PHOSPHORUS FORMS OF PODZOLIC SOILS OF NORTHERN VANCOUVER ISLAND AND THEIR USE BY WESTERN RED CEDAR

by

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M. Sc., The University of British Columbia, 1989

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

Department of Soil Science

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

October 1995

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ABSTRACT

After clear-cutting and slashburning the hemlock-amabilis fir (HA) forest types of northern Vancouver Island support good growth, but the trees on the cedar-hemlock (CH) forest types suffer a growth check which can be overcome with N and P fertilization.

This study focussed on soil phosphorus (P) fractions in CH and HA forests. Extraction methods were evaluated for total, organic and available P. The Parkinson & Allen digest was better than the Saunders & Williams ignition method for total P. Both the Saunders & Williams method and the Bowman & Moir extraction procedure overestimated organic P. The Bray P1 and Mehlich 3 procedures were suitable for available P.

Extracts of forest floor samples by NaOH, NaOH-EDTA and Chelex in both water and NaOH were analysed by ^{31}P NMR spectroscopy. The NaOH-EDTA extracted the greatest portion of the total P and yielded spectra with a greater diversity of P compounds. However, this extractant also maintained other ions in solution which reduced the quality of the spectra.

Evaluation of P status in relation to soil chemistry of mature CH and HA forests revealed that CH forests had higher pH values and C concentrations in the forest floor. The CH forests also exhibited higher loss on ignition, wider C/N and C/P ratios, and increased concentrations of extractable Ca in mineral horizons. The HA forests had higher C concentrations in mineral horizons and higher concentrations of N, extractable Mg, Al and Fe, and more organically complexed Al and Fe. There were no significant differences in P levels between the forest types. P-31 NMR

spectroscopy showed a diversity of compounds, and organic forms throughout the profile. The persistence of labile diester phosphates and wide C/N and C/P ratios suggest slow decomposition.

Comparison of the P status and soil chemistry of mature CH forests to those after burning revealed increases in pH, available P, inorganic P and extractable N, and decreased organic P post-harvest. By 10 years postburn, significant reductions in organic P and organically bound Fe and Al were revealed in mineral horizons. P-31 NMR showed a shift to orthophosphate after burning, but a return to organic forms within 10 years in surface horizons. The results suggest that the burning of organic matter temporarily disrupts illuviation and the P cycle.

In a pot study, cedar grown with high (50 mg P/*l*) or low (10 mg P/*l*) levels of phytic acid, ATP, glycerophosphate, pyrophosphate or KH_2PO_4 showed adequate growth with all P forms but phytic acid, and grew best with the high rate of the P compounds. The poor growth with phytic acid was attributed to its binding of Ca, Zn and Cu. Utilization of organic P compounds was facilitated by various phosphatases, produced by cedar plants, mycorrhizae and/or rhizosphere microbes.

Phosphorus did not appear to play an important role in the growth check problem of CH sites.

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ACKNOWLEDGEMENTS

A project such as this could not have been completed without the help of many people. I am indebted to my committee for their patience: Shannon Berch, for funding through NSERC and the BC Ministry of Forests, and for convincing me that I could master soil chemistry, despite my views to the contrary; Les Lavkulich, for lab space, manpower and Saturday morning chats; Caroline Preston, for countless hours on the NMR, for allowing me to `play' with extractants, and for keeping me abreast of the literature during my pregnancy and after my move; Cindy Prescott, for coordinating the SCHIRP project and for providing considerable input during the write up of this thesis; and to Lawrence Lowe, for his assistance prior to his retirement. I wish to thank Dr. R. A. Bowman, USDA-ARS, Akron, CO, for sharing his NaOH-EDTA extraction technique with me during a 1991 telephone conversation. I am grateful to the staff of Western Forest Products, especially Paul Bavis and Cindy Fox, for providing accommodation and assistance with field work. My sample collection could not have been completed without Buffy, Sandy, Scott, Laura, Shanghou, Chuck and the staff at the Port Hardy Emergency Room. At UBC Laura, Bernie, Lauch, Jane, Amy, Pam, Kim and Amanda helped with analyses, while Kevin, Ann and a host of co-op students assisted me at PFC. I am grateful to Dawn, Jane, Marcia, Pam, Kim and Amanda for help with my trees. I must also thank the people who stocked the vending machine in the basement of McMillan, and the manufacturers of the double chocolate brownies, for helping me through many a long night of lab work.

I wish to thank my parents, Jim and Marilyn Cade, for their love and support through the years (Yes Dad, I'm finally out of school!) and my sister Linda Cade for a warm welcome and accommodation during my Victoria trips. I am grateful to my in-laws, Ted and Eileen Menun, for their support as well. To Jacob Christopher Menun, born during the final year of my thesis work, thank you for being a healthy, happy baby, and for sleeping occasionally so that Mommy could work.

It is difficult to adequately thank my husband, Chuck Menun, for his assistance and support. He helped with sampling and statistics, comforted me during the difficult times and pushed when I got lazy. I love you, Chuck.

And finally, to Oscar, the beloved companion of my graduate school years, Rest in Peace. I miss you.

