and 5-10 cm soil depths, respectively, and less than 40% of total biomass in 10-30 cm depth. Thus, the main functional roots (< 2 mm) were concentrated in the topsoil (0-10 cm) under both grassland and forest, and fine root biomass in the topsoil was approximately 2.5 times greater under grassland compared with forest. In addition, larger roots (> 5 mm) and a greater mass of roots were observed in 10-30 cm soil under forest compared with the grassland (Table 3-1). All these results reflect the differences in root morphology and distribution between two vegetation types in this study.
Table 3-1  Root biomass determined in adjacent soils under grassland and forest †.

<table>
<thead>
<tr>
<th>Vegetation</th>
<th>Depth (cm)</th>
<th>Root weight (dw, kg m⁻³)</th>
<th>&lt; 2mm</th>
<th>2-5m</th>
<th>&gt; 5mm</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland</td>
<td>0-5</td>
<td></td>
<td>14.5</td>
<td>0.9</td>
<td>0.0</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.4)</td>
<td>(0.2)</td>
<td>(0.2)</td>
<td>(0.7)</td>
</tr>
<tr>
<td></td>
<td>5-10</td>
<td></td>
<td>4.3</td>
<td>0.6</td>
<td>0.0</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.2)</td>
<td>(0.2)</td>
<td>(0.2)</td>
<td>(0.2)</td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td></td>
<td>2.1</td>
<td>0.3</td>
<td>0.0</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.2)</td>
<td>(0.1)</td>
<td>(0.2)</td>
<td>(0.2)</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td></td>
<td>1.4</td>
<td>0.1</td>
<td>0.0</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.1)</td>
<td>(0.01)</td>
<td>(0.1)</td>
<td></td>
</tr>
<tr>
<td>Forest</td>
<td>0-5</td>
<td></td>
<td>5.5**</td>
<td>1.0</td>
<td>0.0</td>
<td>6.5**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.1)</td>
<td>(0.2)</td>
<td>(0.2)</td>
<td>(0.2)</td>
</tr>
<tr>
<td></td>
<td>5-10</td>
<td></td>
<td>3.5</td>
<td>2.4*</td>
<td>0.0</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.5)</td>
<td>(0.6)</td>
<td>(0.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td></td>
<td>2.8</td>
<td>2.0</td>
<td>2.2**</td>
<td>6.9**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.2)</td>
<td>(0.4)</td>
<td>(0.7)</td>
<td>(1.2)</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td></td>
<td>2.4</td>
<td>1.4**</td>
<td>2.0**</td>
<td>5.7**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.3)</td>
<td>(0.2)</td>
<td>(0.2)</td>
<td>(0.6)</td>
</tr>
</tbody>
</table>

† a) Data in a column are means (n=4); data in parenthesis are standard errors of the mean;  
   b) * and ** indicate significant differences between grassland and forest soils of the corresponding depth at the 0.05 and 0.01 probability levels respectively.

3.3.2 Soil chemical properties

Soil pH was consistently lower (0.3-0.5 units) under forest compared with grassland, and the pH was lower in the L and H layers under forest than in the underlying mineral soil (Table 3-2). Total organic C, N and P concentrations were generally lower under forest than under the adjacent grassland in topsoil (0-10 cm), but not in 10-30 cm soil (Table 3-2). The C:N ratios were consistently wider in the forest soils than in the corresponding grassland soils, but there were no consistent differences in C:P ratios. Forest floor materials contained substantially higher concentrations of C, N and P, and much wider C:N and C:P ratios than the underlying soil. Moisture content of the soil
<table>
<thead>
<tr>
<th>Vegetation</th>
<th>Depth (cm)</th>
<th>pH</th>
<th>% C</th>
<th>tons ha(^{-1})</th>
<th>% N</th>
<th>tons ha(^{-1})</th>
<th>(\mu g \ g^{-1})</th>
<th>kg ha(^{-1})</th>
<th>C:N</th>
<th>C:P</th>
<th>Soil moisture (%)</th>
<th>Bulk density (g cm(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland</td>
<td>0-5</td>
<td>5.2</td>
<td>7.65</td>
<td>35.53</td>
<td>0.46</td>
<td>2.13</td>
<td>1035</td>
<td>481.2</td>
<td>17 (0.3)</td>
<td>74 (4)</td>
<td>59 (2)</td>
<td>0.93 (0.02)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.06)</td>
<td>(0.51)</td>
<td>(2.4)</td>
<td>(0.02)</td>
<td>(0.13)</td>
<td>(20)</td>
<td>(9.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5-10</td>
<td>5.1</td>
<td>5.73</td>
<td>26.47</td>
<td>0.36</td>
<td>1.66</td>
<td>960</td>
<td>441.8</td>
<td>16 (0.4)</td>
<td>59 (2)</td>
<td>60 (2)</td>
<td>0.92 (0.02)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.03)</td>
<td>(0.42)</td>
<td>(1.95)</td>
<td>(0.02)</td>
<td>(0.11)</td>
<td>(35)</td>
<td>(16.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>5.2</td>
<td>3.89</td>
<td>38.58</td>
<td>0.25</td>
<td>2.44</td>
<td>821</td>
<td>812.8</td>
<td>16 (0.5)</td>
<td>47 (3)</td>
<td>58 (3)</td>
<td>0.99 (0.02)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.06)</td>
<td>(0.43)</td>
<td>(4.29)</td>
<td>(0.02)</td>
<td>(0.22)</td>
<td>(37)</td>
<td>(36.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>5.5</td>
<td>2.08</td>
<td>23.60</td>
<td>0.15</td>
<td>1.67</td>
<td>626</td>
<td>707.4</td>
<td>14 (0.7)</td>
<td>32 (4)</td>
<td>44 (4)</td>
<td>1.13 (0.03)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.1)</td>
<td>(0.40)</td>
<td>(4.61)</td>
<td>(0.02)</td>
<td>(0.24)</td>
<td>(46)</td>
<td>(52.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>124.17</td>
<td></td>
<td>7.90</td>
<td></td>
<td>2443.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest</td>
<td>L</td>
<td>4.0</td>
<td>51.61</td>
<td>10.22</td>
<td>0.50</td>
<td>0.10</td>
<td>639</td>
<td>12.6</td>
<td>103 (3)</td>
<td>812 (34)</td>
<td>26 (4)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>layer</td>
<td></td>
<td>(0.03)</td>
<td>(0.15)</td>
<td>(0.03)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(26)</td>
<td>(0.29)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>4.4</td>
<td>45.38</td>
<td>5.32</td>
<td>0.91</td>
<td>0.11</td>
<td>803</td>
<td>9.4</td>
<td>50 (1)</td>
<td>573 (43)</td>
<td>155 (18)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>layer</td>
<td></td>
<td>(0.03)</td>
<td>(0.82)</td>
<td>(0.10)</td>
<td>(0.03)</td>
<td>(0.01)</td>
<td>(52)</td>
<td>(0.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-5</td>
<td>4.6**</td>
<td>6.31</td>
<td>28.97</td>
<td>0.35*</td>
<td>1.59*</td>
<td>948</td>
<td>436.1</td>
<td>18 (0.4)**</td>
<td>67 (2)</td>
<td>33 (1)**</td>
<td>0.92 (0.02)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.04)</td>
<td>(0.24)</td>
<td>(1.10)</td>
<td>(0.01)</td>
<td>(0.08)</td>
<td>(22)</td>
<td>(10.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5-10</td>
<td>4.8**</td>
<td>4.86</td>
<td>23.38</td>
<td>0.28</td>
<td>1.36</td>
<td>906</td>
<td>434.9</td>
<td>17 (0.4)</td>
<td>53 (2)</td>
<td>33 (1)**</td>
<td>0.96 (0.01)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.07)</td>
<td>(0.34)</td>
<td>(1.66)</td>
<td>(0.02)</td>
<td>(0.11)</td>
<td>(25)</td>
<td>(12.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>4.9*</td>
<td>4.09</td>
<td>41.41</td>
<td>0.24</td>
<td>2.41</td>
<td>836</td>
<td>844.4</td>
<td>17 (0.4)</td>
<td>49 (1)</td>
<td>36 (1)**</td>
<td>1.01 (0.01)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.06)</td>
<td>(0.27)</td>
<td>(2.77)</td>
<td>(0.02)</td>
<td>(0.20)</td>
<td>(37)</td>
<td>(37.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>5.1*</td>
<td>2.58</td>
<td>27.54</td>
<td>0.15</td>
<td>1.58</td>
<td>676</td>
<td>723.3</td>
<td>18 (0.9)*</td>
<td>38 (1)</td>
<td>33 (1)*</td>
<td>1.07 (0.03)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.04)</td>
<td>(0.10)</td>
<td>(1.11)</td>
<td>(0.01)</td>
<td>(0.13)</td>
<td>(25)</td>
<td>(26.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>136.84</td>
<td></td>
<td>7.13</td>
<td></td>
<td>2460.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) ND = not determined; C = organic C; N = total N; P = total P; 
\(^b\) Data in a column are means (n=4); data in parenthesis are standard errors of the mean; 
\(^c\) * and ** indicate significant differences between grassland and forest soils of the corresponding depth at the 0.05 and 0.01 probability levels respectively.
at all depths was consistently and significantly lower under forest than grassland, while soil bulk density was similar under grassland and forest (Table 3-2).

For resin P, the concentrations of both inorganic and organic P were greatest in the L and F layer under forest (Table 3-3). Levels of resin P in mineral soil were generally similar under grassland and forest. However, significant differences in P fractions between grassland and forest were observed in the 0-5 cm and 5-10 cm soil layers where inorganic P in the BPi, N(I)Pi, and HPI fractions was consistently greater under forest than under grassland (Table 3-4). Conversely, concentrations of BPo, N(I)Po, and N(II)Po were consistently lower in soil under forest than under grassland.

Table 3-3  Resin (Lewatit MP500A) extractable P (µg g⁻¹) determined for adjacent soils under grassland and forest †.

<table>
<thead>
<tr>
<th>Vegetation</th>
<th>Depth (cm)</th>
<th>Inorganic P</th>
<th>Organic P</th>
<th>Total P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5</td>
<td>5.2 (0.4)</td>
<td>31 (3)</td>
<td>36 (3)</td>
<td></td>
</tr>
<tr>
<td>5-10</td>
<td>2.3 (0.2)</td>
<td>23 (4)</td>
<td>26 (4)</td>
<td></td>
</tr>
<tr>
<td>10-20</td>
<td>1.1 (0.4)</td>
<td>15 (2)</td>
<td>17 (2)</td>
<td></td>
</tr>
<tr>
<td>20-30</td>
<td>0.3 (0.1)</td>
<td>11 (1)</td>
<td>11 (1)</td>
<td></td>
</tr>
<tr>
<td>Forest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L layer</td>
<td>64 (11)</td>
<td>96 (14)</td>
<td>160 (25)</td>
<td></td>
</tr>
<tr>
<td>F layer</td>
<td>70 (8)</td>
<td>150 (12)</td>
<td>220 (18)</td>
<td></td>
</tr>
<tr>
<td>0-5</td>
<td>7.0 (0.4)*</td>
<td>31 (1)</td>
<td>38 (1)</td>
<td></td>
</tr>
<tr>
<td>5-10</td>
<td>3.1 (0.8)</td>
<td>23 (1)</td>
<td>26 (1)</td>
<td></td>
</tr>
<tr>
<td>10-20</td>
<td>1.2 (0.5)</td>
<td>15 (1)</td>
<td>17 (1)</td>
<td></td>
</tr>
<tr>
<td>20-30</td>
<td>0.2 (0.2)</td>
<td>11 (1)</td>
<td>11 (7)</td>
<td></td>
</tr>
</tbody>
</table>

† a) Data in a column are means (n=4); data in parenthesis are standard errors of the mean; 
b) * indicates a significant (P<0.05) difference between grassland and forest soils of the corresponding depth.
Table 3-4  Phosphorus fractions (µg g⁻¹) determined for adjacent soils under grassland and forest †.

<table>
<thead>
<tr>
<th>Vegetation</th>
<th>Depth (cm)</th>
<th>APi</th>
<th>BPi</th>
<th>BPo</th>
<th>N(I)Pi</th>
<th>N(I)Po</th>
<th>HPI</th>
<th>N(II)Pi</th>
<th>N(II)Po</th>
<th>Res-P</th>
<th>TPi</th>
<th>TPo</th>
<th>TEP</th>
<th>Total P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland</td>
<td>0-5</td>
<td>2.3</td>
<td>5.7</td>
<td>67</td>
<td>121</td>
<td>495</td>
<td>17</td>
<td>59</td>
<td>162</td>
<td>106</td>
<td>205</td>
<td>724</td>
<td>929</td>
<td>1035</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.3)</td>
<td>(0.3)</td>
<td>(7)</td>
<td>(2)</td>
<td>(10)</td>
<td>(1)</td>
<td>(4)</td>
<td>(5)</td>
<td>(1)</td>
<td>(2)</td>
<td>(22)</td>
<td>(20)</td>
<td>(20)</td>
</tr>
<tr>
<td>Forest</td>
<td>0-5</td>
<td>3.3</td>
<td>9.6**</td>
<td>50*</td>
<td>168**</td>
<td>405*</td>
<td>24*</td>
<td>64</td>
<td>144*</td>
<td>81**</td>
<td>269**</td>
<td>598*</td>
<td>867</td>
<td>948*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.6)</td>
<td>(0.3)</td>
<td>(4)</td>
<td>(6)</td>
<td>(23)</td>
<td>(2)</td>
<td>(6)</td>
<td>(4)</td>
<td>(3)</td>
<td>(9)</td>
<td>(28)</td>
<td>(25)</td>
<td>(22)</td>
</tr>
<tr>
<td>Grassland</td>
<td>5-10</td>
<td>2.2</td>
<td>4.6</td>
<td>51</td>
<td>116</td>
<td>460</td>
<td>13</td>
<td>57</td>
<td>165</td>
<td>92</td>
<td>193</td>
<td>675</td>
<td>868</td>
<td>960</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.4)</td>
<td>(0.3)</td>
<td>(6)</td>
<td>(3)</td>
<td>(25)</td>
<td>(2)</td>
<td>(3)</td>
<td>(8)</td>
<td>(2)</td>
<td>(4)</td>
<td>(37)</td>
<td>(36)</td>
<td>(34)</td>
</tr>
<tr>
<td>Forest</td>
<td>5-10</td>
<td>3.4</td>
<td>5.5</td>
<td>40</td>
<td>149**</td>
<td>400*</td>
<td>18</td>
<td>63</td>
<td>147</td>
<td>80*</td>
<td>239**</td>
<td>587*</td>
<td>826</td>
<td>906</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.9)</td>
<td>(0.6)</td>
<td>(3)</td>
<td>(4)</td>
<td>(6)</td>
<td>(3)</td>
<td>(4)</td>
<td>(13)</td>
<td>(5)</td>
<td>(6)</td>
<td>(21)</td>
<td>(22)</td>
<td>(25)</td>
</tr>
<tr>
<td>Grassland</td>
<td>10-20</td>
<td>2.1</td>
<td>4.1</td>
<td>27</td>
<td>109</td>
<td>360</td>
<td>10</td>
<td>53</td>
<td>180</td>
<td>76</td>
<td>178</td>
<td>567</td>
<td>745</td>
<td>821</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.3)</td>
<td>(0.3)</td>
<td>(3)</td>
<td>(5)</td>
<td>(18)</td>
<td>(2)</td>
<td>(2)</td>
<td>(10)</td>
<td>(1)</td>
<td>(7)</td>
<td>(31)</td>
<td>(36)</td>
<td>(37)</td>
</tr>
<tr>
<td>Forest</td>
<td>10-20</td>
<td>3.4</td>
<td>3.3</td>
<td>30</td>
<td>117</td>
<td>370</td>
<td>12</td>
<td>52</td>
<td>173</td>
<td>75</td>
<td>188</td>
<td>573</td>
<td>760</td>
<td>836</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.8)</td>
<td>(0.3)</td>
<td>(3)</td>
<td>(9)</td>
<td>(14)</td>
<td>(3)</td>
<td>(2)</td>
<td>(10)</td>
<td>(3)</td>
<td>(10)</td>
<td>(25)</td>
<td>(35)</td>
<td>(37)</td>
</tr>
<tr>
<td>Grassland</td>
<td>20-30</td>
<td>2.3</td>
<td>3.7</td>
<td>12</td>
<td>104</td>
<td>269</td>
<td>8</td>
<td>41</td>
<td>133</td>
<td>53</td>
<td>159</td>
<td>414</td>
<td>573</td>
<td>626</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.3)</td>
<td>(0.5)</td>
<td>(2)</td>
<td>(6)</td>
<td>(21)</td>
<td>(1)</td>
<td>(4)</td>
<td>(12)</td>
<td>(6)</td>
<td>(6)</td>
<td>(35)</td>
<td>(40)</td>
<td>(46)</td>
</tr>
<tr>
<td>Forest</td>
<td>20-30</td>
<td>2.9</td>
<td>2.1</td>
<td>21</td>
<td>95</td>
<td>310</td>
<td>8</td>
<td>47</td>
<td>135</td>
<td>56</td>
<td>155</td>
<td>466</td>
<td>621</td>
<td>676</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.5)</td>
<td>(0.5)</td>
<td>(4)</td>
<td>(11)</td>
<td>(12)</td>
<td>(2)</td>
<td>(3)</td>
<td>(3)</td>
<td>(1)</td>
<td>(11)</td>
<td>(17)</td>
<td>(24)</td>
<td>(25)</td>
</tr>
</tbody>
</table>

† a) PI = inorganic P; PO = organic P; API = NH₄Cl-Pi; BPI = NaHCO₃-Pi; BPO = NaHCO₃-Po; N(I)Pi = NaOH-Pi (first extraction); N(I)Po = NaOH-Po (first extraction); HPI = HCl-Pi; N(II)Pi = NaOH-Pi (second extraction); N(II)Po = NaOH-Po (second extraction); Res-P = residual (non-extracted) P.

b) TPi = API + BPI + N(I)Pi + HPI + N(II)Pi; TPO = BPO + N(I)Po + N(II)Po; TEP (total extractable P) = TPi+TPo; Total P = TEP + Res-P.

c) Data in a column are means (n=4); data in parenthesis are standard errors of the mean. * and ** indicate significant differences between grassland and forest soils of the corresponding depth at 0.05 and 0.01 probability levels respectively.
3.3.3 Soil microbial biomass C and P
Microbial biomass C and P were significantly higher in 0-5 and 5-10 cm soil under grassland compared with forest (Table 3-5). Concentrations of MBC and MBP in the L and F layers were markedly higher than in the underlying soil, especially in the F layer. The microbial C:P ratio was significantly lower in the 0-5 cm soil under forest compared with grassland (Table 3-5). Microbial biomass C constituted 2.2% of total organic C in 0-10 cm soil under grassland; this value was greater than in the forest soils (1.5%, 0-10 cm). Microbial biomass P constituted 1.6% and 1.3% of total soil P in 0-5 and 5-10 cm soils respectively under grassland; these values were also slightly higher than in the forest soils (1.2% (0-5 cm) and 0.7% (5-10 cm)) (Table 3-5). However, land-use change from grassland to forest did not significantly affect MBC and MBP concentrations in the 10-20 and 20-30 cm soil depths. In forest floor materials, MBC comprised only 0.5% and 1.2% of total C in the L and F layers respectively, while MBP comprised 6.1% (L layer) and 62.4% (F layer) of total P in the L and F layers, respectively. Microbial biomass C in the L layer may be underestimated since low moisture (26%) possibly affected the release of microbial C during the fumigation (Sparling and West, 1989).

3.3.4 Soil respiration and metabolic quotients
After a 4 week incubation period, cumulative CO₂-C production was higher in soil under grassland than under forest at most depths (Figure 3-1). The average respiration rate over 4 weeks was significantly higher in 0-5 cm soil under grassland compared with forest (Table 3-5). Carbon dioxide (CO₂) respired from forest floor material was much higher than from mineral soil, with cumulative respiration being 9.8 mg CO₂-C g⁻¹ and 24.9 mg CO₂-C g⁻¹ in 4 weeks for L and F layers, respectively (Figure 3-2). Results in Table 5 show that the metabolic quotient (qCO₂) was significantly higher in 0-5 cm soil under forest compared with grassland, indicating that more of the C consumed in forest soil was evolved as CO₂ rather than being incorporated to microbial biomass. As expected, qCO₂ values for L and F layer materials (5.89 and 6.87, respectively) were markedly greater than those in the underlying mineral soil (Table 3-5).
<table>
<thead>
<tr>
<th>Vegetation</th>
<th>Depth (cm)</th>
<th>MBC ($\mu$g g$^{-1}$)</th>
<th>MBP ($\mu$g g$^{-1}$)</th>
<th>Microbial C:P</th>
<th>MBC: TOC (%)</th>
<th>MBP: total P (%)</th>
<th>Respiration (mg CO$_2$-C mg$^{-1}$ microbial C h$^{-1}$)</th>
<th>$q$CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland</td>
<td>0-5</td>
<td>1690 (130)</td>
<td>16 (1)</td>
<td>106 (10)</td>
<td>2.2</td>
<td>1.6</td>
<td>1.25 (0.03)</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>5-10</td>
<td>1238 (56)</td>
<td>12 (1)</td>
<td>103 (13)</td>
<td>2.2</td>
<td>1.3</td>
<td>0.86 (0.09)</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>813 (158)</td>
<td>5 (1)</td>
<td>163 (37)</td>
<td>2.1</td>
<td>0.6</td>
<td>0.58 (0.09)</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>319 (51)</td>
<td>1 (0.3)</td>
<td>319 (28)</td>
<td>1.6</td>
<td>0.2</td>
<td>0.52 (0.05)</td>
<td>1.63</td>
</tr>
<tr>
<td>Forest</td>
<td>L layer</td>
<td>2481 (505)</td>
<td>38 (10)</td>
<td>65 (26)</td>
<td>0.5</td>
<td>6.1</td>
<td>14.62 (0.24)</td>
<td>5.89</td>
</tr>
<tr>
<td></td>
<td>F layer</td>
<td>5388 (816)</td>
<td>503 (67)</td>
<td>11 (2)</td>
<td>1.2</td>
<td>62</td>
<td>37.00 (1.97)</td>
<td>6.87</td>
</tr>
<tr>
<td></td>
<td>0-5</td>
<td>949** (78)</td>
<td>12* (0.6)</td>
<td>79* (3)</td>
<td>1.5**</td>
<td>1.2*</td>
<td>0.99* (0.10)</td>
<td>1.04*</td>
</tr>
<tr>
<td></td>
<td>5-10</td>
<td>736** (70)</td>
<td>7* (1)</td>
<td>105 (19)</td>
<td>1.5*</td>
<td>0.7*</td>
<td>0.63 (0.07)</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>530 (69)</td>
<td>5 (1)</td>
<td>106 (40)</td>
<td>1.3</td>
<td>0.6</td>
<td>0.54 (0.11)</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>392 (103)</td>
<td>1 (0.3)</td>
<td>392 (128)</td>
<td>1.5</td>
<td>0.1</td>
<td>0.40 (0.05)</td>
<td>1.02</td>
</tr>
</tbody>
</table>

† a) Data in a column are means (n=4); data in parenthesis are standard errors of the mean;
b) MBC = microbial biomass C; MBP = microbial biomass P; MBC: TOC = the ratio of MBC to total organic C; MBP: total P = the ratio of microbial biomass P to total P; $q$CO$_2$ = metabolic quotient.
c) * and ** indicate significant differences between grassland and forest soils of the corresponding depth at 0.05 and 0.01 probability levels respectively.
Figure 3-1 Evolution of CO$_2$-C from adjacent grassland and forest soils (standard errors shown by vertical bars).
3.3.5 Soil phosphatase activity

The phosphomonoesterase activities for soil and forest floor materials measured at optimum pH are shown in Figure 3-3. Enzyme activities in the 0-5 and 5-10 cm soils under grassland were significantly higher than in the corresponding soils under forest. However, there were no significant differences between the grassland and forest samples in enzyme activity in the 10-20 and 20-30 cm soils. Similar trends were evident for the much lower levels of PDE (Figure 3-4). Activities of both PME and PDE activities in forest floor materials were 2-8 times greater than in the underlying soil (Figures 3-3 and 3-4).
**Figure 3-3** Phosphomonoesterase activity determined for soils and forest floor materials (standard errors shown by horizontal bars).

**Figure 3-4** Phosphodiesterase activity determined for soils and forest floor materials (standard errors shown by horizontal bars).
3.4 Discussion

The assumption that adjoining soils under grassland and plantation forest were initially similar has been the basis for other 'paired-site' studies (Davis and Lang, 1991; Noble et al., 1999; Parfitt et al., 1997; Ross et al., 1999). The nature of the study site meant that the replicate plots under grassland and forest were not randomly distributed ('pseudo-replication' - Yeates and Saggar (1998)), although sampling within each plot was conducted randomly. Therefore, the conclusions drawn from this study relate specifically to this site and must be generalized with caution. In this study, the sites were on the same terrace, had similar land-use history prior to planting of the forest stand, and were within 50 m of each other. Neither had been grazed by livestock after planting, or had received fertilizer. The soils were derived from the same parent material - greywacke sandstone loess, and had similar morphology. In addition, soil texture was similar in the 0-5 cm soil under grassland and forest, with clay, silt and sand contents of 23.5, 39.1 and 36.5% under grassland and 23.5, 38.7 and 37.8% under forest, respectively. Thus, it is reasonable to assume that the observed changes in soil properties were primarily due to the land-use change.

Differences in soil chemical, biochemical and biological properties between the grassland and forest were usually confined to the topsoil (0-10 cm) over the 19-year time frame after establishment of the tree plantation. There were no significant differences in soil properties at greater depths except for pH and moisture (Table 3-2, 3-3, 3-4 and 3-5). These results were probably directly related to root distribution as major functional fine roots (<2 mm) of both grasses and trees occurred mainly in the 0-10 cm layer (Table 3-1).

Results of this study showed that soil pH, TOC, total N and total P in topsoil under forest were generally lower than under the adjacent grassland (Table 3-2). This is consistent with earlier results from this site which examined soil (0-10 cm) taken 10 years after forest establishment (Condron et al., 1996; Davis and Lang, 1991), and with results from other studies conducted in New Zealand (Belton et al., 1995; Parfitt et al., 1997; Alfredsson et al., 1998) and in Canada (Anderson, 1987). Consequently, it seems reasonable to attribute these observed differences to afforestation. No significant difference was found in bulk density between soils under adjacent forest
and grassland, which is also consistent with other studies carried out in New Zealand (Giddens et al., 1997; Parfitt et al., 1997; Yeates et al., 1997).

### 3.4.1 Soil microbial parameters and biological fertility

Microbial biomass is a dynamic component of organic matter and is likely to be more sensitive to land-use change than soil organic matter as a whole. The microbial biomass is actively involved in the assimilation and transformation of organic matter added to soil and also acts as a labile pool of N, P and S (Powlson, 1994). In this study, MBC concentrations in the 0-5 cm soils under grassland (1690 µg g⁻¹) and forest (949 µg g⁻¹) (Table 3-5) were within the range reported for other New Zealand soils (Sarathchandra et al., 1984; Yeates et al., 1997; Ross et al., 1999). The MBP concentration in 0-5 cm soil under grassland and forest (16 µg P g⁻¹ and 12 µg P g⁻¹ respectively) were lower than those reported for other New Zealand soils (32-98 µg P g⁻¹) by Sparling et al. (1994).

Several recent studies have shown that conversion from pasture to radiata pine plantation decreased soil microbial biomass C, N and P together with the number and diversity of nematodes (Yeates et al., 1997; Yeates and Saggar, 1998; Ross et al., 1999). In the present study, land-use change from unimproved grassland to conifers resulted in a similar reduction in soil MBC and MBP, as well as decreases in soil respiration and phosphatase activity and an increase in metabolic quotient (Table 3-5, Figures 3-1, 3-3 and 3-4). These parameters are major biological indicators of soil fertility and their decline tends to indicate an overall reduction in soil quality (Elliott et al., 1996).

Microbial biomass and activity can be affected by tillage practice (Alvarez et al., 1995) and fertilization (Ross et al., 1995). In this study, little physical disturbance occurred during the transition from grassland to forest and no fertilizer was applied. Therefore, the shift in microbial biomass and activity must be related to soil chemical and biological processes associated with plant growth. The lower pH in soil under forest (Table 3-2) may be attributed to a combination of the release of organic acids from tree roots (Giddens et al., 1997) and removal of exchangeable cations by plant uptake (Yeates et al., 1997; Alfredsson et al., 1998; Yeates and Saggar, 1998). A recent study conducted by Blagodatskaya and Anderson (1998) showed that the decreased soil pH...
under North German beech (*Fagus sylvatica*) and spruce (*Picea abies*) not only decreased microbial biomass and the MBC: TOC ratio, but also increased the fungal to bacteria ratio and the metabolic quotient. Therefore, soil pH may be the major driving variable controlling the biomass and composition of microbial communities and their maintenance demand (i.e. metabolic quotient). This, in turn, may partly account for the apparent change in soil microbial properties observed under forest in this study.

Differences in the types and amounts of organic matter added to soil under the grassland and forest could also be responsible for the change in microbial properties. Both the quantity and quality of organic matter returned to soil may affect the microbial biomass (Singh and Singh, 1995), fungal to bacteria ratio (Blagodatskaya and Anderson, 1998) and mineralization of organic matter (Zou et al., 1995). Fine root biomass was approximately two-fold higher in the grassland soil than in the forest soil at 0-5 cm depth (Table 3-1), and input from root detritus may have been larger in the grassland soil than the forest soil, especially during the early stages of stand development (Parfitt et al., 1997). This may have been responsible for the microbial C: TOC ratio being lower in soil under forest compared with grassland (Table 3-5). Moreover, more recalcitrant forms of organic C have been found in mineral soil under coniferous forest compared with grassland in a recent $^{13}$C NMR study, which included samples (0-10 cm) taken from the Cave Stream site 10 years after forest establishment (Condron and Newman, 1998). Differences in the composition (e.g. lignin, cellulose) of organic matter inputs under grassland and forest may also contribute to differences in microbial biomass and activity (Sparling et al., 1994). The C:N and C:P ratios for grassland roots (69 and 750, respectively) were lower than those for pine roots (106 and 840) in this study. Yeates et al. (1997) also suggested that greater release of substrates such as sugars, organic acids and amino acids from roots and foliage in a grassland soil may have been partly responsible for the higher microbial biomass C and N found in another soil under grassland compared with forest.

Although MBC and MBP concentrations in the soils under forest and grassland were within the ranges reported in the literature, the microbial C:P ratios were higher (e.g. 0-5 cm soil: 106 and 79 under grassland and forest respectively; Table 3-5) compared with reported values for agricultural (12-36) and forest (5-26) soils (Brookes et al., 1984; Joergensen et al., 1995). This indicates the relatively high concentrations of MBC determined in this study, while levels of MBP were relatively low (Table 3-5).
The lower microbial C:P ratio in the soil under forest compared with grassland may reflect population changes in the soil microorganisms, with fungal species becoming increasingly dominant over bacteria in the more acid forest soil. Anderson and Domsch (1980) showed that microbial C:P ratios in soil bacteria were higher (18) than in soil fungi (12) grown in identical nutrient media. Another explanation for the lower microbial C:P ratio in the forest soil may be the higher levels of labile inorganic P in this soil compared with the grassland soil (Table 3-4) as microorganisms growing in media with a high P content usually assimilate more P than in media with a low P content (Hedley et al., 1982b).

3.4.2 Soil P fractions

It has been suggested that conifers increase mineralisation of soil organic P and thereby increase plant available inorganic P (Davis 1995; Condron et al., 1996). In the present study, all forms of organic P (BPo, N(I)Po, N(II)Po) in 0-5 cm soil were lower under forest than under grassland while inorganic fractions (BPI, N(I)Pi and HPI) were higher under forest (Table 3-4). In the 0-5 cm soil, total extracted organic P was 17% lower and total extracted inorganic P 31% higher under forest compared with grassland. Similar trends were observed in 5-10 cm soil (Table 3-4). The fact that the relatively chemically resistant organic forms of P (N(II)Po and residual-P) in the 0-10 cm soil were significantly lower under forest than under grassland indicates that conifers were able to utilize not only labile organic P forms (e.g. BPo) but also more recalcitrant (less readily extractable) forms of organic P. This is consistent with $^{31}$P-NMR studies which demonstrated that both orthophosphate monoesters and diesters decreased under recently established conifers compared with adjacent grassland (Condron et al., 1996).

While dynamic interchanges in size among the various soil P pools after the vegetation change were apparent in this study, the changes in BP and N(I)P fractions (both inorganic and organic forms) were the most dramatic. For example, in 0-5 cm soil, the BPo and N(I)Po fractions were 27% and 18% lower, respectively, under forest than under grassland, while BPI and N(I)Pi were 69% and 39% higher, respectively, under forest (Table 3-4). This confirms that soil P extracted by NaHCO$_3$ and NaOH represents most biologically available P (Schmidt et al., 1996).
The residual P fraction contributed approximately 10% of total P in both grassland and forest soils. The proportion of total P included in the residual pool varies with soil type and the P fractionation scheme employed (Tiessen and Moir, 1993). Residual P may be regarded as a combination of occluded inorganic and stable forms of organic P although little is known about the precise nature of this fraction (Magid et al., 1996). Previous studies have shown that residual P in rhizosphere soil can be depleted by plant uptake (Gahoonia and Nielsen, 1992b; Oberson et al., 1996). This, together with the fact that residual P was lower under conifers than under grassland in this study (Table 3-4), suggests that this chemically resistant residual P fraction may not be biologically resistant. While the complexity of organic P makes it difficult to establish a generally applicable operational or theoretical definition for plant-available P (Magid et al., 1996), the nature of residual P clearly requires further study.

Phosphorus extracted by macroporous anion exchange resin (Lewatit MP500A) includes soluble Pi and Po and also Pi and Po released from the solid phase, and has been proposed as a measure of labile P in soil (Rubæk and Sibbesen, 1993). Several studies have demonstrated that levels of resin-extractable P respond to fertiliser addition (Rubæk and Sibbesen, 1995) and changes in land use (Guggenberger et al., 1996). Although significant amounts of resin-P (11-38 μg g⁻¹) were determined at all depths of mineral soil in this study, differences between soils under grassland and forest were small (Table 3-3).

Soil organic P has been found to be very significant in the supply of P to radiata pine in Australia (Turner and Lambert, 1985), while labile organic P (extracted by NaHCO₃) and microbial biomass P have been closely correlated with productivity of eucalypts (Eucalyptus spp.) (Adams et al., 1989). In this study, no fertiliser had been applied to either the grassland or forest and thus the content of labile inorganic P was very low. Organic P is likely to be a major source of P for tree growth, which is supported by the fact that organic P was lower under the forest than under the grassland (Table 3-4).

3.4.3 Soil phosphatase activity
Phosphatase enzymes can be produced by plant roots, microorganisms and other soil organisms (e.g. earthworms) (Speir and Ross, 1978; Satchell and Kyla, 1984; Tarafdar
and Claassen, 1988). Tarafdar et al. (1992) reported that the increase in acid and alkaline phosphatase activities in the rhizosphere of mung bean (*Vigna radiata* (L.) Wilczek) was due to increased microbial and root activities. It has also been found that acid phosphatase activity was positively correlated with microbial biomass P in a humus layer of a spruce forest (Clarholm, 1993). The significant positive correlation found between phosphatase activities and soil organic C, microbial biomass C and P, soil respiration and fine root biomass in this study confirm the microbial and plant-root origin of soil phosphatase under grassland and forest. The positive relationship between phosphatase activity and organic C is possibly due to the binding of the enzyme to the humus-protein complex which protects enzymes from decomposition (Harrison, 1983).

The lower soil phosphatase activity observed under forest compared with grassland may be directly related to the corresponding lower fine root biomass and microbial activities in the forest soil. The labile inorganic P in soil solution was very low (3 µg g⁻¹ for APi and 10 µg g⁻¹ for BPi) under forest in this study, so that it is unlikely that soluble inorganic P inhibited the production of phosphatase by plant roots and soil microbial organisms by a feedback control (Speir and Ross, 1978). However, the lower forest soil pH may also have contributed to the lower phosphatase activity in the forest than in the grassland. Long-term differences in acidity may affect enzyme-mediated reactions by altering the solubility and ionization of enzymes, substrates and cofactors (Dick et al., 1988).

It is possible that the phosphatase enzyme activities measured in this study using artificial substrates (p-nitrophenyl phosphate (PNP); bis-p-nitrophenyl phosphate (bis-PNP)) may not reflect the actual level of phosphatase enzyme produced by ectomycorrhizal fungi in the forest soil. For example, phytates (inositol phosphatases) are a major component of organic P in most soils, and accurate assessment of phytase enzyme activity in soil may require the use of a suitable natural substrate (Leake, 2001).

### 3.4.4 Soil organic P mineralization

The turnover of organic P is essentially microbially mediated (Huffman et al., 1996; Richardson, 1994). Organic P mineralization in soil may occur through a combination
of decomposition of organic matter by microorganisms and biochemical mineralization catalyzed by extracellular phosphatase enzymes (McGill and Cole, 1981). Factors that affect the availability of substrates to microorganisms, such as the quantity and quality of organic matter inputs, substrate solubility, soil pH, moisture and temperature, also influence the mineralization of organic P in soil (Tarafdar and Claassen, 1988; Richardson, 1994). In this study, however, the apparent increase in organic P mineralization in the forest soil, as indicated by lower soil organic P (Table 3-4), is inconsistent with the current lower microbial and enzyme activities in the soil under forest compared with grassland (Figures 3-1, 3-3 and 3-4). We hypothesize that the lower levels of organic P in the forest soil are due to enhanced microbial and phosphatase activity during earlier stages of forest development. Phosphorus uptake from the soil by trees is greatest before maximum tree canopy formation (Harrison, 1989) and the high demand for P during this stage may be the driving force for mineralisation of soil organic P (McGill and Cole, 1981). Ectomycorrhizal fungi associated with the roots of pine trees play a key role in P uptake, not only through their highly developed hyphae allowing effective exploration of the soil volume, but also by enhancing the mineralisation of organic P in soil by extracellular phosphatases (Marschner and Dell, 1994). In addition, the decrease in soil pH under forest might enhance the solubility of organic P (Zou et al., 1995) and increase its susceptibility to microbial attack and enzyme hydrolysis (Adams and Pate, 1992). In the grassland, the comparatively high current levels of microbial and enzyme activities (and hence P turnover) are maintained by continuous organic matter inputs, mainly through greater root turnover.

3.4.5 Role of the forest floor in P cycling
Nutrient turnover in forest litter is an important component of nutrient cycling in forest ecosystems (Attiwill and Adams, 1993; Saggar et al., 1998). In this study, forest floor material (including the L and F layers) contained 15,540 kg C ha\(^{-1}\), 205 kg N ha\(^{-1}\) and 22 kg P ha\(^{-1}\). If forest floor materials were taken into account, there would be little difference in the C, N and P contents of the 0-30 cm soil profile under grassland and forest (data not shown). This result indicates that litterfall may help to balance the C, N and P lost from mineral soil by increased mineralization and tree uptake under forest. Soil microbial biomass C and P, soil respiration and phosphatase activity were 3-6, 3-50, 14-35 and 1-5 fold higher in the forest floor (particularly in the F layer) than the underlying mineral soil, respectively (Table 3-5, Figure 3-3 and 3-4). This in turn
suggests that the forest floor may be a major source of organic P for forest growth. In the forest litter layer, resin-extractable inorganic P and organic P contents were 3-10 times greater than in the mineral soil (Table 3-3). This labile P may be taken up directly by fine tree roots, although some P could also leached into the underlying mineral soil (Attiwill and Adams, 1993).

3.5 Conclusions

The findings of this study clearly demonstrate that concentrations of inorganic P were higher, but concentrations of organic P were lower in topsoil (0-10 cm) under forest compared with adjacent grassland. This, together with the fact that levels of total organic C were lower in soil under grassland than under forest, confirmed that afforestation enhanced mineralization of organic matter and associated nutrient (P). However, levels of microbial biomass C and P, respiration and phosphatase enzyme activity were significantly lower in topsoil under conifers compared with adjacent grassland. It is hypothesized that enhanced mineralization occurred during the early stages of afforestation.
Chapter Four

Long-Term Effects of Afforestation on Soil Sulfur Dynamics and Availability

4.1 Introduction

In New Zealand, plantation forestry based on radiata pine \((Pinus radiata)\) covers approximately 1.7 million ha (7% total land area). Over 50,000 ha of new forest has been planted each year since 1992 (Ministry of Agriculture and Forestry, 1999) and this trend is expected to continue over the next 10 years (Glass, 1997). This expansion has primarily occurred on hill country pastoral farmland, stimulated mainly by declining returns from pastoral farming and expected increases in returns from plantation forestry (Maclaren, 1996). The land-use change from grassland to forest is likely to have significant impacts on soil properties and nutrient cycling, which in turn will influence long-term management of land resources. Previous studies carried out in New Zealand have focused mainly on soil carbon (C), nitrogen (N) and phosphorus (P) in topsoil (e.g., 0-10 cm) (Davis and Lang, 1991; Condron et al., 1996; Yeates et al., 1997; Ross et al., 1999; Chapter 3). Limited work has been done on the effect of this land-use change on soil sulfur (S) dynamics and availability.

Sulfur is an essential nutrient for both agricultural and forest ecosystems. Sulfate-S is the major form of S taken up by plants while organic S comprises most (>90%) of the total S in most soils (Mitchell et al., 1992; Zhao et al., 1996). A number of studies have shown that organic S contributes significantly to plant S uptake through mineralization (Nguyen and Goh, 1990; Sakadevan et al., 1993; Zhao and McGrath, 1994). The mineralization of organic S is mediated by a combination of microbial and enzyme (sulfatase) activities (Zhao et al., 1996).

The objective of this study, which complements that described for P in Chapter 3, was to investigate the effects of land-use change from grassland to forest on the amounts and forms of S in soil and associated microbial and enzyme activities. This was achieved by comparing soils taken from adjacent areas under unimproved grassland and a 19-year-old pine forest in New Zealand.
4.2 Materials and Methods

4.2.1 Site
This study was carried out at the same site as that used in Chapter 3. The site and sampling regime used for this study were also described in Chapters 2 and 3.

4.2.2 Soil analyses
Concentrations of sulfur and water soluble organic C (WSOC) in soil and forest floor materials were determined using the methods described in Chapter 2. Sulfate is the predominant form of inorganic S and the inorganic non-sulfate-S fraction, which includes sulfites and thiosulphates, is negligible in most soil (Landers et al., 1983). In this study, extraction with 500 mg L\(^{-1}\) potassium dihydrogen phosphate (KH\(_2\)PO\(_4\)) at a soil: solution ratio of 1:5 (Ghani et al., 1990) was used to determine potentially available sulfates S, which includes soluble and adsorbed forms of sulfate-S. Phosphate extractable sulfate was assumed to represent ‘inorganic S’ in these soils, and so ‘organic S’ was calculated as the difference between total S and inorganic S.

Microbial biomass S (MBS) was also measured by a fumigation-extraction (1:20 soil and L/F) method using a K\(_s\) factor of 0.35 (Saggar et al., 1981). The total S in 10 mM CaCl\(_2\) extracts of fumigated and non-fumigated soil samples was measured by inductively coupled argon plasma-atomic emission spectrometry (ICAP-AES).

Soil arylsulfatase activity was determined using the method described by Tabatabai and Bremner (1970). In brief, fresh moist soil or forest floor material (1g dry wt equivalent) was added with a 0.25 ml toluene and then incubated with 1 ml 25 mM p-nitrophenyl sulfate solution (Sigma, N3877) in 4 ml 0.5 M acetate buffer (pH 5.8) at 37 °C for 1 hour. The amount of p-nitrophenol released was measured by UV/VIS spectrophotometer (Philips) at 400 nm. Controls (blanks) were also performed for each soil.

4.2.3 Statistical analysis
Descriptive statistics and one-way ANOVA were carried out in Genstat 4.2 (Lawes Agricultural Trust, Rothamsted, UK) to calculate means and standard errors, and test for significant differences between means.
4.3 Results

Data for total organic C and MBC in soils and forest floor materials measured in Chapter 3 were used to calculate soil C:S and microbial C:S ratios in this study. Results from Chapter 3 have shown that soil pH and moisture content were lower under forest than under grassland at all depths, but there were no significant differences in bulk density between soils under forest and grassland (Table 3-2). Results from this study showed that total S (and total organic C) in topsoil (0-5 cm) were lower under forest than grassland (Table 4-1). There were no significant differences in C:S ratio between soils under forest and grassland (Table 4-1). These results were consistent with other similar studies carried out in New Zealand (Davis and Lang, 1991; Condron et al., 1996; Parfitt et al., 1997; Alfredsson et al., 1998), and confirm that afforestation of grassland enhanced mineralization of soil organic constituents. In addition, there were no significant differences observed in concentrations of WSOC between soils at corresponding depths under grassland and forest (except 5-10 cm) (Table 4-1).

4.3.1 Sulfur fractions

Inorganic S concentrations in soil under forest were significantly higher than under grassland at all depths (Table 4-2). Inorganic S concentrations in soil under grassland increased with depth, while levels were similar at different depths under forest (Table 4-2). Organic S in topsoil (0-10 cm) under forest was significantly lower than under grassland (Table 4-2). Organic S was the predominant form of S in soils under both forest (95% of total S for 0-10 cm soil) and grassland (99% of total S for 0-10 cm soil). Forest floor materials contained significant amounts of organic S (546.5 μg g⁻¹ and 738.1 μg g⁻¹ for L and F layer, respectively) compared with the underlying mineral soil, but the inorganic S concentration was very low (Table 4-2).

4.3.2 Microbial biomass S

Microbial biomass S was significantly higher in 0-5 and 5-10 cm soil under grassland than forest, while at greater depths MBS contents in both grassland and forest soils were very low (Table 4-2). Microbial biomass S constituted only 0.3 - 1.4% of total S in soils under both grassland and forest. However, as expected, MBS concentrations in forest floor materials were much greater than in underlying mineral soils, and
constituted 4.7% and 11.4% of total S in the L and F layers, respectively. Microbial biomass C was significantly lower in the 0-10 cm soil under forest than grassland (Table 4-2). There were no significant differences observed in microbial C: S ratios between soils under grassland and forest, although the microbial C: S ratios were lower in topsoil (0-10 cm) under forest than grassland (Table 4-2).

Table 4-1 Organic C, total S and water soluble organic C determined for adjacent soils under grassland and forest.

<table>
<thead>
<tr>
<th>Vegetation</th>
<th>Depth (cm)</th>
<th>Organic C (ton ha⁻¹)</th>
<th>Total S (kg ha⁻¹)</th>
<th>C:S</th>
<th>WSOC (µg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland</td>
<td>0-5</td>
<td>35.5 (2.4)</td>
<td>253 (7)</td>
<td>140 (6)</td>
<td>86 (11)</td>
</tr>
<tr>
<td></td>
<td>5-10</td>
<td>26.5 (1.9)</td>
<td>217 (7)</td>
<td>121 (6)</td>
<td>62 (5)</td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>38.6 (4.3)</td>
<td>343 (19)</td>
<td>111 (7)</td>
<td>36 (7)</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>23.6 (4.6)</td>
<td>287 (21)</td>
<td>80 (10)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Forest</td>
<td>L layer</td>
<td>10.2 (0.1)</td>
<td>10.8 (0.2)</td>
<td>942 (16)</td>
<td>2697 (400)</td>
</tr>
<tr>
<td></td>
<td>F layer</td>
<td>5.3 (0.1)</td>
<td>8.8 (0.2)</td>
<td>602 (6)</td>
<td>3498 (889)</td>
</tr>
<tr>
<td></td>
<td>0-5</td>
<td>29.0 (1.1)*</td>
<td>201 (9)**</td>
<td>144 (5)</td>
<td>84 (14)</td>
</tr>
<tr>
<td></td>
<td>5-10</td>
<td>23.4 (1.6)</td>
<td>204 (11)</td>
<td>114 (5)</td>
<td>42 (7)**</td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>41.4 (2.7)</td>
<td>367 (13)</td>
<td>112 (4)</td>
<td>31 (7)</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>27.5 (1.1)</td>
<td>326 (10)</td>
<td>85 (4)</td>
<td>20 (1)</td>
</tr>
</tbody>
</table>

a) WSOC = water soluble organic C;
b) Data in a column are means (n=4); data in parenthesis are standard errors of the mean.
c) * and ** indicate significant differences between grassland and forest soils of the corresponding depth at the 0.05 and 0.01 probability levels respectively.

4.3.3 Soil arylsulfatase activity

The arylsulfatase activities in 0-5 and 5-10 cm soils under grassland were significantly greater than in corresponding soils under forest (Figure 4-1). However, there were no significant differences observed in enzyme activity between the 10-30 cm soils under grassland and forest. In the forest floor, enzyme activity was greater in the F layer compared with the underlying mineral soil, but lower in the L layer than in the underlying 0-20 cm soil.
Table 4-2  Sulfur fractions and microbial biomass S and C concentrations determined for adjacent soils under grassland and forest 

<table>
<thead>
<tr>
<th>Vegetation</th>
<th>Depth (cm)</th>
<th>Organic S (μg g⁻¹)</th>
<th>Inorganic S (μg g⁻¹)</th>
<th>MBS (μg g⁻¹)</th>
<th>MBS: total S (%)</th>
<th>MBS: total S (μg g⁻¹)</th>
<th>MBC (μg g⁻¹)</th>
<th>Microbial C:S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland</td>
<td>0-5</td>
<td>540.6 (16.6)</td>
<td>3.1 (0.5)</td>
<td>7.8 (0.3)</td>
<td>1.44 (0.1)</td>
<td>1690 (130)</td>
<td>216 (29)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5-10</td>
<td>468.4 (15.8)</td>
<td>2.1 (0.1)</td>
<td>6.0 (0.4)</td>
<td>1.28 (0.34)</td>
<td>1238 (56)</td>
<td>214 (43)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>341.0 (19.7)</td>
<td>4.3 (0.3)</td>
<td>1.2 (0.4)</td>
<td>0.33 (0.28)</td>
<td>813 (158)</td>
<td>689 (185)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>242.1 (19.2)</td>
<td>10.7 (1.1)</td>
<td>1.5 (0.7)</td>
<td>0.53 (0.22)</td>
<td>319 (51)</td>
<td>493 (196)</td>
<td></td>
</tr>
<tr>
<td>Forest</td>
<td>0-5</td>
<td>546.5 (9.9)</td>
<td>0.2 (0.1)</td>
<td>25.8 (5.8)</td>
<td>4.66 (1.95)</td>
<td>2481 (505)</td>
<td>103 (42)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F layer</td>
<td>738.1 (20.1)</td>
<td>1.6 (0.3)</td>
<td>85.6 (6.0)</td>
<td>11.38 (1.82)</td>
<td>5388 (816)</td>
<td>62 (12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-5</td>
<td>418.4** (20.3)</td>
<td>19.6** (2.3)</td>
<td>5.3* (0.1)</td>
<td>1.22 (0.04)</td>
<td>949** (78)</td>
<td>179 (89)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5-10</td>
<td>398.1* (24.5)</td>
<td>25.3** (1.7)</td>
<td>4.2* (0.3)</td>
<td>0.98 (0.24)</td>
<td>736** (70)</td>
<td>183 (36)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>344.0 (16.2)</td>
<td>18.9** (2.9)</td>
<td>1.2 (0.2)</td>
<td>0.35 (0.20)</td>
<td>530 (69)</td>
<td>443 (174)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>285.1 (8.6)</td>
<td>20.3* (4.0)</td>
<td>1.1 (0.3)</td>
<td>0.33 (0.26)</td>
<td>392 (130)</td>
<td>367 (117)</td>
<td></td>
</tr>
</tbody>
</table>

a) MBS = microbial biomass S; MBC = microbial biomass C;
b) Data in a column are means (n=4); data in parenthesis are standard errors of the mean.
c) * and ** indicated significant differences between grassland and forest soils of the corresponding depth at 0.05 and 0.01 probability levels respectively.
4.4 Discussion

In this study, the adjoining soils under grassland and under forest were on a flat terrace, derived from the same parent material (greywacke sandstone loess) and had similar land-use history prior to the planting of the forest stand. Therefore, it is reasonable to assume that adjoining soils were initially similar and that the observed differences in soil properties were primarily due to the change in land-use (see section 3.4). Moreover, the differences in organic S, microbial biomass C and S, and arylsulfatase activity were confined to the topsoil (0-10 cm) over the 19-year time frame after establishment of the tree plantation (Table 4-2; Figure 4-1), which was consistent with results for soil P and associated microbial and enzyme activities described in Chapter 3. These results may be related to the predominance of major functional fine roots (< 2 mm) in the 0-10 cm layer (Table 3-1).

The decrease in organic S content in topsoil under forest indicated that land-use change from grassland to forest enhanced the mineralization of organic S in the upper soil layers (Table 4-2). Release of sulfate-S from the mineralization of organic S may occur by two pathways. One is the oxidation of the organic matter to yield energy and C for biosynthesis by microorganisms and release of sulfate-S as a by-product, while the other is direct hydrolysis of organic sulfate-esters by extracellular enzymes (Dodgson et al., 1982). However, microbial biomass C and S, and arylsulfatase activity were lower in soil under forest than under grassland. This trend is the same as that observed for soil organic P and associated microbial and enzyme activities at this site (Chapter 3). It was previously suggested that the enhanced mineralization of organic P along with organic C might occur at the early stage of forest establishment due to higher demand for nutrients from recently established trees (Chapter 3). The same reasoning would apply to organic S. Ectomycorrhizal association with tree roots may have directly and/or indirectly contributed to the increased mineralization of organic S by improving the uptake, transport and assimilation of sulfate-S by trees (Rennenberg, 2000). Other contributing factors to the observed decrease in soil organic S under forest compared with grassland could be changes in the amounts and forms of organic C (and S) added to soil (Condron and Newman, 1998) together with changes in soil moisture and temperature.
Figure 4-1    Arylsulfatase activity determined for soils and forest floor material under adjacent grassland and forest (standard errors are shown by horizontal bars).

An alternative explanation for the enhanced mineralization of organic S with lower arylsulfatase activity in soils under forest is that the measurement of arylsulfatase activity does not reflect the overall real enzyme activity since there are several types of sulfatase enzyme present in soil (including arylsulfatases, alkylsulfatases, steroid sulfatases, glucosulfatases, chondrosulfatases, and myrosulfatases) (Tabatabai and Bremner, 1970). In addition, the artificial substrate (p-nitrophenol sulfate) used in the measurement may not naturally exist in soil (Zhao et al., 1996). It has also been reported that the activity of arylsulfatase in soil is not consistently correlated with the rate of mineralization of organic S (Speir and Ross, 1978). Ganeshamurthy and Nielsen (1990) suggested that the activity of arylsulfatase in soil was not the rate-limiting factor in the biochemical mineralization of organic S. Instead, they proposed that the rate of the biochemical release of sulfate-S from soil sulfate esters was
primarily governed by dissolution of soil sulfate esters and desorption of the $-\text{OSO}_3\text{H}$ group.

The fact that a greater amount of inorganic S was found in topsoil under forest than under grassland in this study is consistent with other pasture-pine pair studies in New Zealand (Davis and Lang, 1991; Hawke and O’Connor, 1993; Parfitt et al., 1997). In the present study, the major sources of soil inorganic S are likely to be atmospheric input of sulfate-S and mineralization of soil organic S as there was no input of fertilizers, and inputs of S from weathering are likely to be small (Mitchell et al., 1992). It is possible that increased interception of S in aerosol material by the tree canopy (Parfitt et al., 1997) may have contributed to enhanced levels of sulfate-S in soil under forest in the present study, but this is likely to be limited as the annual atmospheric input of S at Broken River near the study site is only 1.6 kg ha$^{-1}$ (Ledgard and Upsdell, 1991). Furthermore, Davis (1998) reported higher sulfate-S levels in soil under pines than under grass after one year in a pot experiment, demonstrating that enhanced sulfate-S may occur under trees in the absence of interception by the forest canopy.

The increase in soil inorganic S with depth under grassland suggests that leaching and plant uptake governed the amount and distribution of inorganic S. On the other hand, under forest inorganic S apparently accumulated in the soil profile in spite of greater uptake of soil S by trees, and there was no significant difference in inorganic S distribution down the soil profile despite the fact that mineralization was restricted to the upper soil layers. This may be partly attributed to a combination of reduced leaching of sulfate-S and increased adsorption due to lower pH (Table 4-1) under trees than grassland.

Extremely low concentrations of inorganic S were found in the forest floor compared with the underlying mineral soils (Table 4-2). This may be due to a combination of factors including leaching (Binkley, 1986) and immobilization of sulfate-S by microorganisms as a result of high organic C to total S ratios (Table 4-1), which is supported by the very high contents of MBS in L and F layers (Table 4-2).

Microbial biomass S is considered to be relatively labile and the most active organic S pool for turnover in soil (Zhao et al., 1996). It has been reported that MBS ranges
from 3 to 300 μg S g⁻¹ dry weight soil (Banerjee and Chapman, 1996). In this study, levels of MBS in soils under both grassland and forest were similar to those found in a New Zealand grassland soil by Ghani et al. (1990).

4.5 Conclusions
The higher levels of inorganic S levels found under forest in this study are consistent with results of earlier studies. The lower organic S levels present under forest indicate that enhanced mineralization of organic S occurred in upper soil layers and this was likely to be the major process responsible for the elevated inorganic S levels under forest at this inland site. Lower arylsulfatase activities under forest compared to grassland at the time of sampling suggest that mineralization of organic S under forest may not be currently mediated primarily by enzyme activity, although enzyme activity may have been important during earlier stages of forest development.
Chapter 5

Seasonal Changes in Soil Phosphorus and Associated Biological Properties under Adjacent Grassland and Forest

5.1 Introduction

It has been reported that conversion from grassland to forest resulted in enhanced mineralization of soil organic matter and higher nutrient availability (Davis and Lang, 1991; Hawke and O'Connor, 1993; Davis, 1994; 1995; Alfredsson et al., 1998), with lower microbial biomass carbon (C), nitrogen (N), phosphorus (P) and nematode populations (Yeates et al., 1997; Yeates and Saggar, 1998). In Chapters 3 and 4, it was confirmed that afforestation mineralized more organic matter and led to higher concentrations of available P and sulfur (S), but lower levels of microbial biomass C, P and S and phosphatase and arylsulfatase activities. However, comparison between adjacent soils under grassland and forest in these studies was based on a single sampling 19 years after forest establishment, and further work was required to investigate seasonal dynamics of organic matter and associated nutrients under the different ecosystems at this site.

Soil P cycling and availability is controlled by a combination of biological processes (mineralization - immobilization) and chemical processes (adsorption - desorption and dissolution - precipitation) (Frossard et al., 2000). The soil microbial biomass plays a central role in nutrient cycling (Jenkinson and Ladd, 1981; Richardson, 1994). A significant amount of P is present in microbial tissues (1.5 – 4 % P of dry weight) (Perrott et al., 1990; Singh and Singh, 1995), and the microbial biomass acts as both a sink and a source of P in soil (Jenkinson and Ladd, 1981; Singh et al., 1989). Extracellular phosphatase enzymes produced by plant roots and soil microorganisms are actively involved in the mineralization of soil organic P (Speir and Ross, 1978; Tarafdar and Claassen, 1988; Häussling and Marschner, 1989). Microbial and enzyme activities are greatly influenced by environmental factors such as soil temperature and moisture content (Rastin et al., 1990; He et al., 1997; Leirós et al., 1999; Piao et al., 2000a, b). Fluctuation in the size and turnover of the soil microbial biomass during the growing season is important in controlling the turnover of C and associated nutrients.
(P and S), which in turn regulates the plant availability of P and S (Patra et al., 1995; He et al., 1997).

The effects of environmental conditions on soil chemical, biochemical and biological properties have been studied in both agricultural and forest ecosystems (Srivastava and Singh, 1989; Perrott et al., 1990; Rastin et al., 1990; Speir and Cowling, 1991; Franzluebbers et al., 1994; Baldocchi et al., 1997; Bardgett et al., 1999). Seasonal changes in soil P fractions may reflect P uptake by plants and mineralization – immobilization processes. However, limited work has been carried out on seasonal dynamics of soil P fractions (Fabre et al., 1996), while the seasonal variations in organic P fractions have been attributed to spatial variation in soils and problems related to P analysis (particularly organic P) (Harrison, 1987). In some studies, clear seasonal variations in some soil P fractions have been observed (Perrott et al., 1990; Tate et al., 1991a, b; Fabre et al., 1996). For example, Perrott et al. (1990) showed that labile organic P, microbial biomass P and organic debris accumulated in winter and declined in spring in a New Zealand pasture soil. Similarly, Fabre et al. (1996) reported that the concentrations of labile P (sodium bicarbonate (NaHCO₃) extractable Po and Pi and sodium hydroxide (NaOH) extractable Po) increased during winter and decreased in spring in a riparian forest (mixture of willow (Salix alba), poplar (Populus alba) and ash (Fraxinus spp.)). These patterns were ascribed to increased mineralization and plant uptake during the growing season and lower microbial activities and mineralization in winter.

Soil biochemical and biological properties, compared with other soil properties, are more sensitive to seasonal environmental changes and frequently show more complicated seasonal patterns (Rastin et al., 1988; 1990). Seasonal trends in soil microbial biomass and enzyme activity vary with soil type (Rastin et al., 1990; Görres et al., 1998; Bardgett et al., 1999), crop species (Patra et al., 1995), land use (Srivastava and Singh, 1989; Luizao et al., 1992) and management practices (Buchanan and King, 1992; Franzluebbers et al., 1994; Campbell et al., 1999a, b). Seasonal variations in these characteristics have been ascribed to a combination of soil moisture, temperature, root activity and organic matter return to soils (litterfall and rhizodeposition) (Rastin et al., 1990; Campbell et al., 1999a, b; Krämer and Green, 2000; Piao et al., 2000).
The specific objectives of this study were to compare chemical, biochemical and biological properties in adjacent unimproved soil (0-5 cm) under grassland and forest over a one-year period (7 samplings), and to determine whether P availability and dynamics and associated biochemical and biological properties in adjacent soil under grassland and forest respond differently to changes in environmental conditions.

5.2 Materials and Methods

5.2.1 Research site
The Craigieburn site used for the studies described in the Chapters 3 and 4 was used for this investigation. Details of the site were described in Chapter 2.

5.2.2 Sampling
Soil samples (0-5 cm) were collected from the subplots by soil corer (6 cm diameter) in the grassland area, and along the tree rows (50-70 cm from tree boles) in the forest stand on 7 occasions between July 1998 and July 1999 (July 21, September 25, November 23, January 25, March 25, May 25 and July 27). The 0-5 cm soil depth selection was based on results obtained from the long-term studies described in chapter 3 and 4. A total of 10 soil cores were taken from each subplot and bulked. Field moist soil was sieved (< 2 mm) and stored at 4 °C during measurement of pH, microbial biomass C and P, respiration, phosphatase activity, resin extractable P and water soluble organic C and P. A subsample of each soil was air-dried and finely ground (< 150 μm) prior to determination of organic C, total N and total P.

Forest floor materials (L and F layers, < 6 mm) were also sampled from 5 areas (10 x 10 cm²) with each subplot. A subsample was cut into 2-4 mm pieces and stored at 4 °C during measurement of pH, water soluble organic C and P, microbial properties and enzyme activities. Another subsample of forest floor material was oven-dried at 70 °C for 72 h prior to determination of total organic C, N and P.

Enzyme activities and soil respiration were measured within 72 h, while pH, water soluble C and P, microbial biomass C and P, resin extractable P were determined within 7 days. These measurements were carried out in the same order after each sampling to avoid the storage effects. The soil moisture content was measured gravimetrically and all results are expressed on an oven-dry basis.
5.2.3 Soil analysis

The pH, total organic C (TOC) and total N concentrations in soil and forest floor materials, and total P concentrations in forest floor materials were measured according to the methods described in Chapter 2.

Soil organic P, inorganic P, and total P were determined by the ignition method of Saunders and Williams (1955) (see Chapter 2). Resin extractable P and sodium bicarbonate extractable P were also determined using the methods described in Chapter 2. However, resin extractable P was only measured in soil samples since resin bags were easily pierced by litter pieces during extraction.

Water soluble organic C (WSOC) and water soluble inorganic P (WSPi) were extracted at a solid: water ratio of 1:2.5 (soil) and 1:5 (forest floor material) on an end-to-end shaker for 1 h, filtered (Whatman No.42 filter paper followed by 0.45-μm membrane (Huang and Schoenau, 1998). The concentration of soluble C in the extracts was determined using a Shimadzu TOC-5000A analyzer, while soluble inorganic P was measured using a malachite green method (Ohno and Zibilske, 1991).

Microbial biomass C (MBC) and P (MBP) were measured by the methods described in Chapter 2. Soil respiration was measured using the method described by Bartha and Pramer (1965). Field moist soil (10 g) and forest floor material (5 g) samples were aerobically incubated in a 1 L sealed glass jar at the respective field temperatures recorded (an average of 24 hours before sampling) for 48 hours and carbon dioxide (CO₂) evolved from soil was trapped in 0.1 M NaOH and measured by titration with 0.05 M HCl.

The measurements of in situ CO₂ efflux in soils under grassland and forest and the forest floor were carried out using the alkaline adsorption method according to Hendrix (1988); PVC cylinders (13 cm diameter and 16 cm long) open at both ends were used as in situ respiration chambers. Three cylinders were inserted 8 cm into the soil in each of four grassland subplots and six cylinders in each of 4 forest subplots. In the forest subplot, the forest floor materials were removed from three of cylinders, and thus the amount of CO₂ evolved from the forest floor was calculated as the difference between respiration rates from cylinders with and without the forest floor materials.
Polyethylene specimen cups (5 cm in diameter and 6 cm long) containing 15 cm 0.05 M NaOH were placed in each cylinder and the cylinders sealed by with tight-fitting plastic tops, covered with the aluminum foil and incubated for 6 h. The residual NaOH was titrated with 0.025 M HCl to determine carbon dioxide evolved.

Phosphomonoesterase (PME) and phosphodiesterase (PDE) activity were determined using the methods described in Chapter 2. However, all these measurements were carried out under the field temperature and soil pH (Harrison and Pearce, 1979).

5.2.4 Rainfall and soil temperature
Daily rainfall was measured at the Landcare Research Station at Craigieburn. Soil (0-5 cm) and forest floor temperatures were measured by thermocouples in two locations randomly chosen in the plots and was logged every 10 minutes (CR-10, Campbell Scientific Instrument Co., Loughborough, UK) over the experimental period (July 1998 – July 1999).

5.2.5 Statistical analysis
Means and standard errors of all data were calculated (n = 4). A two-way ANOVA for repeated measures was performed on the data from mineral soils under grassland and forest to test for significance of effects of seasonality and vegetation type on soil properties using the Genstat 4.2 programme (Lawes Agricultural Trust, Rothamsted, UK). This approach has been used in similar studies of seasonal changes in soil properties (e.g. DeLuca and Keeney, 1994; Diaz-Ravina et al., 1995; Bardgett et al., 1999). Two-way ANOVA was also carried out to test the effects of seasonality and type of forest floor materials on chemical and biological properties of forest floor material. Least significant difference (LSD) test (vegetation x season, P < 0.05) was used to separate the means when the difference was significant. One way ANOVA was carried out for the respective type of vegetation when the interaction between season and vegetation type was not significant, but seasonal effect was significant. In this case, LSD (for the respective vegetations, P < 0.05) was used to separate the means between seasons within the respective vegetation. Correlation coefficients between soil and environmental variables over the experimental period were also calculated using Genstat 4.2.
5.3 Results

5.3.1 Rainfall, soil moisture and temperature
The rainfall data indicated that it was drier in summer (November to January) than any other time during the experimental period (Figure 5-1A). Seasonal patterns for soil moisture were similar for all soil and forest floor samples with highest contents recorded in July (mid-winter) and lowest in January (mid-summer) (Figure 5-1B and C). Soil moisture contents were consistently higher under grassland than forest throughout the experiment. The greatest fluctuation in the moisture content was found in the F layer (Figure 5-1C). The relatively low moisture content in the L layer in May 1998 may be attributed to newly fallen litter. Soil temperature showed the opposite pattern to soil moisture (Figure 5-2A and B). In late autumn and winter (May – August), soil temperature under grassland was lower than under forest, while it was higher under grassland than forest in spring, summer and early autumn (October – March) (Figure 5-2A). The soil moisture contents were negatively correlated with monthly average soil temperature ($r = -0.927$, $P < 0.01$).

5.3.2 Soil pH, C, N and total P
Soil pH values were consistently lower under forest than under grassland for all seasons, but the seasonal patterns were similar (Figure 5-3A). These patterns indicated that soil pH increased slightly over the year. There was no significant seasonal variation observed in pH in F and L layers, while pH values in the L layer were generally lower than those in the F layer throughout the season (Figure 5-3B).