CHAPTER ONE

General Introduction

The forests of northern Vancouver Island can be divided into two distinct types: the CH type, which is composed of western red cedar (*Thuja plicata* Don.) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) stands, and the HA type, made up of stands of western hemlock and amabilis fir (*Abies amabilis* Dougl.) (Lewis 1982). After logging in the late 1970's, these sites were replanted with Sitka spruce (*Picea sitchensis* (Bong.) Carr.). Initially, survival and growth were good, but within five years after planting, the trees on the CH sites suffered a growth check and became chlorotic, while the HA plantations continued to grow well. A study by Germain (1985) indicated that the CH sites were deficient in nitrogen and phosphorus and responded well to fertilization, suggesting a nutrient cycling problem. Preliminary research by Western Forest Products Limited, the University of British Columbia and Natural Resources Canada's Pacific Forestry Centre led to the establishment of the Salal-Cedar-Hemlock Integrated Research Program (SCHIRP), to investigate the many aspects of this regeneration problem (Prescott and Weetman 1994).

Phosphorus (P) is an important element in forest ecosystems. Pritchett and Fisher (1987) suggest that there are more reports of P deficiencies in forests than of deficiencies of any other nutrient, especially in conifer plantations. Produced initially by the solubilization of the soil parent material, P is taken up in

inorganic forms by pioneering organisms and is returned to the soil in organic forms. The actions of bacteria, fungi and fauna allow mineralization and immobilization to occur, and P is cycled through these organisms and higher plants in a variety of organic and inorganic forms. The acidic soils and cool temperatures of the forests of northern Vancouver Island limit the rate of mineralization, so that the majority of soil P is in organic forms. Little is known about the chemistry of organic P in soils, and less than half of the organic P in most soils can be accounted for in known compounds (Stevenson 1982).

Currently, there are no direct methods to measure concentrations of organic P in soils. Two indirect methods are used: ignition and extraction (Olsen and Sommers 1982). In both, organic P is calculated by subtracting the inorganic P from the total P. To determine the forms of organic P in a soil, a number of extractions must be done, each specific to a particular organic P class. The development of ^{31}P NMR spectroscopy as a tool in soil phosphorus research allows a simpler, more direct examination of the forms and amount of organic P in a soil. As a new method, however, refinement of the extraction procedure is still required, as well as testing on a wide range of soil types, because the results with any method for P determination appear to depend on soil type and environment (Anderson 1975). Most soil P methods were developed for agricultural soils, and many may not be appropriate for forested soils. There has been little testing of

soil P methodology to determine which procedures are most suitable for the podzolic soils of Coastal British Columbia.

Not all tree species replanted onto CH sites exhibit as severe a growth check as was initially observed in Sitka spruce. Studies by Weetman *et al.* (1989a, b) suggest that western red cedar trees growing on clearcut and burned CH sites grow better than replanted Sitka spruce and western hemlock, and respond less to P fertilization than do the other species. Western red cedar is an unusual conifer in this region in that it forms vesicular-rbuscular (VA) mycorrhizae, rather than ectomycorrhizae. This symbiotic association may give this species some advantage on these sites, and may allow western red cedar to use nutrient sources unavailable to the other tree species, such as organic P.

Objectives

The general objective of this thesis work was to investigate the concentration and forms of phosphorus in Orthic Ferro-Humic Podzols of the CH and HA forest types on northern Vancouver Island. Specifically, the first objective of this study was to test several methods for total, organic and available P, to find the methods most suited to this type of soil. The second objective was to examine extraction procedures for ^{31}P NMR analysis, to find the most suitable methods for soils of this type, and to further expand the use of this technique as a means by which soil phosphorus forms may be characterized. The third objective was to characterize the soil P forms of mature, uncut CH and HA stands, and to examine some

aspects of the chemistry of these soils which could influence P forms and levels. The fourth objective was to characterize the forms of P and related soil chemistry of CH stands 10 years, 5 years and immediately after clearcutting and slash-burning, comparing these to uncut CH stands, to determine if changes in P after cutting and burning could be producing the observed growth check. The final objective was to examine the use of organic forms of phosphorus by mycorrhizal and non-mycorrhizal western red cedar.

Hypotheses

The hypotheses tested within this thesis include:

1. Some procedures to determine organic, total and available P may be better suited to these forest soils than are other extraction procedures.
2. Some soil extraction procedures for ^{31}P may be better suited to these forest soils than are other procedures.
3. The soils of the mature CH stands contain different P forms from the mature HA stands, as well as differences in other aspects of the soil chemistry.
4. The soils of the CH stands 10 years, 5 years and immediately after burning contain different P forms from one another and from the mature CH stands, as well as differences in other aspects of the soil chemistry.
5. Western red cedar is able to use organic P forms, as well as or instead of inorganic forms, and mycorrhizal trees use different P forms from non-mycorrhizal trees.

Thesis Structure

This thesis consists of nine chapters, including this introduction (Chapter 1). Chapter 2 is a review of the literature relevant to this thesis. Chapter 3 describes the study area and sampling design. Chapter 4 comprises an investigation of the suitability of several methods for total, organic and available P. Chapter 5 is the study of extraction procedures for ^{31}P NMR spectroscopic analysis. In Chapter 6, the study of P forms on mature CH and HA stands is presented, and Chapter 7 details the study of soil P forms on cut and burned CH sites. Chapter 8 describes the greenhouse study in which the ability of western red cedar to use organic P forms is tested. Chapter 9 summarizes the conclusions of this research.