There were no significant differences observed in WSOC between adjacent soils under grassland and forest. Seasonal fluctuations in WSOC were similar in soils under grassland and forest with an obvious peak in summer (January), followed by a decline over late summer and autumn (March, May) and an increase into winter (July 1999) (Figure 5-4A). The WSOC in F and L layers were 50-100 fold higher than in the underlying mineral soils (Figure 5-4B). There was an apparent peak observed in the levels of WSOC for the L layer in March, which may be attributed to the addition of fresh litter (Huang and Schoenau, 1998).

Levels of TOC, total N and total P in soil under grassland were significantly higher than under forest throughout the study period (Figure 5-5; Table 5-1). Seasonal patterns for soil TOC and total N within a respective land-use type (grassland or forest)
Figure 5-1  Distribution of daily rainfall (A) and soil (0-5 cm) and forest floor material moisture contents (B and C) over the period July 1998 to July 1999 at the Craigieburn site (tick positions (A) indicate the middle of the month).
Figure 5-2 Seasonal changes in soil (A) and forest floor (B) temperature over the period July 1998 to July 1999 at the Craigieburn site (tick positions indicate the middle of the month).
Figure 5-3 Changes in the pH of soils (A) and forest floor materials (B) determined over the period July 1998 to July 1999 at the Craigieburn site (standard errors are shown by vertical bars).

Figure 5-4 Changes in water soluble organic C (WSOC) determined for soils (A) and forest floor materials (B) over the period July 1998 to July 1999 at the Craigieburn site (standard errors are shown by vertical bars).
Figure 5-5 Changes in total organic C (TOC), total N, total P, C:N and C:P ratios (C:P for the forest floor material) determined for soils (left) and forest floor materials (right) over the period July 1998 to July 1999 at the Craigieburn site (standard errors are shown by vertical bars).
Table 5-1 F ratios for two-way analyses of variance of seasonal data on chemical, biochemical and biological properties determined for adjacent soils under grassland and forest over the period July 1998 to July 1999 at the Craigieburn site.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Vegetation (grassland, forest)</th>
<th>Season (sampling time)</th>
<th>Vegetation x season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df 1</td>
<td>df 6</td>
<td>df 6</td>
</tr>
<tr>
<td>pH</td>
<td>177.0***</td>
<td>18.6***</td>
<td>1.7</td>
</tr>
<tr>
<td>Soil moisture (%)</td>
<td>331.6***</td>
<td>193.4***</td>
<td>15.8***</td>
</tr>
<tr>
<td>TOC (%)</td>
<td>48.0***</td>
<td>4.2**</td>
<td>4.8***</td>
</tr>
<tr>
<td>N (%)</td>
<td>129.7***</td>
<td>1.08</td>
<td>1.97</td>
</tr>
<tr>
<td>C:N</td>
<td>131.0***</td>
<td>13.0***</td>
<td>6.4***</td>
</tr>
<tr>
<td>C:Po</td>
<td>89.5***</td>
<td>4.7***</td>
<td>3.4**</td>
</tr>
<tr>
<td>WSOC (µg g⁻¹)</td>
<td>0.3</td>
<td>35.1***</td>
<td>2.8*</td>
</tr>
<tr>
<td>WSPI (µg g⁻¹)</td>
<td>23.5***</td>
<td>3.0*</td>
<td>7.2***</td>
</tr>
<tr>
<td>BPI (µg g⁻¹)</td>
<td>298.3***</td>
<td>10.6***</td>
<td>1.4</td>
</tr>
<tr>
<td>BPO (µg g⁻¹)</td>
<td>45.0***</td>
<td>168.6***</td>
<td>5.4***</td>
</tr>
<tr>
<td>Resin-Pi (µg g⁻¹)</td>
<td>6.3*</td>
<td>20.5***</td>
<td>3.9**</td>
</tr>
<tr>
<td>Resin-Po (µg g⁻¹)</td>
<td>25.2***</td>
<td>22.7***</td>
<td>5.1***</td>
</tr>
<tr>
<td>TPi (µg g⁻¹)</td>
<td>406.9***</td>
<td>7.0***</td>
<td>1.1</td>
</tr>
<tr>
<td>TPo (µg g⁻¹)</td>
<td>417.8***</td>
<td>1.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Total P (µg g⁻¹)</td>
<td>208.3***</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>MBC (µg g⁻¹)</td>
<td>428.2***</td>
<td>12.6***</td>
<td>9.3***</td>
</tr>
<tr>
<td>MBP (µg g⁻¹)</td>
<td>51.3***</td>
<td>32.6***</td>
<td>2.0</td>
</tr>
<tr>
<td>Microbial C:P</td>
<td>16.6***</td>
<td>42.2***</td>
<td>1.4</td>
</tr>
<tr>
<td>MBC: TOC (%)</td>
<td>225.3***</td>
<td>9.9***</td>
<td>5.4***</td>
</tr>
<tr>
<td>MBP: Total P (%)</td>
<td>13.7***</td>
<td>35.2***</td>
<td>1.5</td>
</tr>
<tr>
<td>Respiration (µg CO₂-C g⁻¹ h⁻¹)</td>
<td>0.15</td>
<td>190.1***</td>
<td>20.4***</td>
</tr>
<tr>
<td>In situ CO₂ efflux (mg CO₂-C m⁻² h⁻¹)</td>
<td>24.3***</td>
<td>74.6***</td>
<td>22.5***</td>
</tr>
<tr>
<td>PME (p-NP µg g⁻¹ h⁻¹)</td>
<td>165.7***</td>
<td>63.4***</td>
<td>5.0***</td>
</tr>
<tr>
<td>PDE (p-NP µg g⁻¹ h⁻¹)</td>
<td>181.7***</td>
<td>34.0***</td>
<td>3.4**</td>
</tr>
</tbody>
</table>

a) TOC is total organic C; C:Po is the ratio of TOC to organic P; WSOC is water soluble organic C; WSPI is water soluble inorganic P; BPI and BPO are 0.5 M NaHCO₃ extractable inorganic P and organic P, respectively. Resin-Pi and resin-Po are resin (Lewatit MP500A) extractable inorganic P and organic P, respectively. TPi and TPO are the inorganic P and organic P measured by the ignition method of Saunders and Williams (1955). MBC is microbial biomass C; MBP is microbial biomass P; MBC: TOC is the ratio of microbial biomass C to total organic C; MBP: total P is the ratio of microbial biomass P to total P; PME and PDE are phosphomonoesterase and phosphodiesterase activities, respectively.

b) *, ** and *** indicate significant differences at 0.05, 0.01 and 0.001 level (P < 0.05, 0.01 and 0.001), respectively.
were similar although there was no significant seasonal effect on total N and P (Figure
5-5; Table 5-1). Increases in TOC and total N and P and the C:N and C:Po ratios were
apparent in soils under grassland in July 1999 (winter) (Figure 5-5). For the forest
floor materials, the L layer contained higher C and less N and P with higher C:N and
C:P ratios (Figure 5-5; Table 5-2, 5-3). There were no significant seasonal variations
observed in TOC and total N for the L layer (Figure 5-5). In contrast, TOC and total N
in the F layer varied greatly over the year with maximum TOC and minimum total N
(maximum C:N ratio) occurring in January (Figure 5-5). Data for total P in the L and
F layers indicated a distinct seasonal variability with minimum values recorded in
November for the L layer and January for the F layer (Figure 5-5), while variations in
C:P ratios were similar and reflected concomitant changes in TOC and total P over the
year.

5.3.3 Labile P, inorganic and organic P
Compared with values of WSPi in the literature (Huang and Schoenau, 1998; Turner,
2000) levels of WSPi were low with annual average of 0.068 μg g⁻¹ and 0.045 μg g⁻¹ in
soils under grassland and forest, respectively (Table 5-3). Under grassland, WSPi
increased from July 1998 to November 1998, dropped to a minimum in May and
increased again to July 1999 (Figure 5-6A). Concentrations of WSPi in the soil under
forest were less variable over the year than under grassland, with a peak observed in
March. The concentrations of WSPi found in the forest floor materials (F and L
layers) were approximately 100 times higher than the underlying mineral soils (Figure
5-6A and B). There was no significant difference in levels of WSPi between F and L
layers over the year. In addition, the seasonal patterns of WSPi were similar for F and
L layers (except in November 1998).

Sodium bicarbonate extractable Pi (BPi) was consistently higher in soil under forest
than under grassland (Figure 5-7) with annual averages of 4.4 μg g⁻¹ and 1.7 μg g⁻¹,
respectively. The seasonal changes in BPi were similar for both soils with a general
increase over time. The concentrations in the F and L layers were more than 10 times
higher than in the underlying soil (Table 5-3; Figure 5-7). The concentrations of BPi
in the F layer were generally higher than in the L layer, but the seasonal patterns were
similar with a peak in November. Sodium bicarbonate extractable Po (BPo) in soils
under grassland and forest was less variable, although concentrations increased
markedly in winter (July 1999) (Figure 5-7). The concentrations of BPo were
Table 5-2  F ratios for two-way analyses of variance of seasonal data on chemical, biochemical and biological properties determined for forest floor materials (F, L layers) over the period July 1998 to July 1999 at the Craigieburn site.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Litter layer</th>
<th>Season (sampling time)</th>
<th>Litter type x season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent variables</td>
<td>df</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>pH</td>
<td>145.9***</td>
<td>2.16</td>
<td>4.25***</td>
</tr>
<tr>
<td>Soil moisture (%)</td>
<td>876.0***</td>
<td>221.9***</td>
<td>73.4***</td>
</tr>
<tr>
<td>TOC (%)</td>
<td>118.1***</td>
<td>6.9***</td>
<td>7.2***</td>
</tr>
<tr>
<td>N (%)</td>
<td>445.2***</td>
<td>7.2***</td>
<td>5.5***</td>
</tr>
<tr>
<td>C: N</td>
<td>505.0***</td>
<td>5.0***</td>
<td>4.3***</td>
</tr>
<tr>
<td>C: P</td>
<td>287.4***</td>
<td>43.7***</td>
<td>10.7***</td>
</tr>
<tr>
<td>WSO (μg g⁻¹)</td>
<td>30.8***</td>
<td>58.8***</td>
<td>34.6***</td>
</tr>
<tr>
<td>WSPi (μg g⁻¹)</td>
<td>0.11</td>
<td>34.3***</td>
<td>11.8***</td>
</tr>
<tr>
<td>BPi (μg g⁻¹)</td>
<td>80.2***</td>
<td>154.3***</td>
<td>9.9***</td>
</tr>
<tr>
<td>BPo (μg g⁻¹)</td>
<td>413.0***</td>
<td>92.7***</td>
<td>3.64**</td>
</tr>
<tr>
<td>Total P (μg g⁻¹)</td>
<td>256.1***</td>
<td>44.0***</td>
<td>9.7***</td>
</tr>
<tr>
<td>MBC (μg g⁻¹)</td>
<td>400.3***</td>
<td>26.9***</td>
<td>21.0***</td>
</tr>
<tr>
<td>MBP (μg g⁻¹)</td>
<td>731.4***</td>
<td>56.9***</td>
<td>43.7***</td>
</tr>
<tr>
<td>Microbial C:P</td>
<td>23.3***</td>
<td>24.6***</td>
<td>1.77</td>
</tr>
<tr>
<td>MBC: TOC (%)</td>
<td>432.2***</td>
<td>27.5***</td>
<td>19.9***</td>
</tr>
<tr>
<td>MBP: total P (%)</td>
<td>741.7***</td>
<td>62.7***</td>
<td>50.7***</td>
</tr>
<tr>
<td>Respiration (μg CO₂-C g⁻¹ h⁻¹)</td>
<td>10.7*</td>
<td>274.7***</td>
<td>119.6***</td>
</tr>
<tr>
<td>PME (p-PN μg g⁻¹ h⁻¹)</td>
<td>112.7***</td>
<td>10.8***</td>
<td>10.5***</td>
</tr>
<tr>
<td>PDE (p-PN μg g⁻¹ h⁻¹)</td>
<td>113.6***</td>
<td>153.5***</td>
<td>11.8***</td>
</tr>
</tbody>
</table>

* See Table 5-1 for explanation of abbreviations and symbols.
Table 5-3  Range and mean values for chemical properties determined for adjacent soils under grassland and forest and forest floor materials (F, L layers) over the period July 1998 to July 1999 at the Craigieburn site (n = 7)\(^t\).

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Grassland soil</th>
<th>Forest soil</th>
<th>F layer</th>
<th>L layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>pH</td>
<td>5.1-5.6</td>
<td>5.4</td>
<td>4.6-5.2</td>
<td>4.9</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>21-93</td>
<td>57</td>
<td>16-58</td>
<td>39</td>
</tr>
<tr>
<td>TOC (%)</td>
<td>7.14-9.56</td>
<td>7.78</td>
<td>6.02-7.16</td>
<td>6.63</td>
</tr>
<tr>
<td>N (%)</td>
<td>0.413-0.480</td>
<td>0.428</td>
<td>0.322-0.352</td>
<td>0.336</td>
</tr>
<tr>
<td>C:N</td>
<td>17.2-19.8</td>
<td>18.2</td>
<td>18.7-20.8</td>
<td>19.7</td>
</tr>
<tr>
<td>C:Po**</td>
<td>95-117</td>
<td>100</td>
<td>109-130</td>
<td>121</td>
</tr>
<tr>
<td>WSOC (µg g(^{-1}))</td>
<td>41-93</td>
<td>60</td>
<td>41.9-87.3</td>
<td>59.0</td>
</tr>
<tr>
<td>WSPi (µg g(^{-1}))</td>
<td>0.037-0.097</td>
<td>0.068</td>
<td>0.026-0.081</td>
<td>0.045</td>
</tr>
<tr>
<td>BPI (µg g(^{-1}))</td>
<td>0.7-2.4</td>
<td>1.7</td>
<td>3.1-5.5</td>
<td>4.4</td>
</tr>
<tr>
<td>BPo (µg g(^{-1}))</td>
<td>14.7-62.8</td>
<td>25.1</td>
<td>7.8-47.7</td>
<td>19.2</td>
</tr>
<tr>
<td>Resin-Pi (µg g(^{-1}))</td>
<td>1.8-10.7</td>
<td>5.7</td>
<td>4.95-9.46</td>
<td>6.66</td>
</tr>
<tr>
<td>Resin-Po (µg g(^{-1}))</td>
<td>13.1-31.8</td>
<td>21.9</td>
<td>15.1-18.4</td>
<td>24.2</td>
</tr>
<tr>
<td>TPI (µg g(^{-1}))</td>
<td>183-223</td>
<td>202</td>
<td>266-313</td>
<td>287</td>
</tr>
<tr>
<td>Tpo (µg g(^{-1}))</td>
<td>740-814</td>
<td>778</td>
<td>522-578</td>
<td>552</td>
</tr>
<tr>
<td>Total P (µg g(^{-1}))</td>
<td>956-1037</td>
<td>981</td>
<td>835-848</td>
<td>839</td>
</tr>
</tbody>
</table>

\(^t\) See Table 5-1 for explanation of abbreviations. *ND = not determined. **C:P ratio for F and L layers.
generally lower in soil under forest than under grassland. The concentrations of BPo in the F layer were consistently higher than in the L layer over the study period. However, the seasonal changes in BPo were essentially the same in the F and L layers, with peaks in November and July 1999. The levels of resin extractable Pi (resin Pi) were slightly higher in soils under forest than under grassland with annual averages of 6.7 µg g\(^{-1}\) in and 5.7 µg g\(^{-1}\), respectively (Figure 5-7; Table 5-3). Concentrations of resin Pi generally increased with time in soils under grassland and forest. Resin extractable Po was slightly higher in soil under grassland compared with forest with annual averages of 24.2 µg g\(^{-1}\) and 21.9 µg g\(^{-1}\), respectively. The seasonal pattern of changes in resin Po under both grassland and forest were similar to those of BPo (Figure 5-7).

The concentrations of total Pi (TPi) were consistently lower in soil under grassland than under forest with annual averages of 202 µg g\(^{-1}\) and 287 µg g\(^{-1}\), respectively (Figure 5-8A; Table 5-3). The similar seasonal patterns observed under grassland and forest showed that the lowest values of TPi were found in late spring and summer (November, January) while concentrations increased in early autumn (March) (Figure 5-8). The concentrations of TPO were higher in soils under grassland than under forest, although there was no evidence of distinct seasonal fluctuations (Figure 5-8B).
Figure 5-7  Seasonal changes in bicarbonate extractable P (BPi and BPo) and resin extractable P (Pi and Po) determined for soils (left) and forest floor materials (right) over the period July 1998 to July 1999 at the Craigieburn site (standard errors are shown by vertical bars).
5.3.4 Soil microbial biomass and activity

Levels of soil MBC were consistently higher in soils under grassland than under forest, ranging from 1140 to 1705 μg g⁻¹ (average 1417 μg g⁻¹) under grassland compared with 671 to 1027 μg g⁻¹ (average 808 μg g⁻¹) under forest (Figure 5-9; Table 5-4). The seasonal pattern in MBC was also different between soils under grassland and forest (Figure 5-9). In the grassland soil, MBC decreased to its lowest value in mid-summer (January) and then increased between May and July 1999, while MBC in the forest soil remained relatively consistent over the same period. In the forest floor materials, MBC was higher and more variable in the F layer (annual average 7331 μg g⁻¹) than in L layer (annual average 3292 μg g⁻¹). There were two peaks in MBC in the F layer (late spring and late autumn), with the lowest value recorded in late summer (Figure 5-9).

In contrast to soil MBC, seasonal changes in MBP under grassland and forest were generally similar with the lowest values found in late spring (November 1998). Meanwhile, the seasonal patterns for MBP were different from those for MBC. The
concentrations of MBP were consistently higher in soils under grassland than under forest with annual average of 37.4 µg g⁻¹ and 27.4 µg g⁻¹, respectively (Figure 5-9; Table 5-4). The fluctuation in MBP in the F layer was greater than in the L layer (Figure 5-9), and generally similar to that described above for MBC.

Microbial C:P ratios were generally higher in soils under grassland than forest (Figure 5-10; Table 5-4). The seasonal patterns of microbial C:P ratios were similar between soils under grassland and forest with a peak apparent in late spring (November) (Figure 5-10). For the forest floor materials, microbial C:P ratios were higher in the L layer
Table 5-4  Range and mean values for biochemical and biological properties determined in adjacent soils under grassland and forest and forest floor materials (F, L layers) over the period July 1998 to July 1999 at the Craigieburn site (n = 7).

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Grassland soil</th>
<th>Forest soil</th>
<th>F layer</th>
<th>L layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>MBC (µg g⁻¹)</td>
<td>1140-1705</td>
<td>1417</td>
<td>671-1027</td>
<td>808</td>
</tr>
<tr>
<td>MBP (µg g⁻¹)</td>
<td>22.6-51.1</td>
<td>37.4</td>
<td>13.6-42.9</td>
<td>27.4</td>
</tr>
<tr>
<td>Microbial C:P</td>
<td>24-69</td>
<td>42</td>
<td>19-65</td>
<td>35</td>
</tr>
<tr>
<td>MBC: TOC (%)</td>
<td>1.54-2.20</td>
<td>1.83</td>
<td>1.03-1.43</td>
<td>1.22</td>
</tr>
<tr>
<td>MBP: Total P (%)</td>
<td>2.32-5.23</td>
<td>3.80</td>
<td>1.64-5.12</td>
<td>3.26</td>
</tr>
<tr>
<td>CO₂ respiration</td>
<td>0.11-0.84</td>
<td>0.44</td>
<td>0.17-0.65</td>
<td>0.43</td>
</tr>
<tr>
<td>In situ CO₂ efflux (mg CO₂-C g⁻¹ h⁻¹)</td>
<td>3.1-41.6</td>
<td>23.8</td>
<td>11.8-25.4</td>
<td>19.6</td>
</tr>
<tr>
<td>PME (p-PN µg g⁻¹ h⁻¹)</td>
<td>277-596</td>
<td>423</td>
<td>178-486</td>
<td>296</td>
</tr>
<tr>
<td>PDE (p-PN µg g⁻¹ h⁻¹)</td>
<td>7.8-16.9</td>
<td>13.3</td>
<td>5.0-12.3</td>
<td>8.5</td>
</tr>
</tbody>
</table>

¹ See Table 5-1 for explanation of abbreviations. * Values are the amount of CO₂ evolved from the whole forest floor (F + L layers).
Figure 5-10 Changes in microbial C:P, microbial biomass C:total organic C (MBC:TOC) and microbial biomass P:total P (MBP:total P) ratios determined for soils (left) and forest floor materials (right) over the period July 1998 to July 1999 at the Craigieburn site (standard errors are shown by vertical bars).
than in the F layer, although the seasonal pattern was similar to that in the underlying mineral soil (Figure 5-10).

Microbial biomass C constituted 1.54 – 2.20 % and 1.03 – 1.43 % of TOC in soils under grassland and forest, respectively (Table 5-4). Seasonal variations in MBC:TOC ratio were smaller (Figure 5-10; Table 5-2) and were similar to those observed for MBC (Figure 5-9). Microbial biomass C made up 0.67 – 1.95 % and 0.36 – 1.16 % of TOC in the F layer and L layers, respectively (Table 5-4). The seasonal trends in MBC:TOC ratios were similar to those recorded for MBC in the F and L layers (Figure 5-10).

Microbial biomass P comprised 2.32 – 5.32 % of total P in the grassland soils, compared with 1.64 – 5.12 % in the forest soil (Figure 5-10; Table 5-4). Microbial biomass P constituted 13.0 – 72.8 % of total P in the F layer, compared with 5.6 – 20.3 % in the L layer. The seasonal patterns of MBP:total P ratios in both soils under grassland and forest, and in the forest floor material also, were broadly similar to the respective patterns observed for MBP (Figure 5-9).

Soil respiration in the grassland soil increased with time and reached a peak in late spring (November) and declined through autumn and winter (Figure 5-11A). Soil respiration in the forest soil showed a similar pattern. Amounts of CO₂ respired from the grassland soil were higher in late spring and summer (November and January) compared with the forest soil, and lower in late autumn (May 1999) and winter (July 1999). As expected, the levels of respiration measured for forest floor materials were markedly greater than those for the underlying forest soils, and while respiration was greater in the F layer compared with the L layer during spring (July – November), the level of respiration determined in March was substantially greater for the L layer compared with the F layer (Figure 5-11B).

The seasonal trends of in situ CO₂ efflux measured in this study were similar to CO₂ evolution measured in the laboratory under field temperatures (Figure 5-11A and B, Figure 5-12). Seasonal in situ CO₂ efflux ranged from 3.1-41.6 mg CO₂-C m⁻² h⁻¹ in the grassland soil and 11.8 to 25.4 mg m⁻² h⁻¹ in the forest soil (Table 5-4). The CO₂ efflux from the forest floor was much smaller than the underlying soil (Figure 5-12; Table 5-4).
Figure 5-11 Seasonal dynamics of respiration determined for soils (A) and forest floor materials (B) over the period July 1998 to July 1999 at the Craigieburn site (standard errors are shown by vertical bars).

Figure 5-12 Seasonal dynamics of in situ carbon dioxide (CO₂) efflux determined for soils and forest floor materials over the period July 1998 to July 1999 at the Craigieburn site (standard errors are shown by vertical bars). LSD was used only for comparison between soils under grassland and forest.
5.3.5 Phosphatase enzyme activities
Phosphomonoesterase activities were consistently higher in the soil under grassland than under forest, with annual averages of 423 μg p-NP released g⁻¹ h⁻¹ and 296 μg p-NP released g⁻¹ h⁻¹, respectively (Figure 5-13; Table 5-4). Phosphomonoesterase activities fluctuated seasonally and had a similar pattern in both soils (Figure 5-13). Phosphomonoesterase activities in the forest floor materials were higher than in the mineral soils while PME activity in the F layer was greater than in the L layer except in March (Figure 5-13; Table 5-4). Levels of PME activity generally declined with time in the F layer, while PME activity measured in the L layer increased between January and March (Figure 5-13).

Phosphodiesterase activities were 10 – 30 times lower than corresponding PME activities in soils under grassland and forest and forest floor materials (Figure 5-13; Table 5-4). Phosphodiesterase activities in soil under grassland were consistently greater than under forest, while the seasonal trends for these two soils were similar to those described above for PME. In the forest floor material, PDE activities were consistently higher in the F layer than in the L layer, while the seasonal trends were similar in both layers with distinct peaks in late summer (January).

5.3.6 Relationships between soil properties and environmental factors
Statistical analyses were performed to correlate soil environmental factors such as monthly average soil (and forest floor) temperature, monthly rainfall and soil (forest floor) moisture content with selected soil chemical, biochemical and biological properties measured over the period July 1998 to July 1999. The results obtained are summarized in Table 5-5. Monthly average soil temperature was not significantly correlated with any of the measured soil properties under grassland and forest and in the L layer, while in the F layer monthly average soil temperature was directly correlated with TOC and C:P ratio and negatively correlated with TPo (Table 5-5). The limited number of significant relationships observed between monthly average soil temperature and most soil and litter layer properties may be due to the small sample size in the statistical analysis and the interaction with rainfall or soil moisture.
Figure 5-13 Changes in phosphomonoesterase (PME) and phosphodiesterase (PDE) activities determined for soils (left) and forest floor materials (right) over the period July 1998 to July 1999 at the Craigieburn site (standard errors are shown by vertical bars).

Monthly rainfall was found to be positively correlated with MBP and MBP:total P ratios in soils under grassland and forest ($r = 0.825$ and $0.861$ for the grassland soil (P < 0.05) and $0.925$ and $0.931$ for the forest soil (P < 0.01), respectively), although these relationships were not observed for the forest floor materials (Table 5-5).

Soil moisture was positively correlated with TOC, C:Po, BPo, MBC and MBP and negatively correlated with CO$_2$ respiration and PDE activity in the grassland soil (Table 5-5). Similar (but weaker) relationships were evident for the forest soil.
Table 5-5  Correlation coefficients (r) of monthly average soil temperature, rainfall and soil moisture content and selected other soil properties determined for grassland and forest soils and forest floor materials (F, L layers) over the period July 1998 to July 1999 at the Craigieburn site.

<table>
<thead>
<tr>
<th></th>
<th>Grassland</th>
<th>Forest</th>
<th>F layer</th>
<th>L layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp</td>
<td>Rainfall</td>
<td>Moisture</td>
<td>Temp</td>
</tr>
<tr>
<td>WSOC</td>
<td>0.443</td>
<td>-0.603</td>
<td>-0.277</td>
<td>0.54</td>
</tr>
<tr>
<td>TOC</td>
<td>-0.595</td>
<td>0.001</td>
<td>0.637**</td>
<td>-0.655</td>
</tr>
<tr>
<td>C:Po</td>
<td>-0.600</td>
<td>0.041</td>
<td>0.655**</td>
<td>-0.442</td>
</tr>
<tr>
<td>BPo</td>
<td>-0.562</td>
<td>0.045</td>
<td>0.736**</td>
<td>-0.429</td>
</tr>
<tr>
<td>TPo</td>
<td>-0.467</td>
<td>-0.101</td>
<td>0.322</td>
<td>-0.288</td>
</tr>
<tr>
<td>MBC</td>
<td>-0.673</td>
<td>0.268</td>
<td>0.612**</td>
<td>-0.393</td>
</tr>
<tr>
<td>MBP</td>
<td>-0.65</td>
<td>0.825*</td>
<td>0.627**</td>
<td>-0.480</td>
</tr>
<tr>
<td>Microbial C:P</td>
<td>0.257</td>
<td>-0.630</td>
<td>-0.259</td>
<td>0.192</td>
</tr>
<tr>
<td>MBC: TOC</td>
<td>-0.314</td>
<td>0.298</td>
<td>0.188</td>
<td>-0.110</td>
</tr>
<tr>
<td>MBP: total P</td>
<td>-0.617</td>
<td>0.861*</td>
<td>0.581**</td>
<td>-0.481</td>
</tr>
<tr>
<td>CO₂ respiration</td>
<td>0.669</td>
<td>-0.563</td>
<td>-0.704**</td>
<td>0.314</td>
</tr>
<tr>
<td>PME</td>
<td>0.047</td>
<td>0.112</td>
<td>-0.230</td>
<td>-0.306</td>
</tr>
<tr>
<td>PDE</td>
<td>0.383</td>
<td>0.077</td>
<td>-0.552**</td>
<td>0.154</td>
</tr>
</tbody>
</table>

a) Correlation between monthly average soil temperature and rainfall and soil properties were calculated by using the averaged values from 4 plots (n = 7). Correlation between soil moisture content and soil properties were calculated by using data from all plots (replicates) (n = 28).
b) Temp = monthly average soil temperature; rainfall = monthly rainfall.
c) See the Table 5-1 for explanation of abbreviations and symbols. C: P (not C: Po) ratios and total P (not TPo) for F and L layers.
For the forest floor materials, WSOC and TOC were significantly related to moisture content in the F layer ($r = 0.867$ and -0.794, $P < 0.01$), but not in the L layer ($r = 0.337$ and 0.118, ns). Moisture content was also positively correlated with BPo, TPo, MBC, MBP, MBC:TOC and MBP:total P and PME activity, but negatively correlated with C:P ratio and microbial C:P ratios and PDE activity in the F and L layers. Moisture content was not significantly correlated with CO$_2$ respiration in the F and L layers.

Carbon dioxide respiration and in situ CO$_2$ efflux were also plotted against the soil moisture content and actual soil temperature. In the soils under grassland and forest, both CO$_2$ respiration and in situ CO$_2$ efflux decreased with soil moisture content (Figure 5-14A, Fig 5-15) and increased with temperature (Figure 5-16A; Figure 5-17). For the forest floor material, CO$_2$ respiration also decreased with moisture content $\geq$ 57% in the F layer (Figure 5-14B), and a moisture content below 15% constrained CO$_2$ respiration. In the L layer, there was no relationship found between CO$_2$ respiration and moisture content due to the temperature effect (Figure 5-14C). In both the F and L layers, CO$_2$ respiration increased with temperature, but decreased sharply when soil moisture content dropped below 15% (F layer) and 11% (L layer) (Figure 5-16B and C).

5.4 Discussion

While seasonal changes in environmental conditions have been found to affect the soil P dynamics and associated biochemical and biological properties as discussed below, it should be noted that this study was limited to only a one-year period, and environmental conditions may be different from year to year. Thus seasonal studies should ideally be continued for more than one year. The nature of the study site meant that the replicate plots under grassland and forest were not randomly distributed (‘pseudo-replication’ - Yeates and Saggar (1998)), although sampling within each plot was conducted randomly.

5.4.1 Seasonal comparisons of soil characteristics

Results from different sampling dates included in this study clearly demonstrated that levels of soil pH, TOC, total N, total P and selected organic P fractions (BPo, resin Po and TPo) were consistently higher under grassland compared with forest, while inorganic P fractions (BPi and TPi) were lower (Tables 5-1, 5-3; Figures 5-3A, 5-5, 5-
Figure 5-14 Relationships determined between CO₂ respiration and moisture content for soils (A) and the forest floor materials (B, C) over the period July 1998 to July 1999 at the Craigieburn site (n = 28). The data in parenthesis are L layer temperature. The arrow indicates low moisture content in the F or L layer.
This suggests that afforestation has enhanced the net overall mineralization of organic matter and associated organic P as proposed previously (Davis, 1994; 1995; Condron et al., 1996; Alfredsson et al., 1998). On the other hand, the microbial biomass C and P and enzyme activities (PME and PDE) in the soil under grassland were consistently higher than under forest over all seasons, while CO₂ respiration and *in situ* CO₂ efflux under grassland was higher in summer and slightly lower in winter compared with forest (Table 5-4; Figure 5-9, 5-11A, 5-12, 5-13). These support the results from previous studies which involved single sampling (Yeates et al., 1997; Yeates and Saggar, 1998; Ross et al., 1999; Chapter 3). Lower microbial biomass and microbial and enzyme activities in the forest soil compared with adjacent grassland soil reflects a reduction in biological fertility as a consequence of afforestation.
Figure 5-16 Relationships determined between CO$_2$ respiration and the temperature of soils (A) and the forest floor materials (B, C) over the period July 1998 to July 1999 at the Craigieburn site ($n = 7$). The arrows indicate low moisture content.
Figure 5-17 Relationships determined between in situ CO$_2$ efflux and soil temperature over the period July 1998 to July 1999 at the Craigieburn site (n = 7).

5.4.2 Seasonal fluctuation in soil P fractions and soil P recycling

In this study, the substantial increase observed in soil organic C (and to lesser extent total N and total P) under grassland in winter (July 1999) (Figure 5-5) may be attributed to the return of root litter during this period. The increase in soil C:N and C:P ratios in this period was possibly due to the high C:N and C:P ratios apparent in grass roots (69 and 750, respectively, see Chapter 3). Fine root biomass was two-fold higher in the grassland soil than in the forest soil at 0-5 cm depth in this site (Table 3-1, see Chapter 3), which reflects the importance of root turnover in nutrient recycling. No significant seasonal changes in total N and total P (particularly TPo) were found in both soils under grassland and forest, which is consistent with other studies in arable and grassland soils (Magid and Nielsen, 1992; Tate et al., 1991b). These results imply that nutrient demand by plants has been continually met through recycling (root turnover and litterfall), rather than by the further degradation of soil organic matter in these two ecosystems which had not received fertilizer since the forest stand was established 20 years prior to this study.
It has been established that the level of inorganic P in soils depends on a combination of factors including plant uptake, adsorption – desorption, dissolution – precipitation, the mineralization of organic P and microbial immobilization, and fertilizer P addition (Hedley et al., 1982b; Perrott et al., 1990; Frossard et al., 2000). Weaver et al. (1988) found a summer maximum and winter minimum in water soluble Pi in a sandy soil in Australia, while in Denmark Magid and Nielsen (1992) also reported that all soil Pi fractions were at minimum in winter. However, Tate et al. (1991a) in New Zealand and Fabre et al. (1996) in France showed that BPi levels were at their maximum and minimum in winter and summer, respectively. In this study, concentrations of labile inorganic P (WSPi, BPi and resin Pi) were also seasonally variable in grassland and forest soils. In general, the decrease in these inorganic P fractions appeared to be in response to the P uptake while the increase appeared to be due to the mineralization of organic P (BPo and MBP). The increase in WSPi, BPi and resin Pi in early spring was coupled with a decrease in BPo and MBP, which suggested that mineralization of these P pools had occurred, while concentrations of Pi decreased during summer (January), presumably in response to continued plant uptake during the growing season (Figure 5-6A, Figure 5-7, Figure 5-9). The differences in seasonal patterns in inorganic P in different studies may be explained by differences in plant uptake, the extent of fluctuation in soil temperature and moisture over the season and soil types. It is worthwhile noting that the flux (turnover) is probably more important in some cases than the amount of immediately available P (WSPi), since solution Pi taken up by plant and microbes can be rapidly replenished by labile Pi from solid phase (Tate et al., 1991b).

Seasonal changes in organic P pools are considered to be controlled by biological processes in soil which in turn are influenced by soil moisture and temperature and their interaction (Stewart and Tiessen, 1987; Tate et al., 1991b; Fabre et al., 1996). However, Magid and Nielsen (1992) failed to find the effects of soil temperature and moisture and the interaction of these two factors on organic P fractions over a period of 18 months in arable and grassland soils and suggested that the seasonal variation in organic P was caused by changes in solubility rather than by biological transformations.

In this study, the increase in BPo and resin Po observed in late spring was possibly due to mineralization of MBP and/or the breakdown of less labile organic matter and
associated organic P ((moderately labile Po, NaOH extractable (Bowman and Cole, 1978a), not measured in this study). A substantial increase in BPO (and resin Po) in July 1999 (winter) occurred in soils under grassland and forest (Figure 5-7). The increase in BPO in the grassland soil in winter was coupled with a sharp increase in TOC (a smaller increase in total N and total P) (Figure 5-5) and more P retained in microbial biomass (Figure 5-9), which in turn may be attributed to significant returns of root litter. It has been reported that labile organic matter (light fraction), BPO and MBP accumulated in winter and decreased in spring and summer under pasture (Perrott et al., 1990; Tate et al., 1991b; Perrott et al., 1992) and arable soils (Buchanan and King, 1992). These results have been ascribed to increased mineralization of organic matter in spring and immobilization in autumn (Perrott et al., 1990; Tate et al., 1991b). Moreover, reduced microbial activity and associated mineralization in winter also favor the accumulation of organic matter (and Po) (Harrison, 1987).

In the forest soil, contents of TOC, total N and total P remained relatively constant over the year (Figure 5-5), which indicated that organic inputs in winter from root litter were limited. Nonetheless, the sharp increases in BPO and resin Po in the forest soil in winter may be due to a combination of leaching of dissolved organic matter (and P) from the forest floor material and low microbial activity. Fabre et al. (1996) also ascribed the increase in BPi and BPO in winter to the leaching of labile P from the forest litter in a riparian forest. Water soluble organic P was not measured in this study, however, concentrations of labile P (BPO) in the forest floor materials (especially the F layer) were greater than underlying soil (Table 5-3). Most litterfall occurred in autumn, which coincided with a sharp increase in BPO in the forest floor (Figure 5-7). Huang and Schoenau (1998) found that temporal fluxes of soluble N and especially soluble P corresponded to litterfall inputs and they noted a significant correlation between water soluble Po in the forest floor and underlying soil over the year. It has also been reported that organic P is dominant form of dissolved P in the forest floor materials in many forest ecosystems (Qualls and Haines, 1991; Cortina et al., 1995). A number of studies have shown that P in fresh litter (senescent plant tissues) is highly mobile (Qualls and Haines, 1991; Polglase et al., 1992b; Cortina et al., 1995). Qualls and Haines (1991) found that the forest floor in a deciduous forest (Quercus spp.) was an abundant contributor of N and P to the mineral soil in the form of dissolved organic matter. This study also showed that BPO in the forest floor was significantly correlated with BPO in the underlying soil (Figure 5-18). Therefore, it is
likely that leaching of significant amounts of dissolved organic P from fresh litter, enhanced by high rainfall in late autumn and winter, was responsible for the accumulation of labile Po in the forest mineral soil in this study (Figure 5-7).

**Figure 5-18** Relationships determined between bicarbonate extractable Po (BPo) in forest floor materials (F, L layers) and the underlying soil samples (0-5 cm) collected from the Craigieburn site between July 1998 and July 1999 (n = 28).

In summary, seasonal variations in soil P fractions under grassland and forest clearly showed that soil P was taken up by plants and recycled through the roots and leaf litter. The organic inputs from root litter were dominant in the grassland ecosystems while needle litter was dominant in forest ecosystem. Microbial biomass plays an important role and acts as both a P sink (in autumn and winter) and P source (in spring and summer). Labile organic P (MBP and/or BPo) is the most active component of TPo in P recycling. The microbial processes (mineralization – immobilization) that controlled
P recycling were influenced by environmental conditions (precipitation, temperature and moisture).

5.4.3 Seasonal fluctuation in microbial biomass and activity and contribution to P recycling

Seasonal changes in environmental conditions affect microbial biomass and activity either directly by inducing microbial response to soil changes or indirectly by influencing plant metabolism (Díaz-Raviña et al., 1995). Seasonal changes in soil microbial biomass and activity have been ascribed to a combination of factors including substrate availability, root growth, microbial competition and community structure, repression of enzyme activity and the accumulation of recalcitrant and toxic compounds (Bardgett et al., 1999). Seasonal changes in soil biomass C and associated nutrients are important in controlling overall nutrient cycling and availability (He et al., 1997).

In this study, MBC was higher in winter and lower in summer and early autumn in the grassland soil (Figure 5-9), which is consistent with similar studies (Patra et al., 1995; Bardgett et al., 1999; Piao et al., 2000a). In the forest soil, however, MBC was less variable with lowest values observed in summer and highest values in early spring (Figure 5-9) while the MBC in winter (780 µg g⁻¹, an average of two years' values in July) was higher than in summer (690 µg g⁻¹). Díaz-Raviña et al. (1995) also observed that MBC values were highest in spring while MBC in winter was higher than in summer in the forest soil in Spain. It has been suggested that moisture is particularly important in determining MBC of temperate forest soils (Díaz-Raviña et al., 1995) and is a major factor controlling microbial population density and distribution (Aceá and Carballeda, 1990). Death of microbial biomass is clearly related to desiccation and regrowth associated with remoistening (McGill et al., 1986; Pulleman and Tietema, 1999). Soil temperature has been shown to influence microbial activity but not the size of microbial biomass (McGill et al., 1986). In this study, the moisture content was low in summer, particularly in the forest soil (15% of dry weight) and high in winter (93% and 57% for the grassland and forest soils, respectively) (Figure 5-1). Moisture contents were positively correlated with microbial biomass (particularly biomass P) in soils under grassland and forest (Table 5-5), while soil temperature was negatively correlated with microbial biomass (Table 5-5). Therefore, it could be suggested that moisture played a more important role than temperature in determining the size of
microbial biomass and was primarily responsible for the apparent seasonal pattern of changes in microbial biomass. The highest value of MBC in spring in the forest soil may be attributed to an increase in available substrate C derived from root activities (Perrott et al., 1992; Díaz-Raviña et al., 1995). In addition to the effects of soil moisture, the sharp increase in MBC from May (autumn) to July 1999 (winter) in the grassland soil may be attributed to increased supply of soluble organic C (Figure 5-6) derived from fine root turnover (see above).

It was found in this study that MBP was more seasonally variable than MBC (Figure 5-9). Tate et al. (1991b) also found that MBC remained fairly constant throughout the year in a pasture soil, but the P content of the biomass varied over the same period. He et al. (1997) also reported that seasonal changes in soil MBP under pasture were different to those obtained for MBC. In this study, it appeared that MBP was more sensitive to changes in environmental conditions (particularly to soil moisture) and plant growth (P demand) than MBC. Microbial biomass P was better correlated with soil moisture content than MBC in both soils (particularly in the forest soil) (Table 5-5). In addition, MBP and MBP:total P ratios in both soils were significantly and positively correlated with monthly rainfall (Table 5-5). The increased mineralization of P from MBP with increased levels of Bpi (Figure 5-7) in late spring was coupled with higher P demand by plants. Monthly rainfall was lowest in November (36 mm) (Figure 5-1). The decreased moisture content may be partially responsible for P release from biomass in late spring in soils under grassland and forest. The release of P from biomass may also be partly attributed to a combination of the effects of protozoa grazing on bacteria (Perrott et al., 1992), drying - rewetting cycles (Grierson et al., 1998), and increasing soil temperature (Sarathachandra et al., 1988; 1989; Piao et al., 2000a). The observed increase in MBP in autumn and winter was related to a combination of factors including reduced P demand by plants, reduced microbial activity and environmental factors (such as high soil moisture and low temperature) which favor the accumulation of microbial biomass in soil (Perrott et al., 1990; Tate et al., 1991b).

Turnover of nutrients through the soil microbial biomass is an important process which influences overall nutrient availability (Singh et al., 1989; Díaz-Raviña et al., 1995). However, it is difficult to determine the contribution of microbial biomass P to plant P uptake. Brookes et al. (1984) estimated the annual P flux by: biomass P content (kg
biomass P turnover time (2.5 yr), assuming biomass P turnover time was the same as that calculated for biomass C (Jenkinson and Ladd, 1981). They found annual P fluxes through biomass were approximately 2–11 kg P ha$^{-1}$ yr$^{-1}$ for arable soils and 7-40 kg P ha$^{-1}$ yr$^{-1}$ for grassland soils and 21.7 kg P ha$^{-1}$ yr$^{-1}$ for a woodland soil in the UK. In New Zealand, using the same method (assuming annual microbial turnover of 0.3, i.e. turnover time 3.33 yr), Sparling et al. (1994) estimated that annual P fluxes through biomass were 18-22 kg P ha$^{-1}$ yr$^{-1}$ in forest soils and 35-36 kg P ha$^{-1}$ yr$^{-1}$ in pasture soils. However, the turnover time may vary greatly from soil to soil (Brookes et al., 1984), so the annual P flux through biomass estimated by this method must be interpreted with caution when different types of soils are compared. In this study, by calculating the sum of P loss from microbial biomass over the year, it was estimated that annual P release from soil microbial biomass was approximately 29.8 μg P g$^{-1}$ (equivalent to 13.8 kg P ha$^{-1}$ for top 5 cm soil) under grassland and 35 μg P g$^{-1}$ (equivalent to 16.1 kg P ha$^{-1}$ for top 5 cm soil) under forest (Table 5-6). According to the method of McGill et al. (1986), the turnover rate of MBP estimated was also higher in the forest soil (1.28 yr$^{-1}$) than in the grassland soil (0.80 yr$^{-1}$) (Table 5-6). These results clearly demonstrated that MBP plays a key role in P cycling in the grassland and forest ecosystems at the Craigieburn site. In addition, although concentrations of MBP in the forest soil were lower compared with grassland over the year (Figure 5-9), a higher turnover rate of MBP under forest led to greater annual P release from microbial biomass compared with grassland (Table 5-6).

The microbial C:P ratio in pasture soils has been shown to directly related to levels of plant available P (Tate et al., 1991b). Seasonal patterns in microbial C:P ratio were broadly similar in both soils under grassland and forest in this study (Figure 5-10). Lower microbial C:P ratios (20-35) measured in winter (July) indicated a high potential for P release from biomass (Tate et al., 1991b; He et al., 1997), while higher microbial C:P ratios (60-65) found in late spring (November) illustrated the potential for P immobilization in biomass. Changes in microbial C:P ratios with time may also reflect changes in the composition of the soil microbial population. It has been shown that the bacteria contained more N but less P and K than fungi (Anderson and Domsch, 1980). Accordingly, lower microbial C:P ratios found in soils under grassland and forest in winter suggests the fungal population increased relative to bacterial population. The higher soil moisture content observed in winter may have favoured the growth of fungi compared with bacteria (Acea and Carballas, 1990). In addition,
Table 5-6  Calculated seasonal turnover of soil P through the microbial biomass (0-5 cm) under grassland and forest for the period July 1998 to July 1999 at the Craigieburn site †.

<table>
<thead>
<tr>
<th>Season</th>
<th>Sampling period</th>
<th>P released (-) from or stored (+) in microbial biomass over the season (μg g⁻¹)</th>
<th>(kg ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Grassland soil</td>
<td>Forest soil</td>
</tr>
<tr>
<td>Mid-winter – mid spring</td>
<td>Jul 98 - Sep 98</td>
<td>-26.60</td>
<td>-19.50</td>
</tr>
<tr>
<td>Mid-spring - late spring</td>
<td>Sep 98- Nov 98</td>
<td>-2.90</td>
<td>-9.70</td>
</tr>
<tr>
<td>Late spring - mid-summer</td>
<td>Nov 98-Jan 99</td>
<td>+6.70</td>
<td>+6.50</td>
</tr>
<tr>
<td>Mid-summer – mid-autumn</td>
<td>Jan 99 - Mar 99</td>
<td>+9.40</td>
<td>+6.20</td>
</tr>
<tr>
<td>Mid-autumn - late-autumn</td>
<td>Mar 99 - May 99</td>
<td>+8.80</td>
<td>+11.10</td>
</tr>
<tr>
<td>Late-autumn - mid-winter</td>
<td>May 99-Jul 99</td>
<td>-0.30</td>
<td>-5.80</td>
</tr>
<tr>
<td>Total annual release</td>
<td></td>
<td>-29.80</td>
<td>-35.00</td>
</tr>
<tr>
<td>Total annual storage</td>
<td></td>
<td>+24.90</td>
<td>+23.80</td>
</tr>
<tr>
<td>Turnover rate (yr⁻¹)</td>
<td></td>
<td>0.80</td>
<td>1.28</td>
</tr>
</tbody>
</table>

† The amount of turnover of microbial biomass in the unit (kg ha⁻¹) was calculated based on the soil bulk density and the weight of F and L layers obtained in Chapter 3.

Turnover rates of microbial biomass P were calculated as the ratios of total annual biomass P loss to annual average biomass P (McGill et al., 1986).
large amounts of root debris and needle litter returned to the soil in late autumn and winter contain large amounts of cellulose and pectin which also favour the growth of fungi (Sarathchandra et al., 1989).

Water soluble organic C in soil is mainly derived from dead plant (root) material and root exudates (Huang and Schoenau, 1998) and represents readily available C source for microorganisms (McGill et al., 1986). Seasonal patterns for WSOC in both soils were similar (Figure 5-4). An increase in WSOC in spring was possibly due to increased rhizodeposition as plant root grew and continuous increase in WSOC in summer may be attributed to increased turnover of MBC (Piao et al., 2000b). Higher levels of WSOC in winter reflect lower microbial activity and the returns from root litter to the grassland soil and returns from leaf litter in the forest soil.

Both CO₂ respiration determined in the laboratory at field temperature and CO₂ efflux measured in the field should reflect real biological activity in the soil. As expected, CO₂ respiration from soil varied with time throughout the study period (Figures 5-11A, 5-12) and appeared to be inversely related to soil moisture (Figures 5-14A, 5-15) and directly related to soil temperature (Figures 5-16A, 5-17). It has been shown that respiration was positively correlated with temperature in pasture and forest soils when moisture was not limiting (Tate et al., 1993; Fang, 1998). However, in China, Piao et al. (2000b) found that when soil moisture was a limiting factor, CO₂ efflux was directly related to moisture content. In this study, soil moisture content (21-93% under grassland and 16-58% under forest) was generally not limiting, and so CO₂ respiration was primarily controlled by soil temperature. Therefore, the observed negative correlation between soil moisture and respiration may be mainly attributed to significant and inverse correlation appeared between soil temperature and moisture (r = 0.927, P < 0.01). Significant but negative correlation found between CO₂ respiration and MBP in soils under grassland and forest (r = -0.801 (P < 0.01) and -0.516 (P < 0.01), respectively) indicated that increased soil respiration was accompanied by P release from biomass.

It is well established that phosphatase enzymes produced by roots and microorganisms are involved in the mineralization of organic P in soil (Speir and Ross, 1978), and consequently play an important role in P cycling (Magid et al., 1996). In most studies, soil enzyme activity has been measured at optimum pH and temperature (Tabatabai
and Bremner, 1969; Eivazi and Tabatabai, 1977; Adams, 1992), although it has been measured under the field conditions in some studies (e.g. Kang and Freeman, 1999). However, the activity measured under optimum conditions only reflects the potential activity or quantity of active enzymes (Kang and Freeman, 1999). In this study, an attempt was made to correlate phosphatase activity with P availability, and so phosphatase activities were measured under ambient soil pH and temperature conditions. The PME and PDE activities fluctuated throughout the year and the fact that patterns in enzyme activities were similar under grassland and forest indicated that similar processes or factors (root and microbial excretion, soil moisture and temperature etc.) were controlling enzyme activity (Figure 5-13). It has been found that in some cases it is very difficult to interpret seasonal patterns of phosphatase activities (Harrison and Pearce, 1979; Speir and Cowling, 1991). Perrott et al. (1990) failed to find significant seasonal change in phosphatase activity in pasture soils. Large quantities of enzymes are stabilized by interaction with clay mineral and humus and their activity is partly independent of biological regulation (Burns, 1986; Naidja et al., 2000). In this study, while PME activity was not significantly correlated with soil moisture (Table 5-5), PDE activity was negatively correlated with moisture in the grassland and forest soils (r = -0.552 (P < 0.01) for the grassland and -0.369 for the forest, ns). Moisture alone can not explain all the variation in enzyme activity over the season. Significant fluctuations in PME and PDE activity over the year in this study might be due to the combination of the effect of root and microbial activities, moisture and temperature. The PME and PDE activities were significantly and negatively correlated with the level of BPo in soils under grassland (r = -0.560 (P < 0.01) and -0.601 (P < 0.01), respectively) and forest (r = -0.486 (P < 0.05) and -0.521 (P < 0.01), respectively). This suggested that PME and PDE might play a role in the mineralization of organic P.

5.4.4 Role of the forest floor in P recycling in the forest ecosystem
Litterfall represents a major organic input to soil in forest ecosystems, while root turnover can also be substantial (Attiwill and Adams, 1993; Fogel, 1983). The return of leaf litter is important in balancing loss and removal of C, N, P and S from mineral soil by microbial activity and plant uptake (Paffitt et al., 1997; Chapter 3). Nutrients such as N and P in litters are made available to trees following decomposition, which is controlled by soil microflora and fauna interactions. The rate of decomposition
depends on the nature of the litter material and environmental conditions (Attiwill and Adams, 1993).

In this study, seasonal fluctuations in chemical, biochemical and biological characteristics of litter were found to be greater compared with corresponding changes in underlying mineral soil (Figures 5-5, 5-6, 5-7, 5-9, 5-10, 5-11, 5-13). It is important to note that in this study, unlike a litter bag decomposition study, the forest floor materials (both L and F layers) were continuously renewed throughout the year, although most litterfall occurred in autumn (March to May).

Concentrations of TOe and total N showed greater fluctuation with time in the F layer compared with the L layer, while patterns of changes in total P were similar in both materials (Figure 5-5). Changes in C and nutrient contents (N and P) and C:N and C:P ratios in forest floor materials over the season may reflect the extent of decomposition. Several studies have shown that the TOC content and C:N and C:P ratios decrease and nutrient contents (N, P, and S) increase during litter decomposition (Attiwill and Adams, 1993; Gunadi et al., 1998; Kuperman, 1999). The fact that TOC was higher and total N and P were lower in the F layer material in summer compared with late autumn, winter and early spring suggested that F layer materials might be less decomposed in summer (Figure 5-5). This may reflect increased incorporation of L layer materials into the F layer after comminution, fragmentation and oxidation by soil macrofauna - microflora interaction in late spring and summer. This newly incorporated material had relatively high concentrations of TOC and low concentrations of N and P compared with the older materials already present in the F layer.

An alternative explanation for the observed seasonal changes in TOC and associated nutrients in the F layer is that the decomposition process was restricted by environmental conditions during late spring and summer. It has been noted by several workers that temperature and moisture affect litter decomposition via their influence on microbial activity (Attiwill and Adams, 1993; Pulleman and Tietema, 1999). Litter decomposition is primarily controlled by temperature when adequate moisture is available (Witkamp, 1971). However, Gunadi et al. (1998) reported that moisture rather than temperature controlled the decomposition rate of litter materials, while Luizao et al. (1992) also found that microbial activity in litter was greatly influenced
by moisture. In the present study, it appeared that lower temperatures favored the accumulation of MBC and MBP, but restricted microbial activity in both the F and L layers (Table 5-5; Figure 5-16B and C). There was a direct relationship between microbial biomass (especially MBP) and moisture content in the forest floor materials (Table 5-5), while there was also a negative relationship between microbial activity and moisture content ≥ 57% in the F layer and ≥ 15% in the L layer (Figure 5-14B and C). In summer, the temperature in the F layer was approximately 14.5 °C, which did not limit the microbial activity, while low moisture content (approximately 16% of dry weight) in the F layer effectively limited microbial activity and may have also increased microbial death by desiccation (Pulleman and Tietema, 1999), resulting in lower levels of MBC and MBP, together with lower MBC:TOC and MBP:total P ratios (Figures 5-9, 5-10, 5-11, 5-12, 5-14B, 5-16B). This might slow litter decomposition in the F layer during summer.

In the L layer, TOC and total N contents remained relatively constant over the year, but seasonal changes in total P and C:P ratios were similar to those observed in the F layer (Figure 5-5). This is difficult to explain. Seasonal trends in microbial biomass and activity determined in the L layer were similar to those measured in the F layer and were influenced by temperature and moisture content over the season in a similar way (Table 5-5; Figures 5-9, 5-10, 5-11B, 5-14B and C, 5-16B and C). It was possible that continued addition of fresh litter throughout the year might have been responsible for the observed constant TOC and total N contents in the L layer over the year. The lower P contents determined in the L layer in late spring and summer may be attributed to a combination of variations in the P contents of fresh litter (Heng, 1980) and leaching of inorganic P (Quall and Haines, 1991; Polglase et al., 1992b; Attiwill and Adams, 1993).

Large amounts of P were present in the forest floor in this study (Table 5-3). In the F layer, the levels of BPi ranged from 15 to 65 μg P g⁻¹ which were 3-10 times higher than those in the underlying mineral soil, while levels of BPo ranged from 41 to 129 μg P g⁻¹ which were 3-5 times higher than those in the underlying mineral soil (Table 5-3). There were also significant amounts of labile P present in the L layer (Table 5-3). The precise fate of P returned to the forest floor in litter is not clear (Quall and Haines, 1991), although a number of pathways have been suggested. For example, labile P may be directly taken up by fine roots and fungal hyphae present in the forest
floor layers (Quall and Haines, 1991; Vogt et al., 1991a). Numerous fine roots and fungal hyphae were observed in the forest floor in this study and in many other studies (Attiwill and Adams, 1993; Fahey and Hughes, 1994), while the amount of P taken up directly by roots and associated mycorrhizae has yet to be quantified. Recently, Perez-Moreno and Read (2000) found that the mycelium of ectomycorrhizae (P. involutus) could mobilize and transfer a significant proportion of organic P from beech (Fagus sylvatica), birch (Betula pendula) and pine (Pinus sylvestris) litter to birch tree seedlings. In addition, P may be transferred from litter to the underlying mineral soil by leaching either directly or via the microbial biomass (Polglase et al., 1992b). Yanai (1991) indicated that P flux from forest floor to mineral soil ranged from 0.3-1.0 kg P ha\(^{-1}\) yr\(^{-1}\) in a hardwood forest. Quall and Haines (1991) found that annual output from the forest floor in a deciduous forest by leaching in the form of dissolved organic P made up 14% of P input in solid litterfall, while the leaching was not a source of inorganic P for the mineral soil since much of inorganic P from the fresh litter materials and from throughfall was immobilized in the microbial biomass. However, other studies have shown that inorganic P is the dominant form of soluble P lost from litter (Polglase et al., 1992b; Huang and Schoenau, 1998). Furthermore, F layer material undergoes further decomposition and may be directly mixed and incorporated into the mineral soil and become part of organic matter in the mineral soil with the help of soil macrofauna - microflora interaction (Attiwill and Adams, 1993).

Phosphorus transfer from the forest floor to the mineral soil may be enhanced by the rapid turnover of P through microbial biomass. There was a large amount of MBP found in the forest floor materials in the present study (annual average of 250 µg P g\(^{-1}\) in the F layer and 50 µg P g\(^{-1}\) in the L layer). More distinct seasonal changes in MBP were observed in F layer than in the underlying mineral soil (Figure 5-9). The decrease in MBP was often accompanied by the release of labile inorganic P. For example, the reduction of MBP in the F layer in spring (September - November 1998) coincided with a sharp increase in inorganic P (BPi) (Figure 5-7). Table 5-6 shows that annual P release through microbial biomass reached 418 µg g\(^{-1}\) (equivalent to 4.9 kg P ha\(^{-1}\)) in the F layer and 53 µg P g\(^{-1}\) (equivalent to 2.1 kg P ha\(^{-1}\)) in the L layer. This result, together with high turnover rate of MBP (1.67 and 1.06 for the F and L layer, respectively), highlights the important role played by the microbial biomass in P transformations in the forest floor.
As expected, the forest floor contained significant amounts of WSOC (annual averages of 2831 and 4220 µg C g⁻¹ in the F and L layer, respectively). Quall and Haines (1991) also reported that approximately 27% of the C in freshly fallen autumn litter in a deciduous forest was water soluble, and the average annual output of dissolved organic C from the forest floor was about 18% of the input in solid litterfall for organic C. Huang and Schoenau (1998) further confirmed that WSOC can be leached from the forest floor to the mineral soil. It is well known that WSOC is an important energy source for soil microorganisms (McGill et al., 1981). The supply of WSOC from the forest floor to the mineral soil will influence microbial activity in mineral soil which in turn will influence nutrient cycling and availability.

5.5 Conclusions

Results from this study clearly showed that levels of soil pH, TOC, total N, TP and organic fractions of P under grassland were consistently higher, but inorganic P fractions were lower compared with forest over all seasons. These findings support previous suggestions that grassland afforestation enhances the mineralization of organic matter and associated organic P (Davis, 1994; 1995; Condron et al., 1996; Alfredsson et al., 1998; Chapter 3). On the other hand, the microbial biomass C and P and enzyme activities (PME and PDE) in the soil under grassland were consistently higher than under forest, while soil respiration and in situ CO₂ efflux under grassland was much higher in summer and slightly lower in winter compared with forest. This is consistent with results from previous studies carried out in New Zealand plantation forestry (Yeates et al., 1997; Yeates and Saggar, 1998; Ross et al., 1999; Chapter 3).

Based on results from this study, seasonal dynamics of P in both grassland and forest ecosystems are summarized in Figure 5-19. It was suggested that P recycling was mainly driven by plant P demand and sustained by mainly root litter inputs in the grassland ecosystem and needle litter input in the forest ecosystem. Seasonal changes in environmental conditions (rainfall, soil moisture and temperature) influenced the biological and biochemical processes involved in P cycling. In general, organic P (labile Po) was mineralized by the increasing microbial activity to meet the increasing P demand by plant growth in spring and summer, while the accumulation of organic P (labile Po) was increased by organic inputs and slow growth of plants and low microbial activity in late autumn and winter. Labile Po is an important component in P
recycling in grassland and forest soils, while the microbial biomass also plays a pivotal role in P cycling. Annual release of P through the microbial biomass was higher in the forest soil (16.1 kg ha\(^{-1}\)) than in the grassland soil (13.9 kg ha\(^{-1}\)). Turnover rate of biomass P was also higher in the forest soil (1.28 yr\(^{-1}\)) than in the grassland soil (0.80 yr\(^{-1}\)).

Abundant C and P (particularly labile forms) and high microbial and enzyme activities found in the forest floor confirms the importance of the forest floor in P cycling. Labile P in the forest floor can be directly taken up by tree fine roots and mycorrhizal hyphae or transferred to the underlying mineral soil by leaching and/or pedoturbation by earthworms. Water soluble organic C in the forest floor can also be potentially leached to the mineral soil and thus regulates microbial activity and hence P availability.

![Diagram](image_url)

**Figure 5-19** Seasonal soil dynamics in adjacent soils under grassland and forest at the Craigieburn site.
Chapter 6

Effects of Plant Species on Soil Phosphorus Dynamics and Associated Biological Properties

6.1 Introduction

Phosphorus (P) is one of the most important nutrients for plant growth, but low availability of P is a major limiting factor to productivity in many managed ecosystems in the world (Raghothama, 1999). Phosphorus is chemically reactive and has been found in more than 170 minerals (Holford, 1997), while up to 80% of total P found in topsoil is present in organic forms (Magid et al., 1996). Soil P availability has been related to a combination of factors including parent material (Walker and Syers, 1976), soil properties (Harrison, 1979; Krammer and Green, 1999; Samadi and Gilkes, 1999), type of vegetation (Magid, 1993; Davis, 1995; Giardina et al., 1995; Zou et al., 1995; Condon et al., 1996; Cade-Menun and Berch, 1997; Binkley et al., 2000), and management practices (Beck and Sanchez, 1996; Reddy et al., 1996; Oberson et al., 1996; Zhang and MacKenzie, 1997a, b).

Previous studies have suggested that afforestation may increase the level of available P in the topsoil compared with grassland (Davis and Lang, 1991; Belton et al., 1995; Condon et al., 1996; Chapter 3), while effects of plantation forest on P availability varied with soil type, stand age and grassland management history (improved or unimproved) (Belton et al., 1995; Davis, 1995; Condon et al., 1996; Perrott et al., 1999). However, only the quantity of the available P was measured by chemical extraction methods in these studies. A complete understanding of soil P availability requires consideration of quantity, intensity and capacity factors (Beckett and White, 1964; Daughtrey et al., 1973; Frossard and Sinaj, 1997). In recent years, an isotopic exchange kinetics (IEK) technique that measures these three factors has been developed (Fardeau et al., 1985; Frossard and Sinaj, 1997), and used to characterize P availability in soils and composts (Tran et al., 1988; Frossard et al., 1996; Lopez-Hernandez et al., 1998; Traore et al., 1999).
Phosphorus availability is sustained by P cycling in native and managed ecosystems (Attiwill and Adams, 1993; Frossard et al., 2000). Soil P transformations are primarily mediated by microbial activity (Richardson, 1994), which in turn is influenced by a combination of factors including plant species, soil type and environmental conditions. Plants not only take up P from the soil but also exert significant effects on soil P availability and dynamics through litterfall, root turnover and exudation, and specific interactions with microbes in the rhizosphere (Attiwill and Adams, 1993; Lajtha and Harrison, 1995; Magid et al., 1996). However, there is little information available about how plant species affect P availability and associated biochemical and biological properties in different soils.

The objectives of this glasshouse pot experiment were to investigate the effect of two contrasting species (radiata pine and ryegrass) on the nature and availability of P in a range of New Zealand grassland soils as characterized by IEK and chemical extraction methods, and to examine the biochemical and biological processes involved in soil P dynamics under ryegrass and radiata pine.

6.2 Materials and Methods

6.2.1 Soils

The fifteen topsoils described in Chapter 2 (section 2.1.2) were used in this study. Soil samples were air-dried and passed through a 4 mm sieve prior to the glasshouse experiment. Separate air-dried subsamples of each soil were ground and passed through a 2 mm and a 150 μm sieve prior to chemical and physical analyses as described below.

6.2.2 Glasshouse experiment

Each of the soil samples was weighed into 6 small pots (80 mm x 80 mm x 120 mm). Amounts of each soil used are showed Table 6-1 and ranged from 162 to 303 g per pot depending on bulk density as determined by soil properties such as organic matter content. Two plant species, radiata pine (Pinus radiata) (seed lot 92/34 GF12) and perennial ryegrass (Lolium perenne) (cultivar ‘Grasslands Nui’), were used in this glasshouse experiment. Seeds were directly sown in the pot and plants of each species were thinned to five per pot after germination. There were three replicates for each soil and each plant species (total 90 pots). All pots were placed on a capillary mat in
the glasshouse in a completely randomized design, and soil moisture contents were
maintained at 60-70% of field capacity using a water computer (GARDENA 1030,
GARDENA Kress + Kastner GmbH). Radiata pine seedlings were inoculated with
ectomycorrhizae (*Rhizopogon rubescens*) at a rate of $1 \times 10^7$ spores per pot two weeks
after sowing. No nutrients were added to the pots during the experiment. The average
daily temperature was between 12 °C and 25 °C.

Plants were harvested after 40 weeks and separated into root and shoot components.
The ryegrass was not cut during the 40 week growth period in order to simulate
ungrazed conditions (see Chapters 3, 4 and 5), which is consistent with the
methodology used by Davis (1995). Approximately 1 g of fresh fine roots was
 subsampled from three replicates of each plant species for determination of root
phosphatase activity. The shoot material and the remainder of the root material were
oven-dried at 65 °C for 72 h and weighed. These plant samples were finely ground (<
150 μm) prior to chemical analysis (total P).

Fresh soil samples from each pot were thoroughly mixed and a subsample was taken
and sieved through 2 mm, and stored at 5 °C prior to determination of pH, water
soluble organic C (WSOC), sodium bicarbonate extractable Pi and Po (BPi and BPo),
resin extractable Pi and Po (resin Pi and Po), microbial biomass C and P, phosphatase
activity and soil respiration. The remaining soil was air-dried and ground (< 2 mm and
< 150 μm) prior to chemical analysis.

6.2.3 Soil and plant analyses

Soil chemical and physical analysis
Soil pH, total soil organic carbon (TOC), total nitrogen (N), total sulfur (S), dithionite
and oxalate extractable Fe and Al, WSOC, phosphate sorption index (PSI) and texture,
together with plant P, were determined according to the methods described in Chapter
2.

Chemically extractable soil P
Soil inorganic P (Pi), organic P (Po) and total P were determined by the ignition
method (Chapter 2). Sodium bicarbonate and resin extractable Pi and Po were
determined using methods described in Chapter 2.
**Isotopically exchangeable P**

Isotopic exchange kinetics was carried out on < 2 mm sieved soil according to the methodology described by Fardeau et al. (1985) and Frossard and Sinaj (1997). This involved shaking 10 g of soil with 99 ml of deionised water for 16 h prior to the addition of 1 ml of solution containing a known quantity of carrier-free $^{33}$P with continuous agitation on a magnetic stirrer. Solution samples (2-3 ml) were removed from the soil/water suspension after 1, 10, 30, and 60 minutes and filtered immediately <0.20 μm prior to determination of $^{31}$P (malachite green colorimetry (Ohno and Zibilske, 1991; Sinaj et al., 1998)) and $^{33}$P (scintillation counting: 1 ml + 4 ml scintillation cocktail (Packard Ultima Gold) on a Wallac 1415 liquid scintillation counter). When $^{33}$PO$_4^-$ ions are added to a soil solution system at a steady state equilibrium, the radioactivity in solution decreases with time according to the following equation (Fardeau et al., 1985):

$$r(0)/R \equiv \{ r(1)/R \} \times \{ t + [r(1)/R]^{1/n} \}^-n + r(\infty)/R$$  \[6.1\]

where R is the total introduced radioactivity ($\approx 0.1$ MBq); $r(1)$ and $r(\infty)$ are the radioactivity (MBq) remaining in the solution after 1 minute and infinite time, respectively, and $n$ is a parameter describing the rate of disappearance of the radioactive tracer from the solution after 1 minute. The parameter $n$ is calculated as the factor (slope) of the linear regression between log $[r(0)/R]$ and log(t). The ratio $r(\infty)/R$, which is the maximum possible dilution of the isotope, is operationally approximated by the ratio of the water soluble P to the total soil inorganic P ($P_T$, expressed in mg P kg$^{-1}$ soil, Fardeau, 1996). Thus:

$$r(\infty)/R = 10*C_P/P_T$$  \[6.2\]

where $C_P$ is the water soluble P (mg P L$^{-1}$). The factor 10 arises from the fact that, during an isotopic exchange experiment, the soil: solution ratio is 1:10 so that 10*$C_P$ is equivalent to the water soluble P quantity in the soil expressed in mg kg$^{-1}$.