CHAPTER TWO

LITERATURE REVIEW

Forest Soil Phosphorus Cycles

In a broad sense, the soil phosphorus (P) cycle involves the uptake of P by plants and its return to the soil in plant and animal remains (Stevenson 1982). It is a cohesive, dynamic system, which is influenced by both long-term chemical transformations and short-term changes from plant uptake (Tiessen *et al.* 1984). To aid in the management of soil P, many attempts have been made to model the soil P cycle.

Conceptually, this main cycle can be subdivided into two further cycles (Dighton and Boddy 1988). The first is the external (geological) cycle, which involves P inputs to the ecosystem from the atmosphere (including both natural sources and pollutants), from the weathering of soil parent material, and from fertilizer inputs. Losses of P from the external cycle result from leaching, burning and harvesting. The other cycle is the internal (biological) cycle, which involves P exchanges among the soil, plants, fauna and decomposers.

In natural systems, the P cycle is virtually closed, and most plant P is recycled by microbial breakdown of litter and organic matter. In agricultural ecosystems, the P cycle tends to be more open, due to disturbances such as crop removal, ploughing and fertilization (Tate 1984). These are annual disturbances in agriculture, but may only occur in forestry once in fifty years,

and result in higher losses of P from agricultural ecosystems than from forest ecosystems.

The External (Geological Cycle)

The major reserves of P on earth are: marine sediments (840,000 x 10^{12} kg), terrestrial soils (96-160 x 10^{12} kg), dissolved inorganic PO_4^{3-} in the ocean (80 x 10^{12} kg), crushed rocks such as apatite (19 x 10^{12} kg) and the biota or biomass (2.7 x 10^{12} kg) (Stevenson 1986). A very small amount is circulated as dust.

Apatite [$Ca_5(F,Cl,OH)(PO_4)_3$] is the most commonly occurring phosphate mineral in rocks (Wild 1988). It is present as a primary mineral in the sand fraction of soils, especially if the soils are fairly young and are not acidic. Some secondary minerals of phosphate are: wavellite [$Al_3(PO_4)_2(OH)_3.5H_2O$], vivanite [$Fe_3(PO_4)_2.8H_2O$], dufrenite [$FePO_4.Fe(OH)_3$], strengite [$Fe(PO_4).H_2O$] and variscite [$Al(PO_4).2H_2O$] (Stevenson 1986; Wild 1988).

The majority of P compounds have coordination numbers of 3 and 4, although coordination numbers of 1, 3, 4, 5 and 6 are possible (Stevenson 1986). In soil, P is found mainly in its oxidized state, as orthophosphate, and as complexes with Ca, Fe and Al, and silicate minerals.

The forms of the phosphate ion are pH-dependent. In dilute solution, the dissociation of phosphoric acid is:

H_3PO_4 <-------> $H_2PO_4^-$ <-------> HPO_4^{2-} <-------> PO_4^{3-}

At the common soil pH range of 5-8, $H_2PO_4^-$ and HPO_4^{2-} dominate, and the amounts of the other forms are negligible. The usual soil total P (P_T) concentration is in the 500 to 800 ug/g range, on a dry weight basis (Stevenson 1986). In the soil profile, P_T is highest in the upper A horizon and lowest in the lower A and upper B, due to uptake by plants in these lower regions (Stevenson 1986).

Stevenson (1986) recognizes six major groups of soil P compounds. These are:

1. Soluble inorganic and organic compounds in the soil solution.
2. Weakly adsorbed (labile) inorganic phosphates.
3. Insoluble phosphates
   a) of Ca in calcareous and alkaline soils of arid and semi-arid regions
   b) of Fe and Al in acidic soils
4. Phosphates strongly adsorbed and/or occluded by hydrous oxides of Fe and Al
5. Insoluble organic forms
   a) of microbial biomass
   b) in undecomposed plant and animal residues
   c) as part of the soil organic matter

Inorganic Phosphorus

Essentially all of the inorganic soil phosphorus (P_I) is in the form of orthophosphate or a derivative of phosphoric acid (H_3PO_4). Only a small fraction occurs in water-soluble forms at any time (Stevenson 1986). The distribution of P_I into its various forms in soil is controlled by the activities of various ions, including Fe, Al, Mn, Ca and P itself. Among the many factors

controlling the activities of these ions are: soil pH and its effects on the solubility of Fe, Al, Mn and various phosphates; Ca availability; drainage; mineralization; redox potential; the presence of ligands that can replace phosphate when it is in complexes with Ca, Fe, Al, and Mn; and soil weathering and age (Chang and Jackson 1958; Pritchett and Fisher 1987; Fox *et al.* 1990b). In relatively unweathered soils, Ca- and Al-phosphates are more likely to be formed than Fe-phosphates. Fe-phosphates are the least soluble, and with time, Ca- and Al-phosphates will change to Fe-phosphates (Chang and Jackson 1958).