The quantity, $E(0)$ (mg P kg$^{-1}$ soil), of isotopically exchangeable P at a time $t$ can be calculated assuming that (i) $^{31}$PO$_4^-$ and $^{33}$PO$_4^-$ ions have the same fate in the system, and (ii) whatever the time, $t$, the specific activity of the phosphate ions in soil solution is similar to that of isotopically exchanged phosphate ions in the whole system:

$$r(0)/(10*C_P) = R/E(0)$$  \[6.3\]
Therefore,

\[ E(t) = 10^*C_p \times R/r(t) \]  \hspace{1cm} [6.4]

For \( t = 1 \) minute,

\[ E_{1\text{min}} = 10^*C_p \times (R/r_1) \]  \hspace{1cm} [6.5]

\( R/r_1 \) is the ratio of the total introduced radioactivity to the radioactivity remaining in the solution after 1 minute and is considered as an estimation of the P-ions buffering capacity of soils (Tran et al., 1988; Salcedo et al., 1991; Frossard et al., 1992; 1993). The buffering capacity is considered to be high for \( R/r_1 \) values > 5, medium for \( R/r_1 \) values of 2.5 to 5, and low for \( R/r_1 \) values < 2.5.

Isotopic exchange kinetics provided data for the intensity factor (\( C_p \), water soluble P), capacity factors (\( R/r_1 \) and \( n \)) and the quantity factor (\( E \) values, isotopically exchangeable soil P). The quantity factor is presented by a pluricompartmental model that allows for the quantification of the phosphate ions present in compartments of differing mobility (Fardeau et al., 1985) and the amounts of P in the following pools were calculated according to equations 6-1 to 6-5 described previously:

\( (i) \) the pool of 'free' phosphate ions, exchangeable within 1 minute (\( E_{1\text{min}} \)). This pool is composed of ions in the soil solution and those ions that are adsorbed on the solid phase of the soil, but have the same kinetic properties as those in solution (Fardeau et al., 1985). Phosphate ions located in this compartment are completely and immediately plant available. These ions may be directly exchanged with ions presented in the three other pools (Fardeau, 1996);

\( (ii) \) the pool of P exchangeable between 1 minute and 24 hours (\( E_{1\text{min}-24h} \)). This pool corresponds to the quantity of phosphate exchangeable during a period equivalent to the time of active P uptake by a single root or by a root hair.

\( (iii) \) the pool of P exchangeable between 24 hours and 3 months (\( E_{24h-3m} \)). This pool corresponds to the quantity of phosphate exchangeable during a period equivalent to the time of active P uptake by the entire root system of an annual crop.

\( (iv) \) the pool of P which can not be exchanged within 3 months (\( E_{>3m} \)). This pool is determined as the difference between total soil inorganic P (assessed by extraction with 0.5 M sulfuric acid (H\(_2\)SO\(_4\)) (Saunders and Williams (1955)) and the sum of the other Pi pools described above.
**Biochemical and biological analysis**

Microbial biomass C and P, acid phosphomonoesterase (AcPME) and alkaline phosphomonoesterase (AIPME) activities were determined by the methods described in Chapter 2.

Soil respiration was measured using the method described by Bartha and Pramer (1965). Fresh moist soil samples (10 g) were aerobically incubated in a 1 L sealed glass jar at 22 °C for 10 days and carbon dioxide (CO₂) evolved from the soil was trapped in 0.1 M sodium hydroxide (NaOH) and the residual NaOH was titrated to phenolphthalein endpoint with 0.05 M hydrochloric acid (HCl). Carbon dioxide evolved was calculated from the difference in normality between samples and NaOH blanks.

Root phosphatase activity was measured by the modified method of Alexander and Hardy (1981) and Dodd et al. (1987). A preliminary experiment was carried out using a series of buffers ranging from pH 3 to 12 (3-7, citrate buffer; 7.5-12, Tris buffer) to determine the optimum pH for root phosphatase activity of each plant species. Results showed that optimum buffer pH for root surface phosphatase activity was 4.5 for ryegrass and 3.5 for radiata pine (Figure 6-1). All subsequent measurements of root phosphatase activities were made at the determined optima. In brief, 200 mg of fine roots (< 0.5 mm diameter) were incubated for 1 hour at 25 °C in 1 ml of 50 mM p-nitrophenyl phosphate and 4 ml of 1.0 mM sodium citrate buffer. Roots were removed and 5 ml of 0.5 M NaOH were added to the solution and the absorbance read at 400 nm on a UV/VIS spectrophotometer (Philips).

### 6.2.4 Statistical analysis

A two-way ANOVA was carried out using the Genstat 4.2 (Lawes Agricultural Trust, Rothamsted, UK) on the data (root and shoot biomass and P uptake, and soil chemical, biochemical and biological variables) to test significant effects of plant species and soil type. The Least Significant Difference (LSD) test was used to separate differences between species for individual soils when the species x soils interaction was significant. Multiple regression analysis was also carried out using Genstat 4.2; the stepwise estimation procedure was used and only the independent variables with significant correlation with the dependent variable were fitted into the final regression.
Correlation coefficients between soil chemical, biochemical and biological variables, root and shoot biomass, and P uptake were calculated using mean values (n = 15) (Genstat 4.2).

Figure 6-1 Effects of buffer pH on root phosphatase activities of ryegrass and radiata pine roots.

6.3 Results

Chemical and physical properties of the 15 soils before planting are shown in Table 6-1, while the corresponding IEK data for the soils prior to planting are presented in Table 6-2. Soil pH values ranged between 5.0 and 6.0 except for the Himatangi (7.0) and Temuka (6.5) soils. The Stratford, Oruanui, and Patoka soils contained high concentrations of TOC (> 6.5%) and total P (> 1100 µg g⁻¹), while the Mangamahu, Pukaki, Fork and Mapua soils contained lower concentrations of TOC (< 5%) and total P (< 700 µg g⁻¹). Soil organic P made up 35 – 82% of total P (average 61%), while PSI ranged from 3.1 to 59.4 (average 23.6%) (Table 6-1). The Egmont, Stratford, Mangamahu, Oruanui, and Patoka soils had high PSI (> 30), while the Himatangi and Temuka and Okarito soils had low PSI (< 10). As expected, the distribution of P in the different exchangeable pools determined by IEK also varied markedly amongst soils (Table 6-2). For example, the Himatangi and Patoka soils had similar concentrations of inorganic P, but 72% of the inorganic P in the Patoka soil was present in E_{1min-3m} Pi
Table 6-1  Chemical and physical properties determined for the selected New Zealand grassland soils before planting.

<table>
<thead>
<tr>
<th>Soil</th>
<th>pH</th>
<th>Organic C (%)</th>
<th>Total N (μg g⁻¹)</th>
<th>Total P (μg g⁻¹)</th>
<th>Total S (μg g⁻¹)</th>
<th>C/N</th>
<th>C/P</th>
<th>Dithionite Fe%</th>
<th>Oxalate Fe%</th>
<th>P sorption index (PSI) (mg 100g⁻¹) / (μmol L⁻¹)</th>
<th>Soil dry weight (g pot⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egmont</td>
<td>5.7</td>
<td>5.30</td>
<td>0.47</td>
<td>629</td>
<td>74</td>
<td>11</td>
<td>84</td>
<td>1.73</td>
<td>0.62</td>
<td>34.5</td>
<td>226</td>
</tr>
<tr>
<td>Stratford</td>
<td>5.4</td>
<td>6.78</td>
<td>0.70</td>
<td>2746</td>
<td>147</td>
<td>10</td>
<td>25</td>
<td>0.90</td>
<td>0.79</td>
<td>48.2</td>
<td>217</td>
</tr>
<tr>
<td>Mangamahu</td>
<td>5.3</td>
<td>3.93</td>
<td>0.38</td>
<td>401</td>
<td>37</td>
<td>10</td>
<td>98</td>
<td>0.98</td>
<td>0.34</td>
<td>32.0</td>
<td>244</td>
</tr>
<tr>
<td>Himatangi</td>
<td>7.0</td>
<td>2.90</td>
<td>0.30</td>
<td>862</td>
<td>46</td>
<td>10</td>
<td>34</td>
<td>0.36</td>
<td>0.16</td>
<td>3.1</td>
<td>290</td>
</tr>
<tr>
<td>Taupo</td>
<td>5.1</td>
<td>5.54</td>
<td>0.44</td>
<td>1311</td>
<td>63</td>
<td>13</td>
<td>42</td>
<td>0.33</td>
<td>0.36</td>
<td>24.1</td>
<td>187</td>
</tr>
<tr>
<td>Oruanui</td>
<td>5.3</td>
<td>8.89</td>
<td>0.62</td>
<td>1127</td>
<td>90</td>
<td>14</td>
<td>79</td>
<td>0.42</td>
<td>0.55</td>
<td>44.0</td>
<td>162</td>
</tr>
<tr>
<td>Pukaki</td>
<td>5.2</td>
<td>4.91</td>
<td>0.31</td>
<td>663</td>
<td>38</td>
<td>16</td>
<td>74</td>
<td>0.54</td>
<td>0.24</td>
<td>20.8</td>
<td>203</td>
</tr>
<tr>
<td>Fork</td>
<td>6.0</td>
<td>2.52</td>
<td>0.20</td>
<td>690</td>
<td>20</td>
<td>13</td>
<td>37</td>
<td>0.38</td>
<td>0.24</td>
<td>11.7</td>
<td>303</td>
</tr>
<tr>
<td>Hurunui</td>
<td>5.6</td>
<td>7.87</td>
<td>0.67</td>
<td>904</td>
<td>82</td>
<td>12</td>
<td>87</td>
<td>1.21</td>
<td>0.27</td>
<td>14.2</td>
<td>212</td>
</tr>
<tr>
<td>Richmond</td>
<td>5.7</td>
<td>3.65</td>
<td>0.33</td>
<td>813</td>
<td>46</td>
<td>11</td>
<td>45</td>
<td>1.18</td>
<td>0.32</td>
<td>15.6</td>
<td>274</td>
</tr>
<tr>
<td>Temuka</td>
<td>6.5</td>
<td>3.96</td>
<td>0.38</td>
<td>1056</td>
<td>59</td>
<td>10</td>
<td>38</td>
<td>0.41</td>
<td>0.20</td>
<td>3.7</td>
<td>240</td>
</tr>
<tr>
<td>Patoka</td>
<td>5.7</td>
<td>9.34</td>
<td>0.85</td>
<td>1585</td>
<td>123</td>
<td>11</td>
<td>59</td>
<td>0.85</td>
<td>1.22</td>
<td>59.4</td>
<td>170</td>
</tr>
<tr>
<td>Te Kauwhata</td>
<td>5.7</td>
<td>5.00</td>
<td>0.48</td>
<td>938</td>
<td>77</td>
<td>10</td>
<td>53</td>
<td>1.93</td>
<td>0.41</td>
<td>18.8</td>
<td>213</td>
</tr>
<tr>
<td>Mapua</td>
<td>5.2</td>
<td>1.95</td>
<td>0.10</td>
<td>116</td>
<td>16</td>
<td>20</td>
<td>168</td>
<td>0.75</td>
<td>0.12</td>
<td>17.5</td>
<td>279</td>
</tr>
<tr>
<td>Okarito</td>
<td>5.4</td>
<td>13.04</td>
<td>0.73</td>
<td>597</td>
<td>103</td>
<td>18</td>
<td>218</td>
<td>0.10</td>
<td>0.17</td>
<td>7.2</td>
<td>168</td>
</tr>
<tr>
<td>Mean</td>
<td>5.7</td>
<td>5.71</td>
<td>0.46</td>
<td>963</td>
<td>68</td>
<td>13</td>
<td>76</td>
<td>0.80</td>
<td>0.40</td>
<td>23.6</td>
<td>226</td>
</tr>
</tbody>
</table>
Table 6-2  Isotopic exchange kinetics parameters and Pi pools (µg g\(^{-1}\)) determined for the selected New Zealand soils before planting.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Cp (mg L(^{-1}))</th>
<th>R/(r_1)</th>
<th>(n)</th>
<th>(E_{1\text{min}})</th>
<th>(E_{1\text{min}-24h})</th>
<th>(E_{24h-3m})</th>
<th>(E_{&gt;3m})</th>
<th>(E_{1\text{min}-3m})%*</th>
<th>Inorganic P**</th>
<th>Organic P**</th>
<th>Organic P % of total P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egmont</td>
<td>0.08</td>
<td>9.5</td>
<td>0.43</td>
<td>8.4</td>
<td>73.1</td>
<td>48.0</td>
<td>14.1</td>
<td>90.2</td>
<td>144</td>
<td>485</td>
<td>77.2</td>
</tr>
<tr>
<td>Stratford</td>
<td>0.16</td>
<td>8.9</td>
<td>0.46</td>
<td>16.3</td>
<td>321.0</td>
<td>744.6</td>
<td>498.0</td>
<td>68.5</td>
<td>1580</td>
<td>1166</td>
<td>42.5</td>
</tr>
<tr>
<td>Mangamahu</td>
<td>0.02</td>
<td>7.4</td>
<td>0.46</td>
<td>2.1</td>
<td>31.1</td>
<td>38.9</td>
<td>14.8</td>
<td>83.0</td>
<td>87</td>
<td>315</td>
<td>78.4</td>
</tr>
<tr>
<td>Himatangi</td>
<td>0.97</td>
<td>1.1</td>
<td>0.12</td>
<td>13.2</td>
<td>10.0</td>
<td>14.6</td>
<td>493.3</td>
<td>7.1</td>
<td>531</td>
<td>331</td>
<td>38.4</td>
</tr>
<tr>
<td>Taupo</td>
<td>0.26</td>
<td>2.4</td>
<td>0.45</td>
<td>8.9</td>
<td>137.1</td>
<td>367.7</td>
<td>318.8</td>
<td>61.7</td>
<td>832</td>
<td>479</td>
<td>36.5</td>
</tr>
<tr>
<td>Oruanui</td>
<td>0.10</td>
<td>3.1</td>
<td>0.58</td>
<td>4.3</td>
<td>142.3</td>
<td>226.1</td>
<td>53.3</td>
<td>87.5</td>
<td>426</td>
<td>701</td>
<td>62.2</td>
</tr>
<tr>
<td>Pukaki</td>
<td>0.05</td>
<td>2.7</td>
<td>0.50</td>
<td>1.7</td>
<td>35.4</td>
<td>65.7</td>
<td>26.5</td>
<td>79.5</td>
<td>129</td>
<td>534</td>
<td>81.3</td>
</tr>
<tr>
<td>Fork</td>
<td>0.11</td>
<td>1.8</td>
<td>0.43</td>
<td>2.9</td>
<td>37.8</td>
<td>140.2</td>
<td>267.5</td>
<td>40.4</td>
<td>448</td>
<td>242</td>
<td>35.0</td>
</tr>
<tr>
<td>Hurunui</td>
<td>0.81</td>
<td>1.4</td>
<td>0.25</td>
<td>15.9</td>
<td>41.1</td>
<td>59.5</td>
<td>117.1</td>
<td>49.9</td>
<td>234</td>
<td>670</td>
<td>74.2</td>
</tr>
<tr>
<td>Richmond</td>
<td>0.11</td>
<td>3.0</td>
<td>0.42</td>
<td>4.3</td>
<td>53.5</td>
<td>107.7</td>
<td>79.1</td>
<td>67.7</td>
<td>245</td>
<td>568</td>
<td>69.9</td>
</tr>
<tr>
<td>Temuka</td>
<td>1.65</td>
<td>1.3</td>
<td>0.16</td>
<td>29.1</td>
<td>35.8</td>
<td>52.7</td>
<td>429.8</td>
<td>21.5</td>
<td>547</td>
<td>509</td>
<td>48.2</td>
</tr>
<tr>
<td>Patoka</td>
<td>0.04</td>
<td>17.7</td>
<td>0.44</td>
<td>7.6</td>
<td>130.3</td>
<td>246.8</td>
<td>149.8</td>
<td>72.0</td>
<td>534</td>
<td>1051</td>
<td>66.3</td>
</tr>
<tr>
<td>Te Kauwhata</td>
<td>0.19</td>
<td>2.6</td>
<td>0.40</td>
<td>6.9</td>
<td>71.2</td>
<td>139.6</td>
<td>114.7</td>
<td>65.5</td>
<td>332</td>
<td>606</td>
<td>65.6</td>
</tr>
<tr>
<td>Mapua</td>
<td>0.10</td>
<td>2.9</td>
<td>0.31</td>
<td>3.3</td>
<td>12.0</td>
<td>10.8</td>
<td>8.0</td>
<td>76.6</td>
<td>34</td>
<td>82</td>
<td>70.6</td>
</tr>
<tr>
<td>Okarito</td>
<td>1.90</td>
<td>1.0</td>
<td>0.10</td>
<td>21.4</td>
<td>10.4</td>
<td>11.9</td>
<td>74.3</td>
<td>37.1</td>
<td>118</td>
<td>479</td>
<td>80.2</td>
</tr>
<tr>
<td>Mean</td>
<td>0.44</td>
<td>4.5</td>
<td>0.37</td>
<td>9.8</td>
<td>76.1</td>
<td>151.7</td>
<td>177.3</td>
<td>60.5</td>
<td>415</td>
<td>548</td>
<td>61.6</td>
</tr>
</tbody>
</table>

* \(E_{1\text{min}-3m}\)% = \((E_{1m} + E_{1m-24h} + E_{24h-3m}) / \text{inorganic P} \times 100\%.

** Inorganic and organic P were determined by the method of Saunders and Williams (1955).
pool, while only 7.1% of Pi in the Himatangi soil was found in this pool, and up to
93% of inorganic P in this soil was present in $E_{>3m}$ Pi pool.

6.3.1 Plant growth and P uptake
Main effects of plant species and soil type, and the interactions between plant species
and soil type for dry mass of root, shoot and whole plant and shoot: root (S:R) ratio
were all significant. However, effects of the soil type and plant x soil interactions were
small compared with main effects for plant species (Table 6-3). The dry matter (root,
shoot, total) of radiata pine harvested was 1.4 – 5.5 times higher than that of ryegrass
from the 15 soils (Table 6-3). Shoot: root ratio of radiata pine was also generally
higher than that of ryegrass, although significant differences were observed only in 6
soils (Table 6-3).

Phosphorus concentrations in the roots of radiata pine were significantly higher than
those of ryegrass except for the Himatangi soil, while P concentrations in shoots of
radiata pine were significantly lower than ryegrass except in the Egmont, Taupo and
Pukaki soils (Table 6-4). Accordingly, radiata pine generally took up more P (4.5 –
33.5 mg P pot$^{-1}$) than ryegrass (1.1 – 15.6 mg P pot$^{-1}$) (Table 6-4). It should be noted
that the P concentration in the shoots of ryegrass in the Temuka soil was 3.5 times
higher than that of the radiata pine and so P uptake by the ryegrass was greater than
radiata pine in the Temuka soil (Table 6-4). Radiata pine used P more efficiently than
ryegrass in 5 out of 15 soils studied (Hurunui, Temuka, Patoka, Mapua and Okarito)
but less efficiently in 2 soils (Egmont and Pukaki) while there were no differences in P
use efficiency in the remaining soils (Stratford, Mangamahi, Himatangi, Taupo,
Oruanui, Fork, Richmond and Te Kauwhata) (Table 6-4). These differences
contributed to significant plant x soil interactions.

6.3.2 Soil pH and total organic C and total N
There were significant differences observed in soil pH, TOC, and total N between soils
under ryegrass and radiata pine (Table 6-5). The pH values in soils under radiata pine
were 0.2 – 0.8 lower compared with ryegrass ((Table 6-5). There was no significant
plant x soil interactions with soil TOC and concentrations of TOC in soils under
ryegrass were significantly higher compared with radiata pine (Table 6-5).
Concentrations of total N were significantly lower in most soils under radiata pine
compared with ryegrass (Table 6-5).
Table 6-3  Root, shoot and total biomass (dry weight, g pot$^{-1}$), and shoot: root ratios (S:R) of ryegrass and radiata pine determined after a 40-week period of growth.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Root grass</th>
<th>Root pine</th>
<th>Shoot grass</th>
<th>Shoot pine</th>
<th>Total grass</th>
<th>Total pine</th>
<th>S:R ratio grass</th>
<th>S:R ratio pine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egmont</td>
<td>0.94</td>
<td>3.79$^\dagger$</td>
<td>1.80</td>
<td>8.31$^\dagger$</td>
<td>2.74</td>
<td>12.10$^\dagger$</td>
<td>1.94</td>
<td>2.27</td>
</tr>
<tr>
<td>Stratford</td>
<td>2.32</td>
<td>2.59</td>
<td>4.01</td>
<td>6.56$^\dagger$</td>
<td>6.33</td>
<td>9.16$^\dagger$</td>
<td>1.91</td>
<td>2.50</td>
</tr>
<tr>
<td>Mangamahu</td>
<td>0.90</td>
<td>2.31$^\dagger$</td>
<td>1.39</td>
<td>6.73$^\dagger$</td>
<td>2.30</td>
<td>9.03$^\dagger$</td>
<td>1.57</td>
<td>2.93$^\dagger$</td>
</tr>
<tr>
<td>Himatangi</td>
<td>1.62</td>
<td>3.62$^\dagger$</td>
<td>3.55</td>
<td>11.89$^\dagger$</td>
<td>5.16</td>
<td>15.51$^\dagger$</td>
<td>2.21</td>
<td>3.31$^\dagger$</td>
</tr>
<tr>
<td>Taupo</td>
<td>0.96</td>
<td>2.67$^\dagger$</td>
<td>2.78</td>
<td>7.41$^\dagger$</td>
<td>3.74</td>
<td>10.08$^\dagger$</td>
<td>3.03</td>
<td>2.78</td>
</tr>
<tr>
<td>Oruanui</td>
<td>1.74</td>
<td>3.04$^\dagger$</td>
<td>2.91</td>
<td>7.84$^\dagger$</td>
<td>4.64</td>
<td>10.88$^\dagger$</td>
<td>1.67</td>
<td>2.57$^\dagger$</td>
</tr>
<tr>
<td>Pukaki</td>
<td>0.70</td>
<td>1.67$^\dagger$</td>
<td>0.90</td>
<td>5.95$^\dagger$</td>
<td>1.60</td>
<td>7.62$^\dagger$</td>
<td>1.33</td>
<td>3.58$^\dagger$</td>
</tr>
<tr>
<td>Fork</td>
<td>1.06</td>
<td>2.31$^\dagger$</td>
<td>1.64</td>
<td>5.52$^\dagger$</td>
<td>2.71</td>
<td>7.83$^\dagger$</td>
<td>1.55</td>
<td>2.40$^\dagger$</td>
</tr>
<tr>
<td>Hurunui</td>
<td>2.06</td>
<td>2.57$^\dagger$</td>
<td>3.62</td>
<td>7.40$^\dagger$</td>
<td>5.69</td>
<td>9.97$^\dagger$</td>
<td>1.76</td>
<td>2.85$^\dagger$</td>
</tr>
<tr>
<td>Richmond</td>
<td>0.74</td>
<td>2.36$^\dagger$</td>
<td>1.88</td>
<td>7.70$^\dagger$</td>
<td>2.62</td>
<td>10.06$^\dagger$</td>
<td>2.64</td>
<td>3.27</td>
</tr>
<tr>
<td>Temuka</td>
<td>1.14</td>
<td>2.10$^\dagger$</td>
<td>2.92</td>
<td>6.70$^\dagger$</td>
<td>4.05</td>
<td>8.80$^\dagger$</td>
<td>2.58</td>
<td>3.19</td>
</tr>
<tr>
<td>Patoka</td>
<td>0.98</td>
<td>3.25$^\dagger$</td>
<td>1.73</td>
<td>8.31$^\dagger$</td>
<td>2.71</td>
<td>11.56$^\dagger$</td>
<td>1.79</td>
<td>2.62</td>
</tr>
<tr>
<td>Te Kauwhata</td>
<td>2.38</td>
<td>3.28$^\dagger$</td>
<td>3.54</td>
<td>7.60$^\dagger$</td>
<td>5.92</td>
<td>10.88$^\dagger$</td>
<td>2.63</td>
<td>2.33</td>
</tr>
<tr>
<td>Mapua</td>
<td>0.28</td>
<td>1.52$^\dagger$</td>
<td>0.72</td>
<td>3.95$^\dagger$</td>
<td>1.00</td>
<td>5.47$^\dagger$</td>
<td>2.71</td>
<td>2.60</td>
</tr>
<tr>
<td>Okarito</td>
<td>1.61</td>
<td>4.03$^\dagger$</td>
<td>4.22</td>
<td>7.90$^\dagger$</td>
<td>5.83</td>
<td>11.92$^\dagger$</td>
<td>2.63</td>
<td>2.02</td>
</tr>
<tr>
<td>Mean</td>
<td>1.29</td>
<td>2.74</td>
<td>2.51</td>
<td>7.32</td>
<td>3.77</td>
<td>10.06</td>
<td>2.13</td>
<td>2.75</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.16</td>
<td>0.19</td>
<td>0.29</td>
<td>0.44</td>
<td>0.44</td>
<td>0.60</td>
<td>0.14</td>
<td>0.11</td>
</tr>
<tr>
<td>LSD$_{0.05}$</td>
<td>0.73</td>
<td>1.55</td>
<td>2.15</td>
<td>0.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Two-way ANOVA (F ratios):

- Plant species: 233.7***
- Soil type: 10.32***
- Plant x soil: 3.79***

a) grass = ryegrass; pine = radiata pine.
b) $^\dagger$ indicates values for ryegrass are significantly lower than those for radiata pine at the same row (P < 0.05, separated by LSD$_{0.05}$).
c) *, ** and *** indicate significant differences at 0.05, 0.01 and 0.001 level (P < 0.05, 0.01 and 0.001), respectively.
<table>
<thead>
<tr>
<th>Soil</th>
<th>Root P concentration (µg g⁻¹)</th>
<th>P uptake (mg pot⁻¹)</th>
<th>P use efficiency (g biomass /mg P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grass</td>
<td>Pine</td>
<td>Grass</td>
</tr>
<tr>
<td>1 Egmont</td>
<td>663</td>
<td>1096 †</td>
<td>868</td>
</tr>
<tr>
<td>2 Stratford</td>
<td>1247</td>
<td>1635 †</td>
<td>2126</td>
</tr>
<tr>
<td>3 Mangamahu</td>
<td>881</td>
<td>1151 †</td>
<td>1350</td>
</tr>
<tr>
<td>4 Himatangi</td>
<td>2162</td>
<td>1965 †</td>
<td>2895</td>
</tr>
<tr>
<td>5 Taupo</td>
<td>1062</td>
<td>1524 †</td>
<td>2111</td>
</tr>
<tr>
<td>6 Oruanui</td>
<td>970</td>
<td>1269 †</td>
<td>1942</td>
</tr>
<tr>
<td>7 Pukaki</td>
<td>846</td>
<td>1228 †</td>
<td>1260</td>
</tr>
<tr>
<td>8 Fork</td>
<td>1018</td>
<td>1500 †</td>
<td>1785</td>
</tr>
<tr>
<td>9 Hurunui</td>
<td>1056</td>
<td>1510 †</td>
<td>2397</td>
</tr>
<tr>
<td>10 Richmond</td>
<td>908</td>
<td>1789 †</td>
<td>2017</td>
</tr>
<tr>
<td>11 Temuka</td>
<td>1185</td>
<td>1524 †</td>
<td>4875</td>
</tr>
<tr>
<td>12 Patoka</td>
<td>909</td>
<td>1201 †</td>
<td>1798</td>
</tr>
<tr>
<td>13 Te Kauwhata</td>
<td>798</td>
<td>1464 †</td>
<td>1868</td>
</tr>
<tr>
<td>14 Mapua</td>
<td>745</td>
<td>934 †</td>
<td>1274</td>
</tr>
<tr>
<td>15 Okarito</td>
<td>644</td>
<td>1118 †</td>
<td>2053</td>
</tr>
</tbody>
</table>

Mean 1006 1394 2041 1370 1.36 3.84 5.57 10.37 6.93 14.21 0.67 0.77

Standard error 88 68 224 82 0.22 0.31 0.94 1.21 1.10 1.49 0.06 0.04

LSD₁₀₀ 175 333 1.11 2.85 3.65 0.12

Two-way ANOVA (F ratios):
Plant species 292.9*** 243.1*** 296.9*** 170.0*** 238.8*** 32.8***
Soil type 48.1*** 45.9*** 13.9*** 29.6*** 27.9*** 38.2***
Plant x soil 7.09*** 24.7*** 2.4** 9.8*** 7.4*** 7.8***

a) Grass = ryegrass, pine = radiata pine. P use efficiency is the plant biomass production (g) per unit P uptake (mg).

b) † indicates values for ryegrass are significantly higher than those for radiata pine at the same row while ‡ indicates values for ryegrass are significantly lower than those for radiata pine (P < 0.05, separated by LSD₁₀₀).

b) *, ** and *** indicate significant differences at 0.05, 0.01 and 0.001 levels (P < 0.05, 0.01 and 0.001), respectively.
Table 6-5  Selected soil chemical properties determined after a 40-week period of growth under ryegrass and radiata pine.

<table>
<thead>
<tr>
<th>Soil</th>
<th>pH grass</th>
<th>TOC% grass</th>
<th>Total N% grass</th>
<th>pH pine</th>
<th>TOC% pine</th>
<th>Total N% pine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egmont</td>
<td>5.86</td>
<td>5.11</td>
<td>0.417</td>
<td>5.36</td>
<td>4.62</td>
<td>0.391</td>
</tr>
<tr>
<td>Stratford</td>
<td>5.49</td>
<td>6.08</td>
<td>0.580</td>
<td>5.09†</td>
<td>5.88</td>
<td>0.546†</td>
</tr>
<tr>
<td>Mangamahu</td>
<td>5.36</td>
<td>3.96</td>
<td>0.278</td>
<td>5.00†</td>
<td>3.55</td>
<td>0.246†</td>
</tr>
<tr>
<td>Himatangi</td>
<td>6.42</td>
<td>3.13</td>
<td>0.288</td>
<td>6.04†</td>
<td>2.51</td>
<td>0.226†</td>
</tr>
<tr>
<td>Taupo</td>
<td>5.66</td>
<td>5.27</td>
<td>0.387</td>
<td>5.17†</td>
<td>4.63</td>
<td>0.339†</td>
</tr>
<tr>
<td>Ouanui</td>
<td>5.40</td>
<td>8.67</td>
<td>0.546</td>
<td>5.21†</td>
<td>8.09</td>
<td>0.508†</td>
</tr>
<tr>
<td>Pukaki</td>
<td>5.66</td>
<td>4.74</td>
<td>0.299</td>
<td>5.26†</td>
<td>4.24</td>
<td>0.271</td>
</tr>
<tr>
<td>Fork</td>
<td>5.89</td>
<td>2.45</td>
<td>0.175</td>
<td>5.46†</td>
<td>1.89</td>
<td>0.137†</td>
</tr>
<tr>
<td>Hurunui</td>
<td>5.73</td>
<td>7.55</td>
<td>0.598</td>
<td>5.09†</td>
<td>7.59</td>
<td>0.599</td>
</tr>
<tr>
<td>Richmond</td>
<td>5.65</td>
<td>3.75</td>
<td>0.327</td>
<td>5.39†</td>
<td>3.61</td>
<td>0.301†</td>
</tr>
<tr>
<td>Temuka</td>
<td>6.09</td>
<td>3.87</td>
<td>0.363</td>
<td>5.47†</td>
<td>3.67</td>
<td>0.332†</td>
</tr>
<tr>
<td>Patoka</td>
<td>5.68</td>
<td>9.43</td>
<td>0.831</td>
<td>5.42†</td>
<td>8.90</td>
<td>0.783†</td>
</tr>
<tr>
<td>Te Kauwhata</td>
<td>5.85</td>
<td>4.98</td>
<td>0.456</td>
<td>5.26†</td>
<td>4.55</td>
<td>0.421†</td>
</tr>
<tr>
<td>Mapua</td>
<td>5.29</td>
<td>1.97</td>
<td>0.084</td>
<td>4.98†</td>
<td>1.89</td>
<td>0.091</td>
</tr>
<tr>
<td>Okarito</td>
<td>5.48</td>
<td>14.08</td>
<td>0.716</td>
<td>4.71†</td>
<td>13.74</td>
<td>0.672†</td>
</tr>
<tr>
<td>Mean</td>
<td>5.70</td>
<td>5.67</td>
<td>0.423</td>
<td>5.26</td>
<td>5.29</td>
<td>0.391</td>
</tr>
</tbody>
</table>

Standard error: 0.08 0.08 0.82 0.82 0.05 0.05

LSD<sub>0.05</sub> 0.11 N/A 0.026

Two-way ANOVA (F ratios):

- **Plant species**: 935.9*** 61.2*** 91.8***
- **Soil type**: 107.2*** 1153.4*** 951.2***
- **Plant x soil**: 8.6*** 1.3 1.9*

a) TOC = total organic C. N/A = not applicable (due to insignificance in plant species x soil type interactions).
b) see Table 6-4 for explanation of symbols.
6.3.3 Chemically extractable P pools

There were significant effects of plant species and soil type and significant plant x soil interactions for all chemically extractable P fractions determined (Table 6-6). Concentrations of BPi were significantly higher in 9 soils (Stratford, Himatangi, Taupo, Oruanui, Pukaki, Hurunui, Richmond, Temuka and Okarito) under radiata pine than under ryegrass while there were no significant effects of plant species for the remaining soils. Levels of BPo were also significantly higher in 5 soils (Egmont, Stratford, Mangamahu, Hurunui, Patoka) under radiata pine compared with ryegrass, while there were no significant differences between the remaining soils under ryegrass and radiata pine (Table 6-6). Similarly, concentrations of resin extractable Pi and Po were also generally higher in soils under radiata pine compared with ryegrass (Table 6-6).

After 40 weeks, the levels of soil total P were significantly lower in most soils under radiata pine than under ryegrass, but significantly higher in Temuka soil under radiata pine (Table 6-7). Compared with the original soils (Table 6-1), the levels of soil total P decreased by 1.7 – 12.6% in soils under ryegrass and by 4.8 – 16.9% in soils under radiata pine. Concentrations of total inorganic P (TPi) were significantly higher in only two soils (Stratford and Temuka) under radiata pine compared with ryegrass (Table 6-7). However, concentrations of total organic P (TPo) were consistently lower in soils under radiata pine than under ryegrass (Table 6-7).

Specific mineralization rate (SMR) represents the amount of organic P mineralized as a percent of the total organic P present in the original soils prior to planting (Grierson et al., 1999). Results showed that the SMR was significantly lower in all soils under ryegrass (0.5 – 10.8%) than under radiata pine (8.4 – 21.9%) (Figure 6-2), which confirmed that organic P mineralization was greater in soils under radiata pine than ryegrass.

6.3.4 Isotopically exchangeable soil P

Isotopic exchange kinetic parameters and exchangeable P pools in soils under ryegrass and radiata pine are shown in Table 6-8 and 6-9. There were no significant plant species effects on the levels of Cp in most soils while levels of Cp were significantly higher in the Stratford, Himatangi and Hurunui soils but significantly lower in the Temuka soil under ryegrass compared with radiata pine (Table 6-8). Compared with
Table 6-6  Bicarbonate extractable P (BPi and BPo) (μg g⁻¹) and resin extractable P (Resin Pi, Po) (μg g⁻¹) determined in soils after a 40-week period of growth under ryegrass and radiata pine §.

<table>
<thead>
<tr>
<th>Soil</th>
<th>BPi grass</th>
<th>BPi pine</th>
<th>BPO grass</th>
<th>BPO pine</th>
<th>Resin Pi grass</th>
<th>Resin Pi pine</th>
<th>Resin Po grass</th>
<th>Resin Po pine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egmont</td>
<td>1.3</td>
<td>3.4</td>
<td>4.9</td>
<td>19.3†</td>
<td>5.1</td>
<td>7.2</td>
<td>12.8</td>
<td>16.0</td>
</tr>
<tr>
<td>Stratford</td>
<td>24.4</td>
<td>35.1†</td>
<td>26.9</td>
<td>39.2†</td>
<td>29.8</td>
<td>39.9†</td>
<td>23.6</td>
<td>26.1</td>
</tr>
<tr>
<td>Mangamahu</td>
<td>1.0</td>
<td>4.4</td>
<td>11.7</td>
<td>21.7†</td>
<td>3.0</td>
<td>6.7</td>
<td>12.6</td>
<td>16.5†</td>
</tr>
<tr>
<td>Himatangi</td>
<td>13.7</td>
<td>22.1†</td>
<td>3.8</td>
<td>9.7</td>
<td>32.6</td>
<td>37.2†</td>
<td>6.4</td>
<td>10.2†</td>
</tr>
<tr>
<td>Taupo</td>
<td>39.5</td>
<td>47.4†</td>
<td>19.9</td>
<td>20.1</td>
<td>63.8</td>
<td>64.1</td>
<td>22.3</td>
<td>24.4</td>
</tr>
<tr>
<td>Orauaini</td>
<td>21.1</td>
<td>27.0†</td>
<td>26.6</td>
<td>29.9</td>
<td>29.2</td>
<td>37.4†</td>
<td>25.8</td>
<td>22.6</td>
</tr>
<tr>
<td>Pukaki</td>
<td>1.4</td>
<td>5.6†</td>
<td>13.1</td>
<td>17.7</td>
<td>7.5</td>
<td>13.0†</td>
<td>12.7</td>
<td>18.0†</td>
</tr>
<tr>
<td>Fork</td>
<td>6.1</td>
<td>9.1</td>
<td>8.4</td>
<td>15.3</td>
<td>19.5</td>
<td>19.0</td>
<td>12.8</td>
<td>18.1†</td>
</tr>
<tr>
<td>Hurunui</td>
<td>2.7</td>
<td>9.7†</td>
<td>12.5</td>
<td>20.1†</td>
<td>16.0</td>
<td>22.9†</td>
<td>20.2</td>
<td>22.6</td>
</tr>
<tr>
<td>Richmond</td>
<td>1.3</td>
<td>6.8†</td>
<td>8.9</td>
<td>12.8</td>
<td>6.7</td>
<td>15.6†</td>
<td>14.5</td>
<td>16.5</td>
</tr>
<tr>
<td>Temuka</td>
<td>8.4</td>
<td>21.4†</td>
<td>10.4</td>
<td>11.8</td>
<td>29.6</td>
<td>52.5†</td>
<td>17.6</td>
<td>18.1</td>
</tr>
<tr>
<td>Patoka</td>
<td>1.8</td>
<td>5.3</td>
<td>11.8</td>
<td>27.6†</td>
<td>11.8</td>
<td>11.5</td>
<td>19.3</td>
<td>21.6</td>
</tr>
<tr>
<td>Te Kauwhata</td>
<td>4.5</td>
<td>7.0</td>
<td>17.0</td>
<td>15.2</td>
<td>13.0</td>
<td>17.5</td>
<td>16.3</td>
<td>16.0</td>
</tr>
<tr>
<td>Mapua</td>
<td>1.0</td>
<td>0.7</td>
<td>7.4</td>
<td>3.1</td>
<td>3.0</td>
<td>3.1</td>
<td>7.0</td>
<td>12.3†</td>
</tr>
<tr>
<td>Okarito</td>
<td>1.9</td>
<td>6.3†</td>
<td>11.3</td>
<td>14.4</td>
<td>10.8</td>
<td>18.3†</td>
<td>12.0</td>
<td>14.0</td>
</tr>
</tbody>
</table>

Mean 8.7  14.1  13.0  18.5  18.8  24.4  15.7  18.2
Standard error 2.9  3.5  1.8  2.3  4.2  4.6  1.5  1.2
LSD₀.₀₅ 4.1  7.2  4.8  3.8

Two-way ANOVA (F ratios):

- Plant species 75.9*** 35.6*** 159.7*** 20.6***
- Soil type 144.9*** 16.9*** 378.6*** 44.7***
- Plant x soil 4.9*** 2.6*** 11.7*** 3.7***

§ see Table 6-4 for explanation of symbols.
Table 6-7  Concentrations of soil inorganic, organic and total P (µg g⁻¹) determined after a 40-week period of growth under ryegrass and radiata pine.

<table>
<thead>
<tr>
<th>Soil</th>
<th>TPi grass</th>
<th>TPi pine</th>
<th>TPo grass</th>
<th>TPo pine</th>
<th>Total P grass</th>
<th>Total P pine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egmont</td>
<td>117</td>
<td>134</td>
<td>469</td>
<td>408</td>
<td>586</td>
<td>542</td>
</tr>
<tr>
<td>Stratford</td>
<td>1471</td>
<td>1705</td>
<td>1136</td>
<td>911</td>
<td>2607</td>
<td>2615</td>
</tr>
<tr>
<td>Mangamahu</td>
<td>76</td>
<td>105</td>
<td>301</td>
<td>252</td>
<td>377</td>
<td>356</td>
</tr>
<tr>
<td>Himatangi</td>
<td>445</td>
<td>440</td>
<td>309</td>
<td>286</td>
<td>755</td>
<td>725</td>
</tr>
<tr>
<td>Taupo</td>
<td>712</td>
<td>668</td>
<td>461</td>
<td>422</td>
<td>1173</td>
<td>1089</td>
</tr>
<tr>
<td>Oruanui</td>
<td>345</td>
<td>384</td>
<td>694</td>
<td>618</td>
<td>1039</td>
<td>1002</td>
</tr>
<tr>
<td>Pukaki</td>
<td>121</td>
<td>167</td>
<td>531</td>
<td>429</td>
<td>652</td>
<td>596</td>
</tr>
<tr>
<td>Fork</td>
<td>415</td>
<td>403</td>
<td>240</td>
<td>220</td>
<td>655</td>
<td>623</td>
</tr>
<tr>
<td>Hurunui</td>
<td>197</td>
<td>214</td>
<td>617</td>
<td>581</td>
<td>815</td>
<td>795</td>
</tr>
<tr>
<td>Richmond</td>
<td>228</td>
<td>243</td>
<td>547</td>
<td>479</td>
<td>775</td>
<td>722</td>
</tr>
<tr>
<td>Temuka</td>
<td>465</td>
<td>540</td>
<td>454</td>
<td>418</td>
<td>919</td>
<td>958</td>
</tr>
<tr>
<td>Patoka</td>
<td>451</td>
<td>495</td>
<td>1031</td>
<td>927</td>
<td>1482</td>
<td>1422</td>
</tr>
<tr>
<td>Te Kauwhata</td>
<td>295</td>
<td>334</td>
<td>598</td>
<td>519</td>
<td>894</td>
<td>853</td>
</tr>
<tr>
<td>Mapua</td>
<td>30</td>
<td>24</td>
<td>81</td>
<td>75</td>
<td>111</td>
<td>99</td>
</tr>
<tr>
<td>Okarito</td>
<td>64</td>
<td>80</td>
<td>472</td>
<td>423</td>
<td>536</td>
<td>503</td>
</tr>
<tr>
<td>Mean</td>
<td>362</td>
<td>396</td>
<td>529</td>
<td>464</td>
<td>891</td>
<td>860</td>
</tr>
<tr>
<td>Standard error</td>
<td>93</td>
<td>105</td>
<td>71</td>
<td>60</td>
<td>148</td>
<td>149</td>
</tr>
<tr>
<td>LSD₀.₀₅</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Two-way ANOVA (F ratios):

- Plant species: 27.4*** 153.4*** 65.8***
- Soil type: 953.7*** 623.1*** 5979.6***
- Plant x soil: 6.3*** 6.8*** 3.8***

a) TPi = total Pi; TPo = total Po.
b) see Table 6-4 for explanation of other abbreviations and symbols.
the original soils, levels of Cp in soils under ryegrass and radiata pine were generally lower due to plant uptake (Tables 6-2 and 6-8). For the capacity factors (R/r₁, n), the values of R/r₁ were significantly higher in 6 soils (Stratford, Mangamahu, Pukaki, Patoka, Te Kauwhata and Mapua), while there were no significant effects of plant species on the values of R/r₁ found for the remaining soils. The values of R/r₁ for most soils increased under radiata pine and ryegrass compared with the original soils (Tables 6-2 and 6-8). Plant species also affected the value of n, which was higher in 3 soils (Mangamahu, Pukaki and Okarito) and lower in 4 soils (Himatangi, Fork, Te Kauwhata and Mapua) under ryegrass compared with radiata pine (Table 6-8). The n value tended to be higher for most soils after 40 weeks growth compared with the original soils (Tables 6-2 and 6-8).
Table 6-8  Isotopic exchange kinetics parameters (Cp, R/r₁, n) determined in soils after a 40-week period of growth under ryegrass and radiata pine§.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Cp (mg L⁻¹)</th>
<th>R/r₁</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>grass</td>
<td>pine</td>
<td></td>
</tr>
<tr>
<td>Egmont</td>
<td>0.067</td>
<td>0.044</td>
<td>13.22</td>
</tr>
<tr>
<td>Stratford</td>
<td>0.198</td>
<td>0.136†</td>
<td>10.46</td>
</tr>
<tr>
<td>Mangamahu</td>
<td>0.025</td>
<td>0.027</td>
<td>8.97</td>
</tr>
<tr>
<td>Himatangi</td>
<td>1.113</td>
<td>0.875‡</td>
<td>1.04</td>
</tr>
<tr>
<td>Taupo</td>
<td>0.170</td>
<td>0.151</td>
<td>2.93</td>
</tr>
<tr>
<td>Oruanui</td>
<td>0.086</td>
<td>0.078</td>
<td>5.17</td>
</tr>
<tr>
<td>Pukaki</td>
<td>0.035</td>
<td>0.042</td>
<td>3.39</td>
</tr>
<tr>
<td>Fork</td>
<td>0.114</td>
<td>0.089</td>
<td>1.71</td>
</tr>
<tr>
<td>Hurunui</td>
<td>0.450</td>
<td>0.386‡</td>
<td>1.56</td>
</tr>
<tr>
<td>Richmond</td>
<td>0.106</td>
<td>0.122</td>
<td>4.27</td>
</tr>
<tr>
<td>Temuka</td>
<td>0.845</td>
<td>1.263‡</td>
<td>1.6</td>
</tr>
<tr>
<td>Patoka</td>
<td>0.041</td>
<td>0.035</td>
<td>13.02</td>
</tr>
<tr>
<td>Te Kauwhata</td>
<td>0.127</td>
<td>0.107</td>
<td>4.19</td>
</tr>
<tr>
<td>Mapua</td>
<td>0.053</td>
<td>0.061</td>
<td>4.05</td>
</tr>
<tr>
<td>Okarito</td>
<td>1.325</td>
<td>1.326</td>
<td>1.01</td>
</tr>
<tr>
<td>Mean</td>
<td>0.317</td>
<td>0.316</td>
<td>5.11</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.110</td>
<td>0.117</td>
<td>1.09</td>
</tr>
<tr>
<td>LSD₀.₀₅</td>
<td>0.053</td>
<td>1.20</td>
<td></td>
</tr>
</tbody>
</table>

Two-way ANOVA (F ratios):

- Plant species: 0.34 69.6** 0.78
- Soil type: 1099.4*** 272.5*** 200.3
- Plant x soil: 32.1*** 9.2*** 6.8***

§ See Table 6-3 and the text (section 6.2.3) for explanation of abbreviations and symbols.
Table 6-9  Isotopically exchangeable Pi pools ($\mu$g g$^{-1}$) determined in soils after a 40-week period of growth under ryegrass and radiata pine.

<table>
<thead>
<tr>
<th>Soil</th>
<th>$E_{imin}$</th>
<th>$E_{imin-24h}$</th>
<th>$E_{24h-3m}$</th>
<th>$E_{&gt;3m}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>grass</td>
<td>pine</td>
<td>grass</td>
<td>pine</td>
</tr>
<tr>
<td>Egmont</td>
<td>8.8</td>
<td>6.2</td>
<td>64.2</td>
<td>62.2</td>
</tr>
<tr>
<td>Stratford</td>
<td>22.2</td>
<td>19.2</td>
<td>391.7</td>
<td>451.3</td>
</tr>
<tr>
<td>Mangamahu</td>
<td>2.4</td>
<td>3.3</td>
<td>32.3</td>
<td>26.4</td>
</tr>
<tr>
<td>Himatangi</td>
<td>14.9</td>
<td>13.3</td>
<td>14.0</td>
<td>26.1</td>
</tr>
<tr>
<td>Taupo</td>
<td>6.4</td>
<td>7.0</td>
<td>142.8</td>
<td>160.3</td>
</tr>
<tr>
<td>Oruanui</td>
<td>5.2</td>
<td>4.7</td>
<td>120.6</td>
<td>119.3</td>
</tr>
<tr>
<td>Pukaki</td>
<td>1.5</td>
<td>2.4</td>
<td>36.7</td>
<td>38.4</td>
</tr>
<tr>
<td>Fork</td>
<td>2.8</td>
<td>2.5</td>
<td>31.9</td>
<td>39.3</td>
</tr>
<tr>
<td>Hurunui</td>
<td>9.4</td>
<td>8.6</td>
<td>40.6</td>
<td>46.8</td>
</tr>
<tr>
<td>Richmond</td>
<td>5.3</td>
<td>6.4</td>
<td>71.8</td>
<td>77.2</td>
</tr>
<tr>
<td>Temuka</td>
<td>16.1</td>
<td>23.5</td>
<td>31.2</td>
<td>53.9</td>
</tr>
<tr>
<td>Patoka</td>
<td>5.7</td>
<td>7.2</td>
<td>156.9</td>
<td>189.9</td>
</tr>
<tr>
<td>Te Kauwhata</td>
<td>6.3</td>
<td>7.0</td>
<td>86.5</td>
<td>120.5</td>
</tr>
<tr>
<td>Mapua</td>
<td>2.4</td>
<td>3.4</td>
<td>15.6</td>
<td>16.3</td>
</tr>
<tr>
<td>Okarito</td>
<td>14.0</td>
<td>14.2</td>
<td>9.6</td>
<td>6.0</td>
</tr>
<tr>
<td>Mean</td>
<td>8.2</td>
<td>8.6</td>
<td>83.1</td>
<td>95.6</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.5</td>
<td>1.6</td>
<td>25.1</td>
<td>29.0</td>
</tr>
<tr>
<td>LSD$_{0.05}$</td>
<td>1.7</td>
<td>N/A</td>
<td>22.4</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Two-way ANOVA (F ratios):

- **Plant species**: 2.8, 8.7**, 48.9***, 0.1
- **Soil type**: 199.5***, 222.7***, 1222.6***, 102.2***
- **Plant x soil**: 6.9***, 1.6, 12.8***, 1.3

§ See Table 6-4, 6-5 and the text (section 6.2.3) for explanation of abbreviations and symbols.
There were no significant effects of plant species on the levels of $E_{1\text{min}}$ Pi for most soils, although levels of $E_{1\text{min}}$ Pi were significantly greater in the Egmont and Stratford soils and lower in the Temuka soil under ryegrass compared with radiata pine (Table 6-9). Concentrations of $E_{1\text{min}-24\text{h}}$ Pi were significantly higher in the soils under radiata pine compared with ryegrass (Table 6-9), while the values of $E_{1\text{min}-24\text{h}}$ Pi for most soils under ryegrass and radiata pine were higher than those in the original soils (Table 6-2 and 6-9). Concentrations of $E_{24\text{h}-3\text{m}}$ Pi were significantly lower in 5 soils (Stratford, Himatangi, Oruanui, Fork and Temuka) under ryegrass compared with radiata pine, while there were no significant effects of plant species on $E_{24\text{h}-3\text{m}}$ Pi in the remaining soils (Table 6-9). Plant species had no different effects on the levels of $E_{>3\text{m}}$ Pi in soils, while the levels of $E_{>3\text{m}}$ Pi for most soils under ryegrass and radiata pine were generally lower than those in the original soils (Tables 6-2 and 6-9). Concentrations of $E_{1\text{min}-3\text{m}}$ Pi (the sum of $E_{1\text{min}}$, $E_{1\text{m}-24\text{h}}$ and $E_{24\text{h}-3\text{m}}$ Pi) were significantly lower in 6 soils (Stratford, Himatangi, Pukaki, Fork, Patoka, Te Kauwhata) under ryegrass compared with radiata pine while there were no differences in the remaining soils (Table 6-10). The values $E_{1\text{min}-3\text{m}}$ Pi determined for most soils (9) under radiata pine were also greater than those for the original soils (Table 6-10).

### 6.3.5 Microbial biomass and activity

There were significant effects of plant species and soil type, and significant plant x soil interactions on most soil biochemical and biological properties (Table 6-11). Concentrations of MBC were significantly lower in 6 soils (Egmont, Stratford, Himatangi, Taupo, Oruanui and Patoka) under ryegrass compared with radiata pine, while there were no significant effects of plant species on MBC in the remaining soils (Table 6-12). On the other hand, concentrations of MBP were significantly higher in 6 soils (Himatangi, Hurunui, Temuka, Richmond, Patoka and Okarito) under ryegrass compared with radiata pine, while there were no significant effects of plant species on MBP in the remaining soils (Table 6-12). Microbial C:P ratios were significantly lower in 6 soils (Egmont, Mangamahu, Himatangi, Richmond, Temuka and Patoka) under ryegrass compared with radiata pine while there were no differences in these ratios in the remaining soils (Table 6-12). Microbial biomass C made up 0.8 – 2.1% and 0.9-2.7% of TOC in soils under ryegrass and radiata pine, respectively, while the corresponding data for MBP was 0.8 – 15.4% and 0.8-13.8% of total P (Table 6-12). The MBC:TOC ratios were significantly lower in 6 soils (Egmont, Himatangi, Taupo, Pukaki, Fork and Mapua) under ryegrass compared with radiata pine, while there were
Table 6-10  Concentrations of $E_{1\text{min-3m}}$ Pi (sum of $E_{1\text{min}}$ Pi, $E_{1\text{min-24h}}$ Pi and $E_{24h-3m}$ Pi) determined in original soils and soils after a 40-week period of growth under ryegrass and radiata pine $^\S$.

<table>
<thead>
<tr>
<th>Soil</th>
<th>$E_{1\text{min-3m}}$ (µg g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>original soil</td>
</tr>
<tr>
<td>1 Egmont</td>
<td>130</td>
</tr>
<tr>
<td>2 Stratford</td>
<td>1082</td>
</tr>
<tr>
<td>3 Mangamahu</td>
<td>72</td>
</tr>
<tr>
<td>4 Himatangi</td>
<td>38</td>
</tr>
<tr>
<td>5 Taupo</td>
<td>514</td>
</tr>
<tr>
<td>6 Ouanui</td>
<td>373</td>
</tr>
<tr>
<td>7 Pukaki</td>
<td>103</td>
</tr>
<tr>
<td>8 Fork</td>
<td>181</td>
</tr>
<tr>
<td>9 Hurunui</td>
<td>117</td>
</tr>
<tr>
<td>10 Richmond</td>
<td>166</td>
</tr>
<tr>
<td>11 Temuka</td>
<td>118</td>
</tr>
<tr>
<td>12 Patoka</td>
<td>385</td>
</tr>
<tr>
<td>13 Te Kauwhata</td>
<td>218</td>
</tr>
<tr>
<td>14 Mapua</td>
<td>26</td>
</tr>
<tr>
<td>15 Okarito</td>
<td>44</td>
</tr>
</tbody>
</table>

Mean: 238  227  263  
Standard error: 71  72  83  
LSD$_{0.05}$: 27

Two-way ANOVA (F ratios):
- Plant species: 78.5***
- Soil type: 1945.7***
- Plant x soil: 16.5***

$^\S$ Data for original soils are not included in ANOVA, just as reference. See Table 6-4 for explanation of symbols.
Table 6-11  Means and F ratios for two-way analyses of variance of data for various microbial biomass and phosphatase enzyme activities determined in soils after a 40-week period of growth under ryegrass and radiata pine (values are the means for all 15 soils).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mean variables</th>
<th>Plant species</th>
<th>Soil type</th>
<th>Plant species x soil type</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBC (µg g⁻¹)</td>
<td>Grass 651.4, Pine 738.1</td>
<td>df 1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>MBP (µg g⁻¹)</td>
<td>Grass 26.1, Pine 18.8</td>
<td>df 1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Microbial C:P</td>
<td>Grass 32.8, Pine 50.3</td>
<td>df 1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>MBC: TOC (%)</td>
<td>Grass 1.19, Pine 1.60</td>
<td>df 1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>MBP: total P (%)</td>
<td>Grass 3.77, Pine 3.45</td>
<td>df 1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Respiration</td>
<td>Grass 240.8, Pine 262.3</td>
<td>df 1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>qCO₂</td>
<td>Grass 1.565, Pine 1.497</td>
<td>df 1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>WSOC (µg g⁻¹)</td>
<td>Grass 102.4, Pine 177.7</td>
<td>df 1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>AcPME</td>
<td>Grass 585.5, Pine 392.2</td>
<td>df 1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>AIPME</td>
<td>Grass 213.8, Pine 155.0</td>
<td>df 1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>PDE</td>
<td>Grass 31.7, Pine 18.6</td>
<td>df 1</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

a) Grass = ryegrass; pine = radiata pine; MBC = microbial biomass C; MBP = microbial biomass P; MBC: TOC% = the ratio of MBC to total organic C%; MBP: total = the ratio of MBP to total P; qCO₂ = metabolic quotient; WSOC = water soluble organic C; AcPME = acid phosphomonoesterase; AIPME = alkaline phosphomonoesterase; PDE = phosphodiesterase.
b) The unit of respiration is µg CO₂-C g⁻¹ 10 day⁻¹; the unit of qCO₂ is µg CO₂-C mg microbial C⁻¹ h⁻¹.
c) ** and *** indicate significant differences at 0.01 and 0.001 level (P < 0.01 and 0.001), respectively.
### Table 6-12

Microbial biomass and activity determined in soils after a 40-week period of growth under ryegrass and radiata pine.

<table>
<thead>
<tr>
<th>Soil</th>
<th>MBC (µg g⁻¹)</th>
<th>MBP (µg g⁻¹)</th>
<th>Microbial C:P</th>
<th>MBC: TOC (%)</th>
<th>MBP: Total P (%)</th>
<th>Respiration (µg CO₂-C g⁻¹ 10 days⁻¹)</th>
<th>qCO₂ (µg CO₂-C mg microbial C⁻¹ h⁻¹)</th>
<th>WSOC (µg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egmont</td>
<td>499</td>
<td>687</td>
<td>12</td>
<td>7</td>
<td>41</td>
<td>98</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Stratford</td>
<td>590</td>
<td>712</td>
<td>20</td>
<td>20</td>
<td>29</td>
<td>36</td>
<td>0.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Mangamahu</td>
<td>368</td>
<td>395</td>
<td>7</td>
<td>9</td>
<td>7</td>
<td>42</td>
<td>62</td>
<td>0.9</td>
</tr>
<tr>
<td>Himatangi</td>
<td>565</td>
<td>699</td>
<td>30</td>
<td>11†</td>
<td>19</td>
<td>63</td>
<td>1.8</td>
<td>2.7†</td>
</tr>
<tr>
<td>Taupo</td>
<td>550</td>
<td>683†</td>
<td>18</td>
<td>20</td>
<td>32</td>
<td>34</td>
<td>5.0†</td>
<td>1.5</td>
</tr>
<tr>
<td>Opuanui</td>
<td>673</td>
<td>842†</td>
<td>18</td>
<td>23</td>
<td>15</td>
<td>36</td>
<td>0.8†</td>
<td>1.0</td>
</tr>
<tr>
<td>Pukaki</td>
<td>485</td>
<td>579</td>
<td>15</td>
<td>16</td>
<td>34</td>
<td>38</td>
<td>1.0†</td>
<td>1.4†</td>
</tr>
<tr>
<td>Hurunui</td>
<td>1225</td>
<td>1290</td>
<td>66</td>
<td>41†</td>
<td>19</td>
<td>32</td>
<td>1.6†</td>
<td>1.7</td>
</tr>
<tr>
<td>Te Kauwhata</td>
<td>854</td>
<td>882</td>
<td>15</td>
<td>14</td>
<td>56</td>
<td>65</td>
<td>1.7†</td>
<td>1.7</td>
</tr>
<tr>
<td>Mapua</td>
<td>265</td>
<td>349</td>
<td>7</td>
<td>9</td>
<td>38</td>
<td>42</td>
<td>1.3†</td>
<td>1.9†</td>
</tr>
<tr>
<td>Okarito</td>
<td>1244</td>
<td>1231</td>
<td>82</td>
<td>70†</td>
<td>15</td>
<td>18</td>
<td>0.9†</td>
<td>0.9</td>
</tr>
</tbody>
</table>

| LSD₀.₀₅  | 102          | 6            | 17            | 0.3          | 1.0            | 30                      | 0.09                                  | 19          |

*Data in columns are the mean of three replicates; data in brackets are standard deviations. See Table 6-4 and 6-11 for explanation of other symbols and abbreviations.*
no significant effects of plant species on these ratios in the remaining soils (Table 6-12). Plant species also affected MBP:total P ratios, which were significantly greater in 4 soils (Himatangi, Hurunui, Temuka, Okarito) and lower in 1 soil (Mapua) under ryegrass compared with radiata pine (Table 6-12).

Soil respiration was significantly lower in 6 soils (Himatangi, Taupō, Oruanui, Pukaki, Patoka and Okarito) under ryegrass compared with radiata pine, while there were no significant effects of plant species on soil respiration in the remaining soils (Table 6-12). There were no significant effects of plant species on metabolic quotient (qCO₂) for most soils (Table 6-12). Concentrations of WSOC were consistently higher in soils under radiata pine (80–837 μg g⁻¹) than ryegrass (38–260 μg g⁻¹) (Table 6-12).

6.3.6 Soil and root phosphatase activity
There were significant effects of plant species and soil type, and significant plant x soil interactions on soil phosphatase activities (Tables 6-11 and 6-13). All types of soil phosphatase enzyme activities assayed (AcPME, AlPME and PDE) were significantly higher in most soils under ryegrass than under radiata pine (Table 6-13). Root surface phosphatase activities measured at the respective optimum buffer pH were consistently higher in radiata pine (average 1450 μg p-NP g⁻¹ h⁻¹) than ryegrass (average 755 μg p-NP g⁻¹ h⁻¹) for all soils (Figure 6-3).

6.4 Discussion
There were significant plant species x soil type interactions for most soil variables measured in this study (Tables 6-5 to 6-13), resulting from differences amongst soil types in response to different plant species. These interactions possibly arose from the variations in the properties of the original soils (Tables 6-1, 6-2).

6.4.1 Plant P uptake in different soils
It is well known that plant genus, species or even genotypes of the same species may vary in their ability to take up nutrients from soil (Lajtha and Harrison, 1995; Jungk, 1996; Gahoonia and Nielsen, 1996; Otani and Ae, 1996; Gahoonia et al., 1999). This has been related primarily to a combination of factors including root size and distribution (including root hair length and root surface), root exudation (H⁺, HCO₃⁻,
Table 6-13 Phosphatase activities determined in soils after a 40-week period of growth under ryegrass and radiata pine.

<table>
<thead>
<tr>
<th>Soil</th>
<th>AcPME (μg p-NP released g⁻¹ h⁻¹)</th>
<th>AIPME (μg p-NP released g⁻¹ h⁻¹)</th>
<th>PDE (μg p-NP released g⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td>P</td>
<td>G</td>
</tr>
<tr>
<td>Egmont</td>
<td>562 (29)</td>
<td>253 (27)</td>
<td>178 (13)</td>
</tr>
<tr>
<td>Stratford</td>
<td>399 (11)</td>
<td>82 (27)</td>
<td>266 (17)</td>
</tr>
<tr>
<td>Mangamahu</td>
<td>392 (34)</td>
<td>162 (16)</td>
<td>33 (5)</td>
</tr>
<tr>
<td>Himatangi</td>
<td>442 (30)</td>
<td>213 (20)</td>
<td>223 (12)</td>
</tr>
<tr>
<td>Taupo</td>
<td>533 (27)</td>
<td>479 (18)</td>
<td>128 (5)</td>
</tr>
<tr>
<td>Oruanui</td>
<td>629 (21)</td>
<td>627 (44)</td>
<td>280 (27)</td>
</tr>
<tr>
<td>Pukaki</td>
<td>461 (27)</td>
<td>347 (20)</td>
<td>172 (19)</td>
</tr>
<tr>
<td>Fork</td>
<td>168 (3)</td>
<td>159 (25)</td>
<td>17 (1)</td>
</tr>
<tr>
<td>Hurunui</td>
<td>1308 (91)</td>
<td>650 (43)</td>
<td>480 (33)</td>
</tr>
<tr>
<td>Richmond</td>
<td>649 (37)</td>
<td>281 (24)</td>
<td>219 (19)</td>
</tr>
<tr>
<td>Temuka</td>
<td>632 (36)</td>
<td>583 (64)</td>
<td>304 (24)</td>
</tr>
<tr>
<td>Patoka</td>
<td>745 (90)</td>
<td>629 (51)</td>
<td>319 (25)</td>
</tr>
<tr>
<td>Te Kauwhata</td>
<td>690 (53)</td>
<td>299 (15)</td>
<td>294 (27)</td>
</tr>
<tr>
<td>Mapua</td>
<td>183 (13)</td>
<td>141 (28)</td>
<td>42 (4)</td>
</tr>
<tr>
<td>Okarito</td>
<td>990 (27)</td>
<td>483 (37)</td>
<td>412 (10)</td>
</tr>
</tbody>
</table>

LSD<sub>=0.05</sub> 62 51 6

Data in columns are the means of three replicates; data in brackets are standard deviations. See Table 6-4 and 6-11 for explanation of abbreviations and symbols.
Figure 6-3 Root phosphatase activities of ryegrass and radiata pine determined after a 40-week period of growth.
reducing agents, chelates, organic anions, enzymes), mycorrhizal infection, and transpiration rate (Itoh and Barber, 1983; Fohse et al., 1988, 1991; Jungk, 1996; Gahoonia and Nielsen, 1996; Hinsinger and Gilkes, 1997; Subbarao et al., 1997). The interaction of soil and plant properties (nutrient availability and acquisition by plants) determines the rate and the quantity of P transfer into plants (Jungk, 1996).

In this study, radiata pine and ryegrass showed expected differences in plant growth and nutritional characteristics. Radiata pine produced 1.4 – 5.5 times more biomass than ryegrass, while the S:R ratios of radiata pine were also generally higher than those of ryegrass. Moreover, radiata pine generally took up more P in most soils (except for the Temuka soil) compared with ryegrass (Table 6-4). However, P uptake by ryegrass and radiata pine was also greatly influenced by soil type and soil x plant interactions (Table 6-4). Levels of \( E_{\text{min}} \text{Pi} \) in the original soils were negatively correlated with the corresponding P uptake ratios by radiata pine to ryegrass (Figure 6-4). This indicated that radiata pine was able to take up more P than ryegrass when the level of available P was low, which in turn showed that radiata pine was better able to access sparingly soluble sources of soil P. On the other hand, differences in P uptake between the two species declined as soil available P (\( E_{\text{min}} \text{Pi} \)) increased (especially for \( E_{\text{min}} \text{Pi} > 15 \mu g \) g\(^{-1}\)), while ryegrass took up even more P than radiata pine when the levels of available P were high (Figure 6-4). For example, the Temuka soil had a high level of available P (\( E_{\text{min}} \text{Pi} 29 \mu g \) g\(^{-1}\) before planting), and ryegrass took up more P than radiata pine (Tables 6-2, 6-4), which may have resulted in lower levels of available Pi and total P in the soil under ryegrass (Tables 6-6, 6-7). The obvious differences in P uptake between radiata pine and ryegrass may be attributed to the differences in root morphology and associated chemical, biochemical and biological properties.