Another influence on P_I levels in the soil is phosphate adsorption. This can be defined as any process in which phosphate ions in solution react with atoms on the surface of soil particles (Barrow 1978). It is a two-step process, with an initially rapid step followed by a slower step because adsorption increases the negative charge on the surface of the soil, making it more difficult for each additional increment of phosphate to adsorb (Barrow 1978). Adsorption may occur onto clays and sesquioxides in the soil. In mull soils in Quebec, low P_I levels were thought to be due to phosphate adsorption by Al and Fe sesquioxides in Ah horizons, as the Ah was higher in Al and Fe, and lower in P_I, than H horizons (Pare and Bernier 1989). Fernandez and Struchtemeyer (1985) observed similar adsorption in B horizons of podzols under spruce-fir stands. In podzols of Vancouver Island, the P_I of Bhf and Bf horizons is usually sequestered in amorphous sesquioxides

(Sanborn 1987; Yuan and Lavkulich 1994). Phosphate fixation to clays decreases in the order: amorphous hydrous oxides > goethite = gibbsite > kaolinite > montmorillonite, and is pH-dependent (Meuller-Harvey *et al.* 1985; Stevenson 1986). Humic or fulvic acids and low molecular weight aliphatic or aromatic acids may block sites on soil materials to reduce phosphate adsorption (Violante *et al.* 1991). Phosphate which has been fixed or adsorbed in such a way that it is unavailable for desorption or removal is called occluded phosphate (Wild 1988).

The reverse of adsorption is desorption, which also consists of fast and slow reactions (Wild 1988). The fast reaction occurs by ligand exchange, while the slow reaction depends on the dissolving of Ca-phosphates among other processes (Wild 1988).

Organic Phosphorus

The amount of organic P (P_o) in soil is related to the organic matter content of the soil profile, and is quite variable (Wild 1988). The factors which influence soil P_o levels include: P supply, parent material, climate, drainage, cultivation, pH and soil depth. Soils derived from granite tend to have a lower P_o content than do soils from basalt or basic igneous rocks (Stevenson 1982). P_o contents of fine-textured soils are higher than coarse-textured ones, and acidic soils have more P_o than alkaline ones (Stevenson 1982). P_o decreases with depth in the soil profile (Stevenson 1986).

The P_o compounds commonly found in soil, and their approximate recoveries, are: (Stevenson 1982)

inositol phosphate	2-50%
phospholipids	1-5%
nucleic acids	0.2-2.5%
phosphoproteins	trace
metabolic phosphates	trace
phosphonic acid derivatives	trace

Structurally, most of these compounds can be grouped into: monoesters, which include inositol phosphates, sugar phosphates and mononucleotides; and diesters, which include phospholipids and nucleic acids. The monoesters contain one organic moiety per orthophosphate; the diesters two (Fig. 2-1) It would appear that soil P_o compounds are not simply the result of accumulation of decay-resistant Po compounds from plants, but instead are in many cases derived directly, or after biological transformations and synthesis, from organic matter (Wild 1988). Little is known about the chemistry of P_o in soils, and less than half of the P_o in most soils can be accounted for in known compounds (Stevenson 1982).

The types and relative quantities of P_o compounds in soil are related to environmental conditions and soil management. For example, phosphonate P occurs only in soils in which mineralization was constrained by cool, moist conditions (Condron *et al*. 1990b).

Inositol Phosphate

These are esters of hexahydrocyclohexane, commonly called inositol (Fig. 2-2A). Of a variety of possible esters, the most common in soil is the hexaphosphate ester (Fig. 2-2A), which is

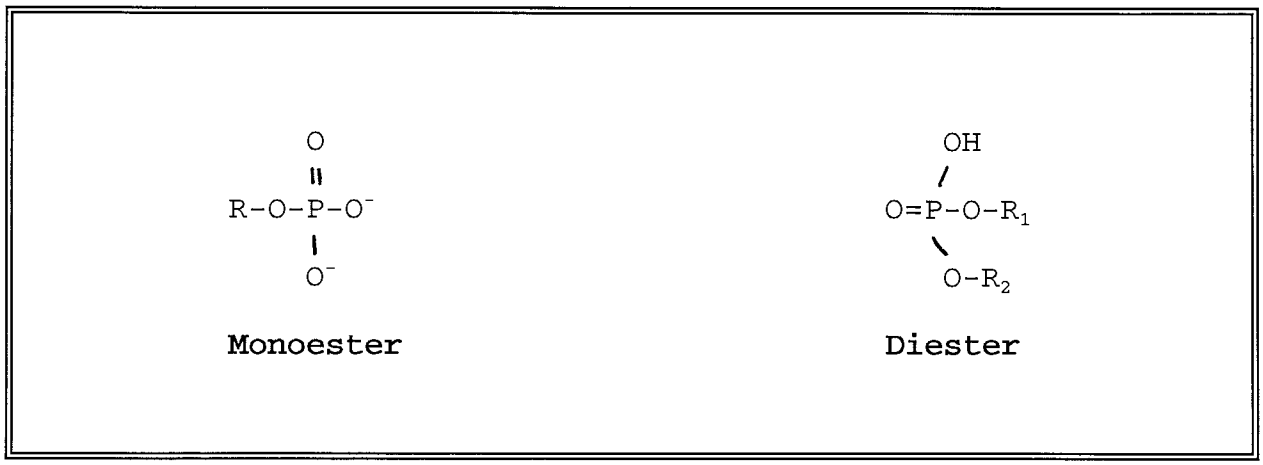


Figure 2-1: General structures of orthophosphate monoesters and diesters. R represents an organic moiety.