In general, vesicular-arbuscular mycorrhizae (VAM) are associated with ryegrass (Powell, 1977), while ectomycorrhizae (ECM) are associated with roots of radiata pine (Chu-Chou, 1979; Chu-Chou and Grace, 1990). The precise nature of mycorrhizae associated with ryegrass and radiata pine were not determined in this study. However, ectomycorrhizal hyphae were clearly visible in soils under radiata pine, and abundant mycorrhizae as characterized by dense mycelial sheaths were observed on roots of radiata pine at the end of experiment. There is a lack of direct comparisons of root-VAM and root-ECM associations in the literature. In general, ECM hyphae are
thought to be more efficient in P uptake and transportation to the host plants than VAM hyphae (Marschner and Dell, 1994). This has been ascribed to a combination of factors including greater extension of ECM hyphae into soils compared with VAM hyphae, low internal Pi concentration in ECM due to formation of polyphosphates, and higher soil volume explored by ECM due to smaller hyphae diameter and the potential role of acid phosphatase and phytase enzymes produced by ECM hyphae (Dighton and Coleman, 1992; Marschner and Dell, 1994; Jones et al., 1998; Leake, 2001). Moreover, ECM hyphae have been found to release significant quantities of low molecular weight organic acids such as oxalic acid which may mobilize P from sparingly soluble Ca-P, Fe-P or Al-P in soil (Malajczuk, 1982; Lapeyrie et al., 1991; Vogt et al., 1991a). Lower pH in soils under conifers such as radiata pine may also enhance the solubility and consequent availability of soil inorganic P and organic P (Gahoonia and Nielsen, 1992b).
In the present study, multiple regression analysis showed that only $E_{1\text{min}}$ Pi, and $R/r_1$ were the most important factors which influenced P uptake by ryegrass. Accordingly, approximately 80% of the variation in P uptake by ryegrass was explained by the following equation:

$$P \text{ uptake (mg pot}^{-1}) = 3.674 + 0.631 \ E_{1\text{min}} \ Pi \ (\text{mg kg}^{-1}) - 0.437 \ R/r_1 \ (n = 15, \ P < 0.001)$$

[6.6]

However, the relationship between P uptake and soil P for radiata pine could not be described by the same multiple regression approach. The Cp, $E_{1\text{min}}$ Pi and $E_{>3m}$ Pi accounted for only 11.6 – 24.4% of the variation in P uptake by radiata pine, while other factors ($R/r_1$, $n$, other exchangeable P pools and TPo) were not significantly correlated with P uptake by radiata pine. These findings strongly indicate that radiata pine was better able to utilize different forms of inorganic and organic P in the soil, compared with ryegrass. In addition, these results also imply that due to enhanced mineralization of organic P, levels of labile P did not appear to limit P uptake by radiata pine. Moreover, variation in P uptake by radiata pine across soil types may also have been influenced by the availability of other nutrients such as N.

Specific mineralization rate has been used as a measure of soil organic P availability (Grierson et al., 1999). In the present study, it was found that SMR varied greatly with soil type and plant species (Figure 6-3). The SMR in soils under ryegrass and radiata pine was not related to TOC ($r = 0.179$ (ns) and $0.077$ (ns) for ryegrass and radiata pine, respectively) or to TPo ($r = -0.045$ (ns) and $0.391$ (ns) for ryegrass and radiata pine, respectively) in the original soils, suggesting that the quality of organic C and P rather than quantity determined the rate of organic P mineralization. The SMR in soils under radiata pine were consistently higher than in soils under ryegrass, indicating that radiata pine could better access forms of soil organic P than ryegrass, and that the growth of radiata pine resulted in increased mineralization of organic P compared with ryegrass. However, P uptake by radiata pine was not significantly correlated with SMR, while there was significant correlation found between SMR and P uptake by ryegrass (Figure 6-5). This result further supported the contention that levels of labile Pi may not have limited P uptake by radiata pine (see above).

Since P in soils is relatively immobile most species take up their P from near the root surface (Harrison, 1989). Accordingly, root surface phosphatase activity may play a
key role in the P uptake by plants, especially when P availability is low (Helal, 1990). The presence of ectomycorrhizae increases root acid phosphatase activity (Pasqualini et al., 1992), while Tarafdar and Claassen (1988) reported that root-derived phosphatase activity was significantly correlated with the amount of soluble organic P hydrolyzed. Antibus et al. (1992) also found that increased $^{32}$P uptake from inositol polyphosphate was related to acid phosphatase activity of ectomycorrhizae. In the present study, P uptake by ryegrass and radiata pine and plant biomass were directly related to the levels of root phosphatase enzyme activity (Figure 6-6). Root phosphatase activity was 1.2 – 13.2 times higher for radiata pine compared with ryegrass, which may contribute to the higher proportion of P obtained from soil organic P sources by radiata pine.

![Figure 6-5](image-url)  
**Figure 6-5** Relationships determined between specific mineralization rate (SMR) and P uptake by ryegrass (left) and radiata pine (right) after a 40-week period of growth for individual replicates (n = 45) (note: correlation coefficients and associated significance were calculated using mean values for each soil (n = 15)).
Figure 6-6 Relationships determined between root phosphatase activity and P uptake of ryegrass (left) and radiata pine (right) after a 40-week period of growth for individual replicates (n = 45) (note: correlation coefficients and associated significance were calculated using mean values for each soil (n = 15)).

6.4.2 Effects of plant species on soil P availability and redistribution

The effects of plant species on soil nutrient availability have been studied in many ecosystems (Nielsen and Dalsgaard, 1987; Walbridge, 1991; Hobbie, 1992; Magid, 1993). Nielsen and Dalsgaard (1987) investigated the effect of invasion of oak (Quercus robur) on a Calluna heath (Calluna vulgaris) in Denmark and found the rate of organic matter decomposition increased under oak. Magid (1993) found that under beech (Fagus sylvatica) a large part of the extractable P was present in labile inorganic fractions (resin Pi, bicarbonate Pi) while under adjacent grass a large part of the extractable P was in labile organic P forms (bicarbonate and fulvic acid Po). This indicated that vegetation affected the nature and distribution of soil P. In the USA, it has been found that red alder (Alnus rubra Bong) increased soil P availability and phosphatase activity (Giardina et al., 1995; Zou et al., 1995), while Binkley et al. (2000) found that available P determined by resin bags was twice as high under Eucalyptus saligna compared with Albizia facaltaria.
Despite the fact that P uptake was greater by radiata pine compared with ryegrass in most soils, concentrations of labile P (BPi, BPo, resin Pi and resin Po, $E_{\text{min}-24h}$ Pi, $E_{24h-3m}$ Pi, $E_{\text{min}-3m}$ Pi) were generally higher in many soils under radiata pine compared with ryegrass (Tables 6-6, 6-9 and 6-10). Furthermore, the higher concentrations of labile P under radiata pine were accompanied by enhanced mineralization of organic P compared with ryegrass (Table 6-7; Figure 6-3). These results are consistent with 'paired-site' comparison studies carried out in New Zealand (Davis, 1994; 1995; Condron et al., 1996; Parfitt et al., 1997; Alfredsson et al., 1998; Chapters 3 and 5). In addition, both plant species were found to have lowered the level of slowly exchangeable Pi ($E_{>3m}$) by approximately 27% compared with original levels (Table 6-2 and Table 6-9). This is consistent with other studies that showed that much of the P taken up by crop plants was derived from the slowly exchangeable Pi pool when no P was applied (Frossard and Sinaj, 1997). The above findings clearly demonstrate that in many soils the growth of ryegrass and radiata pine resulted in the redistribution of P from the slowly exchangeable Pi pool to rapidly exchangeable Pi pools. Radiata pine caused a greater P redistribution from the organic P pools to the inorganic P pools compared with ryegrass. It should be noted that plant species x soil type interactions indicated that effects of plant species on the values of $n$ varied greatly with soil type (Table 6-8). The higher values of $R/r_1$ in soils under radiata pine could be attributed to higher P uptake by radiata pine. Depletion of P from the soil solution is accompanied by an increase in $R/r_1$ and $n$ values (Frossard and Sinaj, 1997). Morel
and Hinsinger (1999) also suggested that enhanced P uptake in rhizosphere soil compared with bulk soil resulted in higher R/r1. In addition, root activity can modify the soil physiochemical properties and thereby affect P exchangeability (Morel and Hinsinger, 1999). Greater root exudation (H+ efflux, organic acids) by radiata pine (as indicated by higher levels of WSOC (see section 6.4.3)) compared with ryegrass may also enhance dissolution of iron (Fe), aluminum (Al) and calcium (Ca) minerals and consequently expose new P exchangeable sites (Bar-Yosef, 1996).

6.4.3 Effects of plant species on soil biochemical and biological properties
Several field studies conducted in New Zealand have shown that afforestation decreased MBC and MBP and soil respiration compared with adjacent grassland soils. Theses findings were attributed mainly to the reduced quantity and lower quality of organic inputs from tree roots compared with grassland species roots (Sparling et al., 1994; Yeates et al., 1997; Yeates and Saggar, 1998; Condron et al., 1998; Chapter 3). However, in the present short-term glasshouse experiment, concentrations of MBC and CO2 respiration were generally higher in soils (significantly in 6 soils) under radiata pine, while levels of MBP were significantly lower in many soils compared with ryegrass (Tables 6-11, 6-12). The higher MBC and microbial activities determined in soils under radiata pine compared with ryegrass may be simply attributed to greater concentrations of WSOC in soils (Tables 6-11, 6-12). Concentrations of WSOC were directly related to the levels of MBC and CO2 respiration in both soils under ryegrass (r = 0.502 and 0.650*, n = 15) and radiata pine (r = 0.823** and 0.554*, n = 15). It has been suggested that soil WSOC is primarily derived from rhizodeposition (including root exudates, root residues) (McGill et al., 1986; Huang and Schoenau, 1998), while other sources for WSOC are microbial debris, decomposition of organic matter and desorption from soil colloids (McGill et al., 1981; Fox, 1995). The quantitative determination of the origins of soil WSOC requires the use of 14C techniques (Rovira, 1969; Helal and Sauerbeck, 1984; Warembourg and Estelrich, 2000). In the present study, concentrations of WSOC were directly related to plant root weight, particularly for radiata pine (Figure 6-7). Moreover, it has been reported that in the ectomycorrhizal association between ponderosa pine (Pinus ponderosa) seedlings and Hebelom crustuliniforme, as much as 41% of the C fixed by photosynthesis was allocated to below-ground parts (Rygiewicz and Andersen, 1994) while only 4-14% of total C photosynthesized by the plant was allocated to the VA
mycorrhizal symbiont (Paul and Clark, 1989). Therefore, it is very likely that enhanced levels of WSOC in soils under radiata pine may mainly have derived from greater rhizodeposition (including root exudation, root residues), which in turn was responsible for higher MBC and microbial activity in soils under radiata pine.

It should be noted that there were significant effects of plant species x soil type interactions for soil MBC, MBP and CO₂ respiration. Differences in the apparent effects of trees on soil MBC and microbial activity observed in this study compared with field experiments (see Chapter 3) may reflect differences in plant C metabolism (at different stages of tree growth) (Richards, 1987), which in turn influences the quantity and activity of microbial biomass in the soil and associated nutrient transformations. This result may also support our previous hypothesis that enhanced mineralization of soil organic C and associated nutrients observed in soil under forest compared with grassland occurred mainly during the early stages of the forest establishment due to higher microbial activity (Chapter 3).
It has been shown that levels of MBP in soil are more seasonally variable compared with MBC (Tate et al., 1991b; Chapter 5). In the present study, lower levels of MBP and higher microbial C:P ratios in many soils under radiata pine indicated a greater release of P from microbial biomass to meet higher P demand by radiata pine, which was also consistent with enhanced mineralization of soil organic P under radiata pine (Table 6-7; Figure 6-3). Large amounts of P were present in the microbial biomass in soils under ryegrass and radiata pine (Tables 6-11, 6-12). It has been found in culture that over 60% of microbial intracellular P is usually in the form of nucleic acid, 20% in acid soluble P-esters and 5% in phospholipids (Hedley and Stewart, 1982; Magid et al., 1996). It has also been suggested that microbial biomass is a major source of P in soil solution (Seeling and Zasoski, 1993; Seeling and Jungk, 1996). However, how P is released from biomass is not well understood (Coleman et al., 1978b; Stewart and Tiessen, 1987). The release of P from microbial biomass may be enhanced by freezing-thawing (Fabre et al. (1996), drying-rewetting (Srivastava, 1998; Pulleman and Tietema, 1999), and by trophic interactions of microflora and microfauna (Cole et al., 1978; Coleman et al., 1978a). However, direct comparison between the effects of VAM-microfauna (amoebae and protozoa)—root exudation and the effects of ECM-microfauna (amoebae and protozoa)—root exudation on nutrient availability has not been carried out. Cole et al. (1978) found in a microcosm study that the release of P from microbial biomass was mainly caused by microfauna (e.g. amoebae) grazing on bacteria and that CO₂ respiration was significantly higher in microcosms containing amoebae and protozoa. This was also supported by results from other similar studies (Kuikman et al., 1990; Darbyshire et al., 1994). Cromack et al. (1988) estimated biomass of soil animals for both fungal mat and non-fungal mat areas in a Douglas-fir (Pseudotsuga menziesii) forest and found that total biomass of soil animals (particularly amoebae and nematodes) was higher in fungal mats which also had greater concentrations of C and N, CO₂ respiration and enzyme activity. Jentschke et al. (1995) also reported that ectomycorrhizal colonization of Norway spruce (Picea abies) significantly increased the abundance of amoebae at the rhizoplane, which was postulated to be related to protozoan grazing on mycorrhizal fungal hyphae. Therefore, it is reasonable to hypothesize that the higher MBC and CO₂ respiration, which was accompanied by the lower levels of MBP in many soils under radiata pine compared with ryegrass in the present study, may be attributed to high microfauna (amoebae and protozoa) biomass and activities related to the presence of ECM and increased root exudation.
Acid phosphomonoesterase (AcPME), AIPME and PDE activities were generally lower in soils under radiata pine compared with ryegrass (Tables 6-11, 6-13), which is consistent with comparable field studies (Perrott et al., 1999; Chapters 3 and 5). However, this is difficult to explain. The extracellular phosphatase activity assay includes enzymes associated with living components (e.g. microbial cells), enzymes attached to entire dead cells and cell debris, and enzymes immobilized on the soil clay and humic colloids (Burns, 1986; Sinsabaugh, 1994). Plant roots and microorganisms respond to demand for available P by releasing phosphatase into the soil environment (Speir and Ross, 1978; Lipton et al., 1987; Abd-Alla, 1994; Li et al., 1997). However, the fate and regulation of these released enzymes is not well understood. The extracellular enzymes may be subject to adsorption, inhibition, stabilization and humification depending on soil properties (e.g. heavy metal, clay and organic C contents) (Sinsabaugh, 1994). Thus enzyme activity measured in the soil generally reflects the interactions of biological components (plant and microorganisms) and chemical components (e.g. clay, organic matter and heavy metal). In the present study, lower phosphatase activity in soils under radiata pine may be associated with the different modification of soil properties by radiata pine compared with ryegrass. Radiata pine reduced the soil pH by 0.4 compared with ryegrass (Table 6-5). Decreased pH in soil under radiata pine have been shown to increase concentrations of available Copper (Cu$^{2+}$), Zinc (Zn$^{2+}$), Fe$^{3+}$ and Al$^{3+}$ ions which in turn may have inhibited phosphatase activity (Tyler, 1976; Juma and Tabatabai, 1977). Moreover, an increase in the number of enzyme adsorption sites caused by the lower pH and increased levels of organic exudates may also partly account for the lower phosphatase activity in soils under radiata pine (Burns, 1986; Naidja et al., 2000). Alternatively, the lower phosphatase activity under radiata pine may be attributed to feedback control (i.e. increased concentration of immediately available Pi) (Speir and Ross, 1978; Fox and Comerford, 1992). However, this feedback control mechanism does not explain the higher root phosphatase activity in radiata pine compared with that in ryegrass (Figure 6-4), since it has been shown that root phosphatases are more sensitive to high concentrations of Pi than soil phosphatases (Adams and Pate, 1992). In addition, levels of immediately available P (Cp and E$_{imin}$ Pi) may not have been high enough to repress the synthesis and then activity of soil phosphatase enzyme since no significant relationships or positive relationships were observed between phosphatase activity and concentrations of Cp and E$_{imin}$ Pi in soils under ryegrass and radiata pine (r = 0.032
(ns) – 0.707**, n = 15). However, more recently, Olander and Vitousek (2000) found that in forest soils phosphatase activity was inhibited by P fertilization in the long term (4 years), but not inhibited in the short term (1 month). This suggests that it takes time before a negative feedback results in lower levels of enzyme activity. Therefore, it is still unclear from this short-term study whether the feedback control mechanism plays a major role in determining enzyme activity.

6.4.4 Chemical, biochemical and biological processes involved in soil P transformations under different plant species

Phosphorus mineralized from organic sources is an important factor in determining overall P availability in soil (Stewart and Tiessen, 1987; Fox and Comerford, 1992; Magid et al., 1996). In general, organic P comprises 30-80% of total P in soil, while up to 50% of organic P remains unidentified (McGill and Cole, 1981; Magid et al., 1996; Frossard et al., 2000). Most of the identified organic P in soil is present in the form of phosphate esters (C-O-P), although small amounts of organic condensed phosphates (P-O-P) and phosphonates (P-O-P, P-N and P-S) are also present (McGill and Cole, 1981; Magid et al., 1996; Turner, 2000). The availability of soil organic P to plants is determined by its solubility and susceptibility to degradation / hydrolysis by extracellular enzymes and/or direct microbial assimilation (Harrison, 1983; Polglase et al., 1992a; Hayes et al., 2000).

It has been suggested that mineralization of organic P is mainly through biochemical pathways (phosphatase hydrolysis), independent of C mineralization, and is controlled by demand for P (McGill and Cole, 1981; Smeck, 1985), while P can also be released as a by-product from organic matter by biological mineralization and driven by the demand for energy (Stewart and Tiessen, 1987). Biological transformations of soil organic P are mainly mediated by a combination of plant root activity and microbial activity (Hedley et al., 1982b, c; Stewart and Tiessen, 1987; Richardson, 1994; Magid et al., 1996). It is well established that root activities of different plant species selectively stimulate growth of different microflora in the rhizosphere via root exudation (e.g. sugars, amino acids, organic acids, hormones and vitamins, and phosphatase enzymes) (Rovira, 1969; Zwart et al., 1994). Root exudates are sources of as much as 30% to 40% of organic inputs to the below-ground portion of terrestrial ecosystems (Coleman et al., 1978a), and thereby represent an important source of organic C for microflora in the rhizosphere. Root exudates (including organic acids
and phosphatase enzymes) react with soil components in solution and the solid phase, and therefore modify soil chemical, biochemical and biological properties (Hedley et al., 1982b, c; Fox, 1995; Bar-Yosef, 1996).

In the present study, different impacts on soil chemical, biochemical and biological processes exerted by different root activities of ryegrass and radiata pine are responsible for apparent differences in soil P transformations. Water soluble organic C is mainly derived from root exudation (see section 6.4.3) and is considered to be labile and play an important role in soil processes (Fox, 1995). Higher levels of WSOC measured in soils under radiata pine may have enhanced the growth and activity of microorganisms (particularly ectomycorrhizae) and may consequently have resulted in more rapid turnover of soil organic P under radiata pine seedlings compared with ryegrass.

Low molecular weight organic acids (e.g. oxalate, citric and maleic acids) can have a significant impact on soil chemical and biological processes although they usually comprise less than 10% of WSOC (Fox and Comerford, 1990; Fox, 1995). Significant amounts of organic acids have been found to be produced by ectomycorrhizae associated with radiata pine (Malajczuk, 1982) and many other forest soils (Smith, 1976; Fox and Comerford, 1990; Fox, 1995). Solubilization of mineral inorganic P by organic acids in relation to soil P availability has been the focus in many studies in recent years, while possible dissolution of soil organic P by organic acids has received little attention (Fox, 1995; Bar-Yosef, 1996; Jones, 1998; Hinsinger, 2000; Crowley, 2000). However, it has also been demonstrated that organic acids such as oxalic acid greatly enhance the solubility of organic P in soil (Comerford and Skinner, 1989; Fox et al., 1990a, b). While mechanisms responsible for enhanced dissolution of organic P are not clear, it has been suggested that organic acids dissolved Al (Fe)-organic P complexes by chelation, thus releasing organic P (Fox and Comerford, 1990). In addition, increased \( H^+ \) efflux in the pine soils as indicated by increased pH may also contribute to increased solubility of organic P (Gahooonia and Nielson, 1992b). In summary, it is suggested that increased root exudation of organic acids, together with lowered soil pH, might increase solubilization of organic P, and thus contribute to the enhanced mineralization of organic P observed in soils under radiata pine compared with ryegrass in this study.
Phosphatase is essential to the mineralization of soil organic P (Speir and Ross, 1978; Sinsabaugh, 1994). However, the relationship between phosphatase enzyme activity and mineralization of organic P is poorly understood despite extensive investigation (Stewart and Tiessen, 1987; Magid et al., 1996). It has been reported in many studies that soil phosphatase activity was not related to organic P mineralization (Speir and Ross, 1978; Adams, 1992), and solubility of organic P rather than soil phosphatase activity has been suggested to determine the rate of organic P mineralized in soils (Tarafdar and Claassen, 1988; Adams, 1992, Adams and Pate, 1992). Moreover, the mineralization of organic P is also linked to the mineralization of organic C (Dalal, 1979; Gressel and McColl, 1997), and is probably the result of interactions involving a variety of structural and functional elements (Sinsabaugh et al., 1991).

Dalal (1979) found in a study in which non-labelled plants were grown in soil incorporated with radioactively-labeled clover residues, that the uptake of $^{32}$P by plants was strongly related to release of $^{14}$CO$_2$, indicating P availability was coupled with residue decomposition. Linquist et al. (1997) also suggested that NaHCO$_3$ extractable Po declined due to crop uptake at the same rate as soil organic C, indicating that NaHCO$_3$ extractable P and C mineralization may be coupled. Gressel et al. (1996) used $^{13}$C NMR and $^{31}$P NMR spectroscopy and found a positive correlation between monoester P and alkyl C in the forest floor, indicating that mineralization of organic P is linked to breakdown of the associated C structure. This result also suggests that P release from organic sources depends on conditions that are conducive to decomposition of the C structure occluding P rather than optimal conditions for phosphatase activity and can explain the inconsistent relationships found between organic P mineralized and phosphatase enzyme activity in many studies (Tarafdar and Claassen, 1988; Adams and Pate, 1992).

In the present study, the higher rate of mineralization of organic P is inconsistent with the lower soil enzyme activity under radiata pine compared with ryegrass (Tables 6-7 and 6-13, Figure 6-3; section 6.4.3). Moreover, soil phosphatase enzyme activities were directly correlated with SMR in soils under ryegrass ($r = 0.342 - 0.641^*, n = 15$), while no such relationship was found in soils under radiata pine ($r = -0.070 - 0.227$ (ns), $n = 15$). In addition, SMR was significantly correlated with the turnover rate of organic C (C mineralized as percent of the amount of C originally) ($r = 0.515^*, n = 15$) in soils under radiata pine while there was no such relationship observed in soils under
ryegrass \( (r = -0.149 \text{ (ns), } n = 15) \) (Figure 6-8), indicating that mineralization of organic P may be linked to that of organic C in soils under radiata pine, but not in soils under ryegrass. These results strongly showed that there might be different pathways

![Figure 6-8](image)

**Figure 6-8** Relationships determined between the specific mineralization rate of organic P (SMR) and the turnover rate of organic C in soils under ryegrass and radiata pine after a 40-week period of growth for individual replicates \( (n = 45) \) (note: correlation coefficients and associated significance were calculated using mean values for each soil \( (n = 15) \)).

(biochemical and/or biological pathways) involved in mineralization of organic P in soils under ryegrass and radiata pine. In soils under ryegrass, the biochemical pathway (hydrolysis by extracellular enzymes) might be dominant with phosphatase enzymes playing a crucial role in the mineralization of organic P. On the other hand, in soils under radiata pine both biochemical and biological pathways might be involved in mineralization of organic P and interactions of these two pathways determine the overall rate of mineralization. The involvement of a biochemical pathway in the mineralization of organic P in soils under radiata pine is also supported by the inverse correlation observed between PDE activity and resin extractable P (Table 6-15, also see below), together with significantly higher root surface phosphatase activity (Figure 6-3).
6.5 Conclusions

Results from this glasshouse experiment clearly showed that compared with ryegrass, radiata pine took up more P from the soil particularly when the level of available P (e.g. $E_{1\text{min}}$ Pi) was low. The growth of ryegrass and radiata pine resulted in the redistribution of P the from the slowly exchangeable Pi pool ($E_{>3\text{m}}$ Pi) to the rapidly exchangeable P ($E_{1\text{min}-24\text{h}}$ Pi, $E_{24\text{h}-3\text{m}}$ Pi) pools in most soils, while radiata pine caused greater P redistribution from organic P to inorganic P compared with ryegrass.

The growth of radiata pine resulted in higher concentrations of MBC and CO$_2$ respiration, and lower levels of MBP and phosphatase enzyme activities in many soils compared with ryegrass. Increased root exudation as indicated by higher levels of WSOC in soils under radiata pine seedlings might have enhanced microbial activity and improved the solubility and utilization of soil inorganic and organic P. It is suggested that biochemical hydrolysis by phosphatase enzymes is the dominant mechanism for mineralization of organic P in soils under ryegrass, while both biochemical and biological processes determine the mineralization of organic P in soils under radiata pine.
Chapter 7

Phosphorus Dynamics in the Rhizosphere of Ryegrass and Radiata pine

7.1 Introduction

7.1.1 The rhizosphere
The rhizosphere is the most chemically, biochemically and biologically active microsite in soil and the ecological importance of the rhizosphere is widely recognized (Lynch et al., 1982; Toal et al., 2000). The term ‘rhizosphere’ was originally used to describe a zone of bacterial growth which was stimulated near the roots of legumes by the release of nitrogen (N) compounds by the nodules (Lynch, 1982; Rovira, 1991). The definition of the rhizosphere has since been modified to the zone of soil that is affected by the root activity of any plant species (Lynch, 1982; Martin, 1983). Its extent varies and there is no sharp boundary between it and the neighboring soil (Martin, 1983; Richards, 1987; Campbell and Greaves, 1990). It is generally considered to be within 0-1500 \( \mu \text{m} \) from the root surface (Foster et al., 1983), although stimulation of microflora can occur up to 5000 \( \mu \text{m} \) from the root surface (Martin, 1983). The rhizoplane is defined as ‘the external surface of plant roots together with any closely adhering particles of soil or debris’ (Lynch, 1982; Richards, 1987). However, it is often difficult to distinguish microbial colonization across the regions of the soil-root interface (rhizosphere to rhizoplane to epidermis/cortex), and it appears more reasonable to consider it as a continuum (Lynch, 1982; Campbell and Greaves, 1990).

7.1.2 Impacts of root-induced changes on rhizosphere P dynamics
Plant-derived organic matter in the rhizosphere modifies its chemical, biochemical and biological processes (Lynch, 1982; Richards, 1987; Rovira, 1991). This organic matter includes mucilages (high molecular weight polysaccharides and polygalacturonic acids), sloughed off root cap cells, and root exudates (sugar, amino acids, low molecular weight organic acids, nucleotides and enzymes) (Rovira, 1969; Richards, 1987; Jungk, 1996). These organic materials enhance microbial activity and thus influence nutrient transformations (especially N, phosphorus (P) and sulfur (S)) in the
rhizosphere (Marschner, 1991; Jungk, 1996). In particular, root exudates have been implicated in many soil processes including mobilization and uptake of nutrients by plants and microbes, and detoxification of metals by plants (Jungk, 1996; Jones, 1998). The amounts and types of compounds exuded have been shown to vary with plant species, age and environmental conditions, and account for approximately 12-40% of plant photosynthate (Rovira, 1965; Martin, 1983; Campbell and Greaves, 1990).

It is well established that the presence of organic acids (e.g. maleic, citric and oxalic acids) in the rhizosphere soil enhance the solubility of mineral P by ligand exchange and complexation of metal ions such as aluminum (Al$^{3+}$), iron (Fe$^{3+}$) and calcium (Ca$^{2+}$) (Bar-Yosef, 1996; Jones, 1998). It also has been suggested that organic acids such as oxalic acid greatly enhance the solubility of organic P in soil (Comerford and Skinner, 1989; Fox et al., 1990a, b), although the precise mechanisms involved are not well understood (see Chapter 6). In addition, increased levels of carbon dioxide (CO$_2$) in the rhizosphere may also enhance the solubility of inorganic P and organic P (Campbell and Greaves, 1990). Root-induced pH changes also affect soil P solubility (Hedley et al., 1982a; Gillespie and Pope, 1990a, b; van Diest, 1991).

Organic P is an important source of P for plants (Stewart and Tiessen, 1987; Fox and Comerford, 1992; Magid et al., 1996). Root-borne acid phosphatase enzymes, together with fungal acid phosphatase and bacterial alkaline phosphatase are believed to be responsible for hydrolysis of organic P in the rhizosphere (Speir and Ross, 1978; McGill and Cole, 1981; Tarafdar and Jungk, 1987; Tarafdar and Claassen, 1988). It has been reported that phosphatase activities were higher in rhizosphere soil compared with the adjacent bulk soil (Tarafdar and Jungk, 1987; Häussling and Marschner, 1989; Asmar et al., 1995; Joner et al., 1995). It has also been observed that depletion of organic P was correlated with acid phosphatase activity in the rhizosphere of Norway spruce (Picea abies) (Häussling and Marschner, 1989). However, phosphatase activity was not related to mineralization of organic P in the rhizosphere in some other studies (Joner et al., 1995; McDowell et al., 1996), so the importance of the precise role of phosphatase enzymes in soil organic P mineralization has yet to be verified (Jungk, 1996).
7.1.3 Rhizosphere study technique

The quantitative understanding of rhizosphere processes is poor, since the rhizosphere is a difficult system to physically sample and manipulate (Toal et al., 2000). Currently there are two broad kinds of methodology available to physically separate rhizosphere soil from bulk soil. One is the hand-shaking method described by Hendriks and Jungk (1981), whereby the rhizosphere soil is separated from the bulk soil by gentle shaking. The soil adhering to the roots after shaking is taken as the rhizosphere soil. This method is simple and convenient, and has been used in field experiments (Häussling and Marschner, 1989; Gobran et al., 1998).

The second approach involves direct (in situ) sampling of soil adjacent to roots by thin sectioning and/or placement of different sized mesh materials around the roots. Farr et al. (1969) used two soil blocks to hold onion (Allium cepa) roots and then froze the soil block using liquid N\textsubscript{2} and thin sectioned the rhizosphere soil (at a distance of 20 – 1500 \textmu m from the root surface). This method worked well only for plant roots with root hairs. Helal and Sauerbeck (1983; 1984) used nylon or stainless steel fine mesh (31.5 \textmu m) fabric to separate the soil into 3 zones (a central root zone (20 mm wide) and two outer zones at 10 and 20 mm from the root surface) to study carbon (C) dynamics in the rhizosphere using \textsuperscript{14}C. McLaughlin and James (1991) and Teng and Timmer (1995) also used nylon mesh (5 \textmu m) to simply separate rhizosphere soil from bulk soil.

Kuchenbuch and Jungk (1982) developed an improved method to sample rhizosphere soil at a known distance from the root surface. Plants are grown in a small container in which the roots were separated from the soil by a nylon mesh (30 \textmu m). Root hairs (but not roots) penetrate the mesh into the soil. After a period of growth, the soil is frozen using liquid N\textsubscript{2} and sliced into 0.06 mm thick layers by a refrigerated microtome. This method is restricted to young seedlings due to the small container used. Based on the principle of this method, Gahoonia and Nielsen (1991; 1992a) developed a system in which water and nutrients were supplied. This method can be used to study the rhizosphere processes at the advanced stages of plant growth and pH can be controlled. In recent years, the method developed by Kuchenbuch and Jungk (1982) has been modified to study various aspects of rhizosphere nutrient dynamics (Dijkstra et al., 1987; Li et al., 1991; Hinsinger and Gikes, 1995; 1997; Zoysa et al., 1997; 1998).
Previous studies described in this thesis have investigated the influence of contrasting plant species/populations on soil P dynamics in relation to time (Chapters 3 and 5) and soil properties (Chapter 6). The specific objective of this experiment was to focus on the effects of ryegrass and radiata pine on soil P dynamics in the rhizosphere of selected soils.

7.2 Materials and Methods

7.2.1 Soil sample and plant species
The Fork and Hurunui soils described in Chapter 2 and used in Chapter 6 were used in this study. The Hurunui soil had higher levels of available inorganic P and organic P compared with the Fork soil. The soils were air-dried and passed through a 1 mm sieve prior to use. Plant species used in this study were perennial ryegrass (*Lolium perenne*) (cultivar ‘Grasslands Nui’) and radiata pine (*Pinus radiata*) (seed lot. 92/34 GF12).

7.2.2 Glasshouse experiment

*Rhizosphere study technique*
The thin slicing technique described by Kuchenbuch and Jungk (1982) and Zoysa et al. (1997) was modified and used to sample rhizosphere soil in this study. The equipment used for this included plant growth containers (PGC), an automatic irrigation system, and thin sectioning microtome with a custom adapter. The PGC is a two-compartment PVC cylinder device (Figure 7-1), and the two compartments were separated by a 25 μm nylon mesh (Schweiz Seidengazefabrik AG, Thal, Switzerland). The upper compartment was 40 mm high with an internal diameter of 40 mm, while the lower compartment comprised two cylinders of the same diameter (40 mm) with heights of 13 mm (top) and 27 mm (bottom). The irrigation system was also shown in Figure 7-1. Water potential in the soil in the compartment was maintained at approximately –5.0 kPa by fixing the hydraulic head at 50 cm. The rotary microtome and purpose built steel adapter are shown in Figure 7-2.

*Experimental procedure*
The upper compartments were packed with 54 g soil (Fork, bulk density 1.102 g cm⁻³) and 36 g soil (Hurunui, bulk density 0.735 g cm⁻³), while the lower compartments were
Figure 7-1  Schematic representation of the plant growth container (PGC) and irrigation system.
packed with 60 g soil (Fork, bulk density 1.224 g cm$^{-3}$) and 39 g soil (Hurunui, bulk density 0.775 g cm$^{-3}$). There were three treatments for each soil, including control (without plants), ryegrass and radiata pine. These treatments were replicated 4 times and each replicate consisted of 4 individual PGCs. Seeds of each species were directly planted in the upper compartment and thinned to three per pot after germination. Radiata pine seedlings were inoculated with ectomycorrhizae (*Rhizopogon rubescens*) at a rate of $1 \times 10^7$ spores per pot two weeks after sowing. When a root mat had developed after 90 days growth, the plants along with the upper compartment were placed on the lower compartment. Root hairs and hyphae, but not roots, were able to penetrate the 25 μm mesh and enter the soil in the lower compartment and thus create the rhizosphere. This experiment was carried out in a glasshouse with an average daily temperature of between 12 °C and 25 °C.