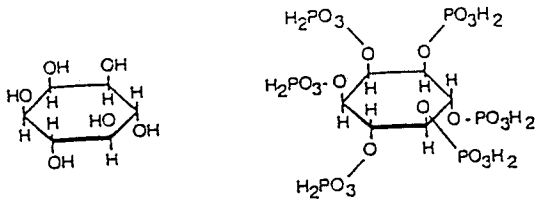
primarily derived from soil microbes. Although inositol compounds are produced in low amounts by living organisms, they are the most abundant of the P_o compounds in soil due to stabilization via the formation of inositol complexes with metal ions and other organic substances (Wild 1988; Stewart and Tiessen 1987)and from adsorption of inositol phosphates onto surface hydroxyls of soil colloids (Ognalaga *et al.* 1994). In addition to hexaphosphate, lower phosphates such as mono-, di-, tri-, tetra- and pentaphosphates may also be found in soil (Stevenson 1994). Inositol hexa- and pentaphosphates can comprise up to 60% of soil P_o (Tate 1984). Nine positional stereoisomers of inositol are possible, depending on the arrangements of H and OH groups. These include seven optically inactive forms and one pair of optically active isomers. Of these, the best known is myo-inositol, which is widely found in

nature and from which phytic acid (*myo*-inositol hexaphosphate) is derived (Stevenson 1986). Other naturally occurring isomers are d-chiro-, 1-chiro-, scyllo-, and neo-inositol. These are limited in distribution, and are found in microbes but not in higher plants, The microbial synthesis of these other isomers could occur by: the cyclization of carbohydrates; the direct phosphorylation of free inositol from soil organic matter; or from the formation of epimers from myo-inositol or myo-inositol hexaphosphate (L'Annunziata 1975).

Phospholipids

These are esters of fatty acids and alcohols, and contain a phosphate group (Fig. 2-2B). They are soluble in fat solvents such as ether, benzene or chloroform. Because they contain a hydrophobic (glyceride) group and a hydrophilic (phosphate) group in one molecule, they are not particularly stable (Baker 1975). They are the second most abundant group of soil P_o compounds, comprising up to 5% of P_o (Stevenson 1982). Included in this group are the glycerophosphatides, such as phosphatidyl choline or lecithin, phosphatidyl serine, phosphatidyl ethanolamine and phosphatidyl inositol. Phosphatidyl choline is the most abundant (Emsley and Niazi 1983). Most soil phospholipids are of microbial (bacterial and fungal) origin.

A)



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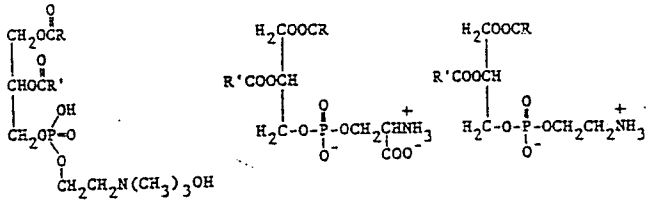

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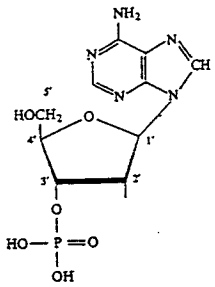

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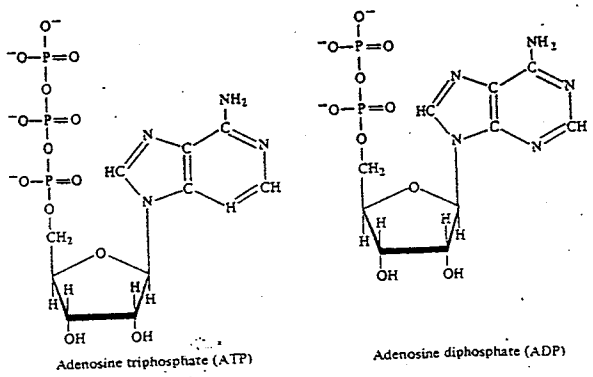


Figure 2-2: Structures of soil organic P compounds. A) myoinositol and phytic acid, the hexaphosphate ester of myoinositol B) the phospholipids lethicin, phosphatidyl serine and phosphatidyl ethanolamine C) a nucleotide, 3'-AMP D) adenosine triphosphate (ATP) and adenosine diphosphate (ADP). (A and B from Stevenson (1994); C and D from Suttie (1972).

Nucleic Acids

Nucleic acids are found in all living cells and are released in soil by the microbial decomposition of plant and animal remains. Approximately 3% of P_o in soils is in nucleic acids or derivatives, and they are easily decomposed by microorganisms (Stevenson 1986). The two known types are RNA (ribonucleic acid) and DNA (deoxyribonucleic acid). Each of these consists of a chain of nucleotides, and each nucleotide contains a pentose sugar, a purine or pyrimidine base, and phosphoric acid residue which links adjoining pentose units (Fig. 2-2C). Anderson (1970) isolated two nucleoside phosphates from NaOH extracts of soil. One appeared to contain thymine, the other uracil.

Other P_o Compounds

Other P_o compounds which have been isolated from soils include: ATP (Fig. 2-2D) and several high molecular weight P_o compounds which have not yet been identified (Emsley and Niazi 1983). They were all in low abundance. Teichoic acid has also been identified in native Grey Luvisols under aspen forest by Condron *et al.* (1990b). Teichoic acid is an orthophosphate diester form of P_o, consisting of sugar units linked by phosphate groups such as polyribitol phosphates. Naturally occurring teichoic acid is not a single compound but a complex, found only in bacterial cell walls, and its composition changes with environmental conditions and nutritional status of the bacterial population. Condron *et al.* (1990b) found it in natural but not cultivated

soils, and suggested that its occurrence in the L horizon of the moder which they studied indicated that the leaf litter in this horizon had undergone substantial bacterial decomposition.

Losses from the External Cycle

Burning, either from prescribed burning or forest fires, reduces litter and produces small short-term available P increases, but large long-term total P losses (DeBano and Klopatek 1988; Saa *et al.* 1993; Romanya *et al.* 1994). The heat of the fire, if intense enough, may kill beneficial fungi such as those forming mycorrhizae. The remaining P in the ash following a fire is susceptible to wind erosion and to fixation to sesquioxides (DeBano and Klopatek 1990).

Harvesting removes P and other nutrients from the ecosystem but the net effect depends on the balance of P gains and losses and the forms in which P is retained in the soil (Turner and Lambert 1986). The above-ground stand is estimated to contain about 25% of the "available" P pool in a forest. Harvesting of logs, with branches and slash left behind, removes about 4% of the available pool, or 2% if the bark were also left behind (Turner and Lambert 1986). The equilibrium between P_I and P_O in the soil is also upset by harvesting. The soils most affected by harvesting losses are those lowest in P_T at the start.

Tsubota (1959) claimed to have observed the volatilization of P through the microbial reduction of phosphate to phosphine (PH_3). However, extensive testing by Burford and Bremner (1972) showed

that any phosphine formed in soils would be adsorbed by soil constituents and thus would not be lost to the atmosphere.

Although leaching of phosphates is generally low, it can occur following fertilization, especially in agricultural soils (Stevenson 1986). In forest soils, Schoenau and Bettany (1987) observed leaching of labile P-rich compounds from surface horizons to lower horizons, where they became fixed and unavailable to plants. Leaching is also thought to be common in some coastal lands and organic soils of the southeastern USA (Pritchett and Fisher 1987), and may be most common in more weathered soils (St. Arnaud *et al.* 1988). The extent of P leaching, and subsequent fixation in lower horizons or loss to the ground water, depends on the nature of the mineral surfaces throughout the soil profile (Frossard *et al.* 1989). Wind erosion may also remove soil P (Stevenson 1986).

Internal (Biological) Cycle

The internal or biological cycle of soil phosphorus involves exchanges among plants, fauna and decomposers. The most important processes in this cycle are mineralization and immobilization. These are companion processes: P_O is converted to P_I by mineralization, while P_I is converted to P_O by immobilization. Because immobilized nutrients cannot be taken up by plant roots, this can be considered a temporary form of P fixation in the soil (Baath and Soderstrom 1979). The importance of P_O mineralization

in providing plant-available P_I is well-established (Stevenson 1986). Generally, the $C:P_o$ ratio determines which process will occur. Stevenson (1982) suggests that when the $C:P_o$ ratio is 300 or more, net immobilization will occur, and when the ratio is 200 or less, there will be net mineralization. Both of these processes are mediated by soil microbes, and thus are governed by the factors affecting microorganisms, such as temperature, moisture, aeration, pH and energy supply (Tate 1984). There do not appear to be any specific bacterial or fungal groups involved in either of these processes; they are mediated by a wide range of organisms instead.

Some purely biochemical mineralization may also occur via the hydrolysis of esters by extracellular enzymes present in the soil or released by plants and microbes in response to low P_I availability in the soil solution (Stewart and Tiessen 1987).

Bacteria

The soil bacteria represent a relatively labile P pool. Besides transforming P, soil microbes are an important source and sink for P (Van Veen *et al.* 1987). Microbial biomass P is determined by the change in P_o and P_I following chloroform treatment of the soil, and may be complicated by spatial and temporal variations in soil microbial populations (Stewart and Tiessen 1987). It is influenced by the many factors which affect mineralization and immobilization, such as temperature, moisture, aeration, and C and N supply. The amount of P which is sequestered

in biomass may also be influenced by soil texture, with more in clay than in sand (Van Veen *et al.* 1987).

Of microbial intracellular P, over 60% is in nucleic acids, 20% is in acid-soluble phosphate esters, and 5% is in lipids, with variations (Stewart and Tiessen 1987). The most common phospholipid in bacteria is phosphatidyl ethanolamine (> 50%), followed by phosphoglycerol, phosphatidic acid, and phosphoinositols (Stewart and Tiessen 1987). Teichoic acids may be present in bacterial walls, and surplus P may accumulate in bacterial cells as polyphosphates (Stewart and Tiessen 1987). Biomass P_o can be taken up directly by predators or by saprophytes and incorporated into new consumer biomass, or may be released back into the soil solution by grazers such as protozoa and nematodes (Dighton and Boddy 1989).