At the end of 60 days, the lower compartment was carefully separated from the upper compartment. Plant shoots were cut 5 mm above the soil surface and oven-dried at 65 °C for 72 hours, weighed and finely ground for P analysis. A 2.0-mm-thick section was taken from the upper compartment immediately above the inter-cell boundary (25 μm nylon mesh) and root length was measured by the line intersect method of Tennant (1975). The root volume was determined by the amount of water displaced when roots were immersed in water. The root surface area was calculated using the following formula:
Root surface area = 2\sqrt{\pi LV},
where \( \pi = 3.1416 \), \( L \) is the length of roots, and \( V \) is the volume of roots. The equation assumes that the root can be regarded as a cylindrical tube with a constant radius (Zoysa et al., 1998). The lower surface of the roots in the soil section (0-2 mm) immediately above the mesh was assumed to be accountable for the changes in the rhizosphere below. Roots in this section (after measurements of root length and volume as above) and in soil above this slice were oven-dried at 65 °C, weighed and finely ground for P analysis.

The top cylinder in the lower compartment was carefully removed and the bottom part of the lower cylinder was fitted into the microtome adapter. The exposed soil was then sliced perpendicular to the root mat into thin sections starting at the inter-cell boundary at 0.5, 1, 1.5, 2, 3, 5 and 13 mm from the root surface. Slices from four identical soil columns of the same treatment (i.e. one replicate) were bulked to obtain the 3-5 g soil required for analysis. All analyses were carried out on the fresh soil while results are expressed on an oven-dry basis.

7.2.3 Soil and plant analysis

Soil pH values were measured after shaking 0.5 g moist soil with 10 ml deionized water for an hour in an end-to-end shaker. After the measurement of soil pH suspension, the extract was then filtered (< 0.45-μm) and the filtrate was used to determine water soluble organic C (WSOC) using a Shimadzu TOC-5000A analyzer.

The soil P fractionation scheme described by Condron et al. (1996) was modified for use in this study. This involved sequential extraction of fresh soil (0.5 g oven-dry equivalent) with 30 ml 1 M ammonium chloride (NH₄Cl) (APi), 30 ml 0.5 M sodium bicarbonate (NaHCO₃) (pH 8.5) (BPi, BPo), 30 ml 0.1 M sodium hydroxide (NaOH) (NPi, NPo), and 30 ml 0.5 M sulfuric acid (H₂SO₄) (H₂SO₄-Pi). Residual P was determined following HNO₃-HClO₄ digestion. Concentrations of inorganic and organic P in the extracts were determined using the method described in Chapter 2.

Microbial biomass C (MBC) was measured using the method described in Chapter 2 except that 0.5 g soil (oven-dry equivalent) and 10 ml 0.5 M K₂SO₄ extractant were used. Acid phosphatase (AcPME), alkaline phosphatase (AIPME) and
phosphodiesterase (PDE) activities were determined using the methods described in Chapter 2 except that 0.25 g fresh soil was used.

7.2.4 Statistical analysis
A one-way ANOVA was carried out using Genstat 4.2 (Lawes Agricultural Trust, Rothamsted, UK) on data for root and shoot weight and plant P contents within each soil to test for significant differences between plant species. A two-way ANOVA was also carried out using Genstat 4.2 on the data for soil properties within each soil to test significant effects of plant species and sampling zone (distance from the root surface). Least significant difference (LSD) (plant species x sampling zone, \( P < 0.05 \)) was used to separate the means when the difference was significant. This approach was also employed in a similar rhizosphere study by Dormaar (1998). Correlation coefficients between soil chemical, biochemical and biological variables, root and shoot biomass, and P uptake were calculated using mean values (SigmaPlot, SPSS Inc.).

7.3 Results
Table 7-1 shows that root, shoot and total dry weights of radiata pine were significantly greater in both soils compared with ryegrass. Moreover, P contents in root, shoot and the whole plant of radiata pine were higher compared with ryegrass. Root surface areas in the 0-2 mm section above the inter-cell boundary were approximately 1-1.5 times greater under ryegrass compared with radiata pine (Table 7-1).

Soil pH and most P fractions were significantly influenced by plant species, sampling zone and plant species x sampling zone interactions (Table 7-2). During the short-term experiment (60 days) soil pH decreased by 0.1-0.4 units in the Fork and Hurunui soils near the roots of radiata pine compared with bulk soil, while the pH reduction zone extended up to 5 mm from the root surface (Figure 7-3). However, no significant changes in the pH values were observed in the ryegrass and control treatments (Figure 7-3).

Ryegrass caused greater depletion of APi in the zone 0- 2 mm from the root surface compared with radiata pine in both soils (Figure 7-4). The depletion zone of APi in both soils under ryegrass and radiata pine extended up to 13 mm from the root surface.
Maximum depletion of BPi by ryegrass in the rhizosphere was 16 and 33 μg g⁻¹ in the Fork and Hurunui soils, respectively, compared with 9 and 22 μg g⁻¹ in the Fork and Hurunui soils under radiata pine (Figure 7-4). On the other hand, the depletion zones for BPi extended up to 5 mm from the root surface in the Fork and Hurunui soils under radiata pine, compared with only up to 3 mm under ryegrass. In general, differences in concentrations of H₂SO₄-Pi between treatments were not significant for most of zones determined (Figure 7-4). Maximum depletion of NPi was 16 and 23 μg g⁻¹ in the Fork and Hurunui soils under ryegrass, respectively, compared with 10 and 17 μg g⁻¹ under radiata pine (Figure 7-4). However, the depletion zone for NPi was extended up to 5 mm from the root surface in soils under radiata pine, compared with only up to 3 mm under ryegrass.

**Figure 7-3** Effect of plant species on pH determined in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days.
Table 7-1  Dry matter yield, root surface area and P contents determined for ryegrass and radiata pine after 60 days †.

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Plant species</th>
<th>Dry weight (g unit⁻¹)</th>
<th>Root surface area (cm² unit⁻¹)</th>
<th>P content (mg unit⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td>Total</td>
</tr>
<tr>
<td>Fork soil</td>
<td>Ryegrass</td>
<td>0.60 (0.06)</td>
<td>0.44 (0.03)</td>
<td>1.05 (0.06)</td>
</tr>
<tr>
<td></td>
<td>Radiata pine</td>
<td>1.02 (0.14)</td>
<td>1.13 (0.11)</td>
<td>2.15 (0.22)</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Hurunui soil</td>
<td>Ryegrass</td>
<td>1.35 (0.08)</td>
<td>2.16 (0.11)</td>
<td>3.51 (0.19)</td>
</tr>
<tr>
<td></td>
<td>Radiata pine</td>
<td>1.74 (0.09)</td>
<td>3.50 (0.21)</td>
<td>5.23 (0.23)</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

† Data in parenthesis are the standard deviations (n =4). Root surface areas are the surface areas of roots in 0-2 mm slices above the inter-cell boundary (mesh). One unit = 4 PGCs (plant growth container).
Table 7-2 F ratios for two-way analyses of variance of data for pH and P fractions of rhizosphere Fork and Hurunui soils under ryegrass and radiata pine determined after 60 days.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Fork soil</th>
<th>Hurunui soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>Plant species</td>
</tr>
<tr>
<td>pH</td>
<td>2</td>
<td>50.9***</td>
</tr>
<tr>
<td>API</td>
<td>2</td>
<td>529.6***</td>
</tr>
<tr>
<td>BPI</td>
<td>2</td>
<td>147.9**</td>
</tr>
<tr>
<td>H2SO4-Pi</td>
<td>2</td>
<td>3.8*</td>
</tr>
<tr>
<td>NPI</td>
<td>6</td>
<td>43.6***</td>
</tr>
<tr>
<td>BPO</td>
<td>6</td>
<td>5.6**</td>
</tr>
<tr>
<td>NPO</td>
<td>6</td>
<td>30.3***</td>
</tr>
<tr>
<td>Residual P</td>
<td>6</td>
<td>0.22</td>
</tr>
<tr>
<td>TEPi</td>
<td>6</td>
<td>75.6***</td>
</tr>
<tr>
<td>TEPO</td>
<td>6</td>
<td>21.6***</td>
</tr>
<tr>
<td>Total P</td>
<td>6</td>
<td>36.2***</td>
</tr>
</tbody>
</table>

† a), ** and *** indicate significant differences at 0.05, 0.01 and 0.001 level (P < 0.05, 0.01 and 0.001), respectively.
b) API = NaHCl extractable Pi; BPI, BPO = 0.5 M NaHCO3 (pH 8.5) extractable Pi and Po; H2SO4-Pi = 0.5 M H2SO4 extractable Pi; NPI, NPO = 0.1 M NaOH extractable Pi and Po; TEPi = API + BPI + NPI; TEPO = BPO + NPO. Total P = TEPi + TEPO and residual P.
Figure 7-4 Effects of plant species on inorganic P fractions determined in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days.
Bicarbonate organic P (BPo) actively accumulated in rhizosphere soil (up to 3 mm from the root surface) under ryegrass and radiata pine compared with the control treatment, with the extent of accumulation being greater under radiata pine (Figure 7-5). On the other hand, significant depletion of NPo occurred up to 5 mm from the root surface in both soils under radiata pine. However, no significant depletion of NPo was apparent in the Fork soil under ryegrass, while some depletion of NPo was observed up to 2 mm from the root surface in the Hurunui soil under ryegrass (Figure 7-5). There was no apparent depletion of residual P observed in soils under ryegrass and radiata pine compared with the control treatment (Figure 7-5).

The depletion zone for TEPi extended up to 5 mm from the root surface in both soils under radiata pine compared with only up to 3 mm under ryegrass (Figure 7-6). The TEPo was significantly depleted up to 5 mm from the root surface in both soils under radiata pine, while accumulation of TEPo was observed in the rhizosphere of ryegrass in the Fork soil. Accordingly, total P depletion was generally greater and extended further under radiata pine compared with ryegrass (Figure 7-6).

Soil biochemical and biological properties were significantly influenced by plant species, sampling zone and plant species x sampling zone interactions (Table 7-3). Both ryegrass and radiata pine significantly increased levels of MBC in the rhizosphere up to 5 mm from the root surface in both soils (Figure 7-7). In general, concentrations of MBC were greater in between the 1 mm and 3 mm zones in the Fork soil and between the 1 mm and 5 mm zones in the Hurunui soil under radiata pine compared with ryegrass (Figure 7-7). Accumulation of WSOC was observed in the rhizosphere (up to 5 mm from the root surface) of both ryegrass and radiata pine compared with the control treatment, while concentrations of WSOC were significantly greater in the rhizosphere of radiata pine compared with ryegrass in both soils (Figure 7-7).

As expected, phosphatase enzyme activity increased in the rhizosphere of both ryegrass and radiata pine in the Fork and Hurunui soils compared with the control treatments (Figure 7-8). There were no significant differences in AcPME activity in the rhizosphere found between ryegrass and radiata pine in the Fork and Hurunui soils, while AIPME and PDE activities were generally higher in the rhizosphere of radiata pine compared with ryegrass in both soils (Figure 7-8).
Figure 7-5 Effects of plant species on organic P and residual P fractions determined in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days.
Figure 7-6 Effects of plant species on total extractable Pi (TEPi), Po (TEPo) and total P determined in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days.
Table 7-3  F ratios for two-way analyses of variance of data for biochemical and biological properties of rhizosphere Fork and Hurunui soils under ryegrass and radiata pine determined after 60 days †.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Fork soil</th>
<th>Hurunui soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plant species x sampling zone</td>
<td></td>
</tr>
<tr>
<td>Dependent variables</td>
<td>df 2</td>
<td>df 6</td>
</tr>
<tr>
<td>MBC</td>
<td>190.3***</td>
<td>64.8***</td>
</tr>
<tr>
<td>WSOC</td>
<td>582.0***</td>
<td>116.5***</td>
</tr>
<tr>
<td>AcPME</td>
<td>66.5***</td>
<td>82.0***</td>
</tr>
<tr>
<td>AlPME</td>
<td>33.1***</td>
<td>23.0***</td>
</tr>
<tr>
<td>PDE</td>
<td>43.9***</td>
<td>39.7***</td>
</tr>
</tbody>
</table>

† a), ** and *** indicate significant differences at 0.05, 0.01 and 0.001 level (P < 0.05, 0.01 and 0.001), respectively.

b) MBC = microbial biomass C; WSOC = water soluble organic C; AcPME = acid phosphomonoesterase; AlPME = alkaline phosphomonoesterase; PDE = phosphodiesterase.
Figure 7-7 Effects of plant species on microbial biomass C (MBC) and water soluble organic C (WSOC) determined in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days.

7.4 Discussion

Depletion of P fractions in the rhizosphere varies with plant species and soil type (Gahoonia and Nielsen, 1995; 1996; Jungk, 1996). Mechanisms for P depletion by different plants have been related to differences in root morphology (root density, root surface area, root hair length and density), root-induced chemical and biochemical and biological changes, and root-soil interactions in the rhizosphere (Fohse et al., 1988; 1991; van Diest, 1991; Jungk, 1987; 1996). In the present short-term study, both
Figure 7-8 Effects of plant species on acid phosphatase (AcPME), alkaline phosphatase (AIPME) and phosphodiesterase (PDE) activities determined in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days.
ryegrass and radiata pine significantly depleted all Pi fractions (except $\text{H}_2\text{SO}_4$ –Pi), although the depletion patterns were different for ryegrass and radiata pine (Figure 7-4). The API fraction includes Pi in the soil solution and some weakly adsorbed Pi. The extended depletion zone of API (up to 13 mm) by both ryegrass and radiata pine may be attributed to significant P movement by diffusion and mass flow in response to continued plant P uptake under the high soil moisture conditions (~5.0 kPa) employed (Gahoonia et al., 1994; Jungk, 1996).

Depletion profiles for BPI and NPi were different under ryegrass and radiata pine (Figure 7-4). The greater depletion of BPI and NPi near the roots (< 1mm from the root surface) of ryegrass in the Fork and Hurunui soils was possibly due to greater root surface areas (Table 7-1) and density of root hairs, compared with radiata pine (Jungk, 1987). The extent of depletion zones for BPI and NPi in soils under ryegrass may reflect the length of root hairs (Bhat et al., 1976; Jungk, 1987; Jungk, 1996). The greater extension of depletion zones of BPI and NPi in soils under radiata pine may be ascribed to the effects of ectomycorrhizal hyphae, which could be clearly seen in soil in the bottom of the lower compartment although they were not quantified. This is consistent with other studies (e.g. Marschner and Dell, 1994).

Root-induced chemical changes in the rhizosphere may involve mobilization of sparingly soluble forms of soil P (Neumann and Römheld, 1999). It has been reported that the acid extractable inorganic P pools can be depleted by plant species such as rape (Brassica napus), groundnut (Arachis hypogaea), pigeonpea (Cajanus cajan), and tea trees (Camellia sinensis) (Hedley et al., 1983; Suzuki and Hirata, 1997; Zoysa et al., 1997; 1998). These results have been ascribed to increased solubility of inorganic P due to decreased pH and/or increased exudation of organic acids in the rhizosphere. However, in the present study, $\text{H}_2\text{SO}_4$-Pi was not significantly affected by plant uptake (Figure 7-4), despite the significant amounts of $\text{H}_2\text{SO}_4$-Pi present in the Fork and Hurunui soils and the fact that a reduction in rhizosphere pH occurred under radiata pine (Figure 7-3). Different results between this and other studies may be attributed to the diverse nature of the acid extractable inorganic P pool in different soils and/or soil –plant interactions.
Transformation of organic P in the soil is mediated by plant root and microbial activity (Richardson, 1994; Magid et al., 1996). It is well known that root-derived organic C stimulates the growth of microorganisms and increases microbial activity in the rhizosphere (Martin, 1983; Helal and Sauerbeck, 1989; Bazin et al., 1990; Toal et al., 2000). The amount of root-derived C flow through the rhizosphere has a significant impact on transformations of soil organic P (Lynch, 1982; Helal and Sauerbeck, 1989).

It has been suggested that WSOC is mainly derived from root exudates and root residues (McGill et al., 1986; Huang and Schoenau, 1998; Chapter 6). In the present study, WSOC accumulated in the rhizosphere of ryegrass and radiata pine, while concentrations of WSOC were significantly greater in the rhizosphere of radiata pine compared with ryegrass (Figure 7-7). These results were consistent with previous studies which showed that concentrations of WSOC were greater in soils under radiata pine compared with ryegrass (Chapter 6). There were significant relationships observed between MBC and WSOC in soils under ryegrass and radiata pine (Figure 7-9), indicating that greater concentrations of MBC in the radiata pine rhizosphere may be partly attributed to increased levels of WSOC.

It has been suggested that AcPME and PDE are produced by both plant roots and microorganisms, while AlPME is produced by microorganisms (e.g. bacteria) but not plant roots (Speir and Ross, 1978; Tarafdar and Claassen, 1988). In the present study, it was found that AcPME, AlPME and PDE activities were higher in the rhizosphere of ryegrass and radiata pine compared with bulk soil (Figure 7-8). These results are consistent with many other studies (Helal and Sauerbeck, 1984; Tarafdar and Jungk, 1987; Häussling and Marschner, 1989; Asmar et al., 1995). Moreover, AcPME, AlPME and PDE activities were directly related to concentrations of MBC in soils under ryegrass and radiata pine, except for relationships between AlPME and MBC in the Fork soil under ryegrass and in the Hurunui soil under radiata pine (Figure 7-9). This confirms that these enzymes were at least partly of microbial origin. Therefore, increased root exudation in the rhizosphere of radiata pine compared with ryegrass may account for the enhanced levels of microbial biomass and enzyme activity and consequent rapid turnover of soil P.

It was found that BPo accumulated in the rhizosphere soil of ryegrass and radiata pine, while the extent of the accumulation was greater under radiata pine (particularly in the
Figure 7-9 Relationships determined between microbial biomass C (MBC) and water soluble organic C (WSOC), and between MBC and acid phosphatase (AcPME), alkaline phosphatase (AlPME) and phosphodiesterase (PDE) activities in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days (r<sub>o</sub> = correlation coefficient (Ryegrass); r<sub>p</sub> = correlation coefficient (Radiata pine); n = 7).
Hurunui soil) (Figure 7-5). Armstrong and Helyar (1992) also reported that BPo accumulated in the rhizosphere of many grass species (*Aristida armata, Cenchrus ciliaris, Digitaria ammophilla, Thyridolepis mitchelliana*). Moreover, Helal and Sauerbeck (1984) found that increased levels of BPo and phospholipids in the rhizosphere of many grass species (Aristida armata, Cenchrus ciliaris, Digitaria ammophilla, Thyridolepis mitchelliana). In this study, levels of MBC were also greater in the rhizosphere of radiata pine compared with ryegrass (Figure 7-7). Therefore, it is likely that the greater accumulation of BPo in the radiata pine rhizosphere was related to the increased concentration in MBP.

Relationships between phosphatase activity and the amount of organic P mineralized in the rhizosphere are poorly understood despite extensive investigation. Tarafdar and Jungk (1987) reported that depletion of organic P in the rhizosphere of rape, onion, wheat (*Triticum aestivum*) and clover (*Trifolium alexandrinum*) were directly related to higher acid and alkaline phosphatase activities. Asmar et al. (1995) also found that depletion of BPo by barley (*Hordeum vulgare*) genotypes was positively related to phosphatase activity in the rhizosphere. Moreover, Häussling and Marschner (1989) revealed that in a 80-year-old Norway spruce (*Picea abies*) forest, lower levels of readily hydrolyzable organic P were accompanied by higher acid phosphatase activity in the rhizosphere. However, Hedley et al. (1983) suggested that the increase in rhizosphere phosphatase activity appeared to be a response to increasing root density of rape and decreasing concentrations of soluble inorganic P in the soils, and was not related to changes in the rhizosphere organic P. Boero and Thien (1979) also found that increased levels of phosphatase activity in the rhizosphere of corn (*Zea may*) were not related to mineralization of organic P. They suggested that organic P moieties accessible for enzymatic hydrolysis might have been already saturated with indigenous soil enzymes and the dominant factor controlling mineralization of organic P may have been their susceptibility to enzyme hydrolysis.

In the present short-term study, NPo remained unchanged or decreased slightly in the rhizosphere of ryegrass, while greater depletion of NPo occurred in the rhizosphere of radiata pine (Figure 7-5). This confirmed that soil organic P is an important P source for radiata pine. Meanwhile, AlPME (in the Fork soil) and PDE activities in the radiata pine rhizosphere were greater compared with ryegrass, while there were no differences in AcPME activity (Figure 7-8). Depletion of NPo under radiata pine was significantly related to increased activities of all phosphatase enzymes measured
(Figure 7-10), but no significant relationships were found between NPo and phosphatase activities (except AIPME activity in Hunurui soil) under ryegrass. These findings suggest that phosphatase activities play an important role in the mineralization of soil organic P in soils under radiata pine. Moreover, concentrations of WSOC and MBC were also negatively correlated with levels of NPo in the Fork and Hurunui soils under radiata pine, but not under ryegrass (Figure 7-11). This indicates that in addition to enhancing microbial activity in the rhizosphere, root exudation might improve solubility and thus availability of organic P (Comerford and Skinner, 1989; Fox et al., 1990a, b, Chapter 6).

The discrepancy between various studies in respect to relationships between phosphatase enzyme activity and mineralization of organic P in the rhizosphere may be attributed to the complex interaction of many factors including plant species, soil properties (form and level of P, pH, soil moisture), and time (e.g. different stages of plant growth) (McKenzie et al., 1995). Accordingly, appropriate interpretation of rhizosphere processes requires a detailed knowledge of soil properties, physiology of plant roots and their interaction (Dormaar, 1988).

It should be noted that in the present study some analytical methods were modified due to limited amounts of soil samples collected in this and many other similar studies (e.g. soil: extractant ratios were increased from 1:4 to 1:20 for the measurement of MBC. The validity of these modifications requires further examination, and appropriate analytical methods for small amounts of soil sample are also needed.

7.5 Conclusions

Results from this study have clearly demonstrated that significant amounts of inorganic P (API, BPI and NPI) in the rhizosphere were depleted by both ryegrass and radiata pine, while depletion zones were extended further from the root surface in soils under radiata pine. Accumulation of BPO was observed in rhizosphere soil under both ryegrass and radiata pine, which may be attributed to increased levels of MBP in the rhizosphere soil. Significant depletion of NPo was observed in the rhizosphere of radiata pine, which in turn, may be attributed to a combination of enhanced root exudation and consequently higher levels of MBC and phosphatase enzyme activities.
Figure 7-10 Relationships determined between phosphatase activities and concentrations of sodium hydroxide extractable organic P (NPo) in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days (see Figure 7-9 for explanation of abbreviations; n = 7).
Figure 7-11 Relationships determined between water soluble organic C (WSOC) and microbial biomass C (MBC) and concentrations of NPo in the rhizosphere of Fork (left) and Hurunui (right) soils after 60 days (see Figure 7-9 for explanation of abbreviations; n = 7).
Chapter 8

Summary, Conclusions and Recommendations for Future Work

8.1 Summary

The major aspects of soil phosphorus (P) and sulfur (S) dynamics and grassland afforestation relevant in New Zealand were briefly reviewed. The current expansion of plantation forestry in New Zealand is expected to have significant impacts on soil chemical, biochemical and biological properties, which in turn will determine the long-term sustainability of plantation forestry. Despite the fact that a number of studies have been carried out in the past decade, the impacts of grassland afforestation on the soil nutrient dynamics (nitrogen (N), P and S) are not well understood. Further detailed studies were clearly required to determine the effects of grassland afforestation on specific chemical, biochemical and biological processes involved in organic matter and nutrient transformations. The major focus of this study was on soil P dynamics although soil S dynamics were also investigated. It was hypothesized that grassland afforestation caused marked changes in soil carbon (C) inputs, rooting characteristics, microenvironment and specific plant root-soil microbe associations, which in turn would affect chemical, biochemical and biological processes involved in soil P and S dynamics.

Results from a paired-site comparison of a 19-year old forest stand (mixture of *Pinus ponderosa* and *Pinus nigra*) and adjacent unimproved grassland at Craigieburn demonstrated that concentrations of organic C, P and S were lower but levels of labile inorganic P and S were higher in topsoils (0-10 cm) under forest compared with grassland. This confirmed that grassland afforestation enhanced the mineralization of organic matter and associated nutrients. However, these findings were inconsistent with lower concentrations of microbial biomass C, P and S and microbial and enzyme activities found in soils under forest compared with grassland. It was hypothesized that enhanced mineralization of soil organic C and associated P and S under forest may have been due to greater microbial and enzyme activities during the early stage of forest establishment. Moreover, the effects of afforestation on soil properties were
confined to topsoil (0-10 cm), which was attributed to a predominance of functional fine roots (< 2mm) of grasses and trees.

Results from a seasonal study (7 sampling occasions over a year) conducted at the same site (Craigieburn) also showed that soil total organic C, total P and selected organic P fractions (sodium bicarbonate (NaHCO₃) and macroporous resin extractable) were consistently lower under forest compared with grassland, further confirming that afforestation enhanced mineralization. On the other hand, microbial biomass C and P and phosphatase enzyme activity were also lower in soil under forest compared with grassland, which was consistent with previous studies. Results from this current study also showed that seasonal changes in soil moisture and temperature influenced the various biological and biochemical processes involved in P recycling. It was suggested that P recycling was primarily driven by plant P demand and sustained mainly by root litter inputs under grassland and by leaf litter inputs under forest. In general, organic P (in particular labile organic P) was mineralized by the microbial activity to meet P demand by plants in spring and summer under grassland and forest, while organic P accumulated in response to a combination of increased organic inputs, reduced plant growth, and low microbial activity in late autumn and winter. Microbial biomass P played a pivotal role in P recycling, and annual release of P through microbial biomass was higher under forest (16.1 kg ha⁻¹) than under grassland (13.9 kg ha⁻¹).

Findings from this study confirm that forest floor materials (F and H layers) play an important role in nutrient cycling and balancing the C, N, P and S loss from soil by enhanced mineralization and tree uptake. The forest floor at Craigieburn contained 15.54 tonnes C ha⁻¹, as well as 205, 22 and 20 kg ha⁻¹ of N, P and S, respectively. Moreover, higher microbial and enzyme activities were also found in the forest floor compared with the underlying mineral soil. Labile P in the forest floor can be directly taken up by tree roots and associated mycorrhizal fungi or be transferred to the underlying mineral soil by leaching and/or pedoturbation. Water soluble organic C in the forest floor can also be leached to the mineral soil and thus regulate microbial activity, which in turn influences nutrient cycling and availability.

Effects of grassland afforestation on the dynamics and availability of P are influenced by soil properties. Accordingly, a short-term (40 weeks) glasshouse experiment was
carried out using chemical extraction and isotopic exchange kinetics to investigate the effects of two contrasting plant species (radiata pine (Pinus radiata) and perennial ryegrass (Lolium perenne)) on the nature and availability of P in 15 different soils collected under grassland around New Zealand. Compared with ryegrass, radiata pine generally took up more P from most soils and was better able to access different forms of soil P (especially organic P), particularly when the level of soil available P was low. Phosphorus uptake by ryegrass and radiata pine was directly related to the levels of root phosphatase enzyme activity which were markedly higher for radiata pine than for ryegrass. Thus, higher root phosphatase activity may have partly been responsible for the higher proportion of P obtained from soil organic P by radiata pine compared with ryegrass.

In most soils, the growth of ryegrass and radiata pine resulted in the redistribution of P from the slowly exchangeable Pi pool (E>3m Pi) to more rapidly exchangeable Pi pools (E1min-24h Pi, E24h-3m Pi), while radiata pine also caused greater P redistribution from organic P to inorganic P compared with ryegrass. The enhanced mineralization of organic P observed in many soils under radiata pine was accompanied by higher concentrations of water soluble organic C, microbial biomass C and carbon dioxide respiration. This partly supports the previous hypothesis that enhanced mineralization of organic P may occur during the early stages of forest establishment. However, soil phosphatase enzyme activities were lower in most soils under radiata pine compared with ryegrass. Moreover, specific mineralization rates of organic P were directly related to turnover rates of organic C in soils under radiata pine but not under ryegrass. Therefore, it was suggested that biochemical hydrolysis by phosphatase enzymes might control the mineralization of organic P in soils under ryegrass, while a combination of both biochemical and biological processes determined mineralization of soil organic P under radiata pine. In addition, lower levels of microbial biomass P were found in soils under radiata pine compared with ryegrass, which may be ascribed to P release from microbial biomass by microfaunal grazing to meet higher P demand by radiata pine. Lower phosphatase activities in soils under radiata pine may also be partly attributed to increased adsorption of enzymes and inhibition by increased concentrations of available copper, zinc, iron and aluminum ions. Increased root exudation as indicated by higher levels of water soluble organic C in soils under radiata pine seedlings may have enhanced microbial activities and improved the solubility of soil organic P, which in turn lead to enhanced mineralization.
In a further investigation of specific rhizosphere processes involved in soil P dynamics under ryegrass and radiata pine, it was found that significant amounts of ammonium chloride (NH₄Cl), NaHCO₃ and sodium hydroxide (NaOH) extractable inorganic P were depleted by ryegrass and radiata pine. Depletion zones extended further from the root surface of radiata pine, which may be attributed to the presence of ectomycorrhizal hyphae. Greater depletion of NaOH extractable organic P in the rhizosphere of radiata pine was observed compared with ryegrass, while microbial biomass C, water soluble organic C, alkaline phosphatase and phosphodiesterase activities were also greater in the rhizosphere of radiata pine. Moreover, the amounts of NaOH extractable organic P in the rhizosphere of radiata pine were negatively related to levels of microbial biomass C, water soluble organic C and phosphatase activities. These findings indicated that greater mineralization of organic P in the rhizosphere of radiata pine compared with ryegrass was primarily mediated by a combination of higher microbial and enzyme activities.

8.2 Conclusions

The following are the major conclusions of this study:

(i) Grassland afforestation enhanced mineralization of organic matter and associated nutrients (P and S) and consequently resulted in greater levels of available inorganic P and S in topsoils, which may have occurred due to greater microbial and enzyme activities during the early stages of forest establishment in response to higher P demand by trees (Chapters 3 and 4). This was partly supported by the results from short-term glasshouse studies which showed that greater mineralization of organic P in soils under radiata pine compared with ryegrass was accompanied by higher levels of microbial biomass C, carbon dioxide respiration and water soluble organic C (Chapter 6).

(ii) Soil moisture and temperature influenced the biological and biochemical processes involved in P recycling that was driven by plant P demand and sustained mainly by root litter inputs under grassland and by leaf litter inputs under forest. Organic P was mineralized by the increasing microbial activity to meet the increasing P demand by plant growth in spring and summer under ryegrass and radiata pine, while organic P accumulated in response to
increased organic inputs, reduced plant growth and low microbial activity in late autumn and winter.

(iii) Significant amounts of C, N, P and S, and high microbial and enzyme activities were found in the forest floor compared with the underlying mineral soil, confirming that the forest floor plays an important role in nutrient cycling and balancing the C, N, P and S loss from soil by enhanced mineralization and plant uptake (Chapters 3, 4 and 5).

(iv) Results from a glasshouse experiment (Chapter 6) showed that biochemical hydrolysis of soil organic P by enzymes might be dominant under ryegrass, while both biochemical and biological pathways might be linked and involved in mineralization of organic P in soils under radiata pine. Furthermore, the findings from a rhizosphere study (Chapter 7) also clearly showed that mineralization of organic P was better related to microbial biomass and enzyme activity in soils under radiata pine compared with ryegrass.

(v) Radiata pine root phosphatase activity was markedly higher than ryegrass in a range of soils. This may also be partly responsible for enhanced mineralization of organic P in soils under radiata pine compared with ryegrass.

(vi) Increased root exudation as indicated by higher levels of water soluble organic C in soils under radiata pine seedlings compared with ryegrass were observed in the short-term glasshouse studies, but not in the field study. This may reflect differences in C metabolism at different stages of tree growth. Moreover, the higher levels of root exudates in soils under radiata pine seedlings may have enhanced microbial activity and improved the solubility of organic P, which in turn may have contributed to enhanced mineralization of organic P.
8.3 Future work

The findings from this study have improved our understanding of the effects of grassland afforestation on soil organic matter and associated nutrient dynamics. However, there is a need for further investigation in the following important areas:

- Long-term field experiments with randomized plots are required to fully investigate chemical, biochemical and biological processes involved in the transformations of soil P under grassland and forest at different stages of forest growth. Such experiments may help to explain the apparent contradiction between greater mineralization of organic P but lower microbial and enzyme activity in soils under forest compared with grassland. Similar trials are also needed to evaluate the long-term effects of grassland afforestation on soil quality, since it has been demonstrated here and elsewhere that afforestation reduces microbial biomass and microbial and enzyme activities and thus soil biological fertility. Such trials would in turn provide useful information for ongoing assessment of the sustainability of plantation forestry in New Zealand.

- The study on the effects of seasonal changes in environmental factors on the dynamics of P in soils under forest and grassland was carried out only for one year. However, environmental conditions may be different from year to year. An extended seasonal study is needed to improve the understanding of the effects of environmental conditions on P dynamics in soils under adjacent grassland and forest.

- Since root exudation plays an important role in the transformations of organic matter and associated nutrients, it is necessary to quantify and qualify plant root exudation at different stages of growth. Moreover, the role that root exudates (particularly low molecular weight organic acids such as oxalic acid) play in the solubilization of soil inorganic and organic P requires further study.

- The mineralization of soil C and P are clearly linked (Gressel et al., 1996; Gressel and McColl, 1997). It is necessary to investigate what fractions of soil C and P are linked by using $^{13}$C and $^{31}$P nuclear magnetic resonance
(NMR) techniques to understand the differences in the dynamics of soil C and P under grassland and forest.

- Vesicular-arbuscular mycorrhizae (VAM) and ectomycorrhizae (ECM) play an important role in plant P uptake and the effect of different plant species on the nature and availability of soil P. It is necessary to quantify ryegrass root-VAM and radiata pine root-ECM associations in relation to the production of phosphatase enzymes and organic acids, which would be helpful in understanding their roles in the mineralization of soil organic P under ryegrass and radiata pine.
References