Mineralization of P is highest in the rhizosphere, where substances from root exudates, sloughed-off root cells, tissues and mucigels sustain a larger and more active microbial population than in the rest of the soil (Tate 1984). Phosphorus may be more plant-available in the rhizosphere (Gillespie and Pope 1990a, 1990b), due to the production of phosphatases, either by the microbial population or by the plant itself (Stewart and Tiessen 1987). Organic acids, which may also be produced by the plant and/or the microbial population, will also increase the availability of phosphate in the rhizosphere (Comerford and Skinner 1989). Phosphate is released by these acids through ligand-exchange

reactions, the dissolving of metal-oxide surfaces which adsorb P, or the complexing of metals in solution to prevent the precipitation of metal phosphates (Fox *et al.* 1990a). Acids involved include oxalic, formic, citric, malic and acetic, all of which are commonly found in soils (Fox *et al.* 1990b).

Fungi

Fungi play a crucial role in the cycling of P, and in mor humus of temperate coniferous forests may have the greatest biomass of all organisms (Baath and Soderstrom 1979). Fungi, as saprophytes, are major decomposers of soil organic matter, and are responsible for the return of the P which is immobilized in dead plant, animal and microbial tissue to the soil P pool, solubilizing organic P forms through the production of organic acids and phosphatases (Dighton and Boddy 1988). Fungi may also obtain P from parasitic associations on living organisms (Dighton and Boddy 1988). Phosphorus is concentrated in the mycelium, and when the fungi are alive, is released by excretion, secretion of extracellular enzymes, leaching and grazing by animals (Swift 1977), and on their death by other fungi and organisms (Stark 1972). Systems of hyphae may move P considerable distances, drawing on areas of high P concentration to assist in areas of low P concentration (Dighton and Boddy 1989). Spore dispersal will also relocate P.

Plants

Most of the movement of P to plant roots occurs by diffusion, and depends on the concentration gradient between the soil and the root surface, and on the diffusion coefficient (Bhadoria *et al.* 1991). Mass flow is a very minor mechanism for P transport to roots. Phosphorus is absorbed by plant roots as the negatively charged primary and secondary orthophosphate ions ($H_2PO_4^-$ and $HPO_4^=$), which are present in the soil solution. The concentration of P at the root surface regulates the quantity of P absorbed by the root (Gillespie and Pope 1990b), and decreases as the buffering capacity of the soil for phosphate increases (Barrow 1978). Most of the P in soil is in forms which are not readily available to plants, such as organic P forms (Stevenson 1986).

In response to a need for P, plants may produce extracellular phosphatases (Tate 1984). They also form mycorrhizae, which are symbiotic associations of higher plants and fungi (Harley and Smith 1983). Ectomycorrhizae, in which the fungi do not enter the cortical cells, are the most common associations for forest trees (Isaac 1992). Many of the host plants belong to the families Pinaceae, Fagaceae, Betulaceae and Myrtaceae; the fungi are ascomycetes and basidiomycetes. In other types of mycorrhizae, the fungi penetrate the cortex of the host plant. These types of mycorrhiza can be subdivided into orchid, ericalean and vesicular-arbuscular (VA) mycorrhizae. Orchid mycorrhizae are symbioses involving members of the Orchidaceae and basidiomycetes or

ascomycetes. Ericalean mycorrhizae are formed by plants of the order Ericales (ericoid, arbutoid and monotropoid), and are often found on acid soils of northern temperate forests (Isaac 1992). The fungi of ericalean mycorrhizae are basidiomycetes and ascomycetes. The VA mycorrhizae are the most widely occurring of all mycorrhizae, and are particularly common in crop plants, herbs and tropical trees. They are found in temperate coniferous forests less frequently than ectomycorrhizae and ericalean mycorrhizae, but they are formed with some conifers such as western red cedar (*Thuja plicata* Don). Many plant species form VA mycorrhizae; the fungi are all zygomycetes (Harley and Smith 1983).

Mycorrhizae assist the plant in the uptake of nutrients, especially P (Harley and Smith 1983). They improve the uptake efficiency of plants by increasing the absorptive surface area in the soil, thus allowing the plant access to pools of P which were unavailable or less available to the non-mycorrhizal plant. Ectomycorrhizae may produce acid phosphatases (MacFall *et al.* 1991; Lapeyrie 1991), protease, cellulase, phenol oxidase (Entry *et al.* 1991) and phytase (Dighton 1983). Cromack *et al.* (1979) and Alexander and Hardy (1981) reported the exudation of oxalic acid by ectomycorrhizal fungi in forest litter. These enzymes and acids allow the plant to use P forms not available to non-mycorrhizal plants, particularly organic P. The P content of the hyphae of ectomycorrhizal fungi often exceeds that of plant roots (Fogel and Hunt 1983), and mycorrhizae may accumulate polyphosphate for

storage or hyphal P transport (Martin *et al.* 1983; MacFall *et al.* 1992). Phosphatase production has also been demonstrated in ericoid mycorrhizae (Mitchell and Read 1981; Straker and Mitchell 1986; Shaw and Read 1989; Dighton and Coleman 1992) and orchid mycorrhizae (Antibus and Lesica 1990).

It is generally believed that VA mycorrhizae obtain their P from the same soil pool as non-mycorrhizal plants (Bolan 1991; Jennings 1995). However, recent work suggests that VA mycorrhizae may produce phosphatase to mineralize organic P forms (Jayachandran *et al.* 1992; McArthur and Knowles 1993; Thiagarajan and Ahmad 1994; Tarafdar and Marschner 1994).

Within higher plants, the three main types of P compounds are: inositol hexaphosphates, particularly phytin; phospholipids; and nucleic acids (Ting 1982). Other plant P compounds include ATP, NAD, NADP and mono-, di- and triphosphates of inositol (Stevenson 1994; Ting 1982). The central role of P in plants is in energy transfer. In P-deficient plants, growth is stunted and maturity is delayed.

Plants return P to the soil cycle in many forms. Foliage dominates the above-ground return of P in litter to the forest floor, while the contribution of woody litter to P inputs is minor (Fogel and Hunt 1983). Many deciduous species conserve P by translocating it into perennial parts before leaf drop (Morrison 1991). Decomposition of below-ground biomass may return as much as 80% of the total tree P in Douglas-fir to the soil (Fogel and Hunt

1983). Pollen rain is an important P source in forest soils because it provides an input of nutrients during the summer when there is little leaching of nutrients out of the dry surface L layer, but when the F layer is still moist enough for considerable decomposition and biological activity at summer temperatures (Stark 1972).

Fauna

Soil fauna affect P cycling by fragmenting litter, grazing on bacteria and fungi, and improving soil structure, which in turn improves conditions for microbes (Reichle 1977; Dighton and Boddy 1989). Soil fauna may contain higher concentrations of P than plant litter, most of which was obtained from grazing on bacteria and fungi (Pokarzherskii and Gordienko 1985). Grazing may therefore result in the release of nutrients, in addition to altering the relative contributions of different fungal and bacterial species to the decomposition process (Dighton and Boddy 1989). After death, soil fauna are themselves attacked by decomposers, returning P to the bacterial and fungal pool. Earthworms enrich surface soils with P, and increase the amount of readily exchangeable inorganic P as well as the rate of mineralization of organic P by the improvement of soil structure (MacKay *et al.* 1982).

Soil Phosphorus Methodology

Many methods are currently used to determine total (P_T), organic (P_O) and available (P_A) phosphorus concentrations in soils. There does not appear to be an ideal method to determine any of these soil P fractions, and the results appear to depend on soil type and environment (Anderson 1975). Most soil P tests have been developed for agricultural soils. Some methods may be better suited to forest soils than others.

Total P

Total P (P_T) analysis requires the conversion of insoluble material to soluble forms, followed by colorimetric analysis (Olsen and Sommers 1982). The most commonly used methods are fusion with Na_2CO_3 and digestion with $HClO_4$ (Syers *et al.* 1967; Syers *et al.* 1968; Sommers and Nelson 1972; Dick and Tabatabai 1977; Olsen and Sommers 1982). Other P_T methods in use include: ignition of the soil followed by extraction with H_2SO_4 (Syers *et al.* 1967; Olila and Reddy 1995); digestion with H_2SO_4 and H_2O_2 (Thomas *et al.* 1967; Roberts *et al.* 1985; Schoenau and Bettany 1987; Xiao *et al.* 1991; Compton 1994; Fyles and Cote 1994; Hanafi and Syers 1994); digestion with H_2SO_4, H_2O_2 and HF (Bowman 1988); digestion with H_2SO_4, H_2O_2, Li_2SO_4 and Se (Parkinson and Allen 1975; Edmonds 1980; Rowland and Grimshaw 1985; Tiedemann and Klemmedson 1986; Prescott *et al.* 1993; Silver *et al.* 1994); and digestion with HCl and HNO_3 (*aqua regia*) (Crosland *et al.* 1995). The Na_2CO_3 fusion method gives the highest recovery of P_T from soil, but it is tedious and

time consuming (Dick and Tabatabai 1977; Bowman 1988). The other P_T procedures are simpler for routine laboratory analysis.

Available P

Labile phosphorus is related to the quantity of P available to plants growing in a soil - so-called "available" P (P_A) (Thomas and Peaslee 1973). Soil testing procedures for P_A, developed for agricultural soils, can provide an accurate "relative index" of the quantity of P that plants may utilize from the soil, and an index of the quantity of fertilizer P required for some range of soil-crop-climate combination (Thomas and Peaslee 1973). These procedures should be tested and calibrated for each soil, crop and environment. The main extractants used to determine P_A are grouped by the soil pH range at which they are most effective. For alkaline soils, the Olsen *et al.* (1954) extraction using $NaHCO_3$ is most suitable. The most common P_A methods for acid soils are the Bray P1 (Bray and Kurtz 1945) and the Mehlich 3 (Mehlich 1984) methods, which use fluoride as a complexing ion in dilute acid.

Organic P

A direct method to determine the total organic P (P_o) concentration of soil has not yet been devised (Olsen and Sommers 1982). Soil P_o is estimated by either of two indirect methods: ignition or extraction. Ignition methods utilize ashing at either low temperature (Legg and Black 1955) or high temperature (Saunders and Williams 1955) to oxidize the soil organic matter prior to acid extraction. An unignited sample is concurrently extracted with

acid, and the soil P_o concentration is the difference between the P contents of the ignited and unignited extracts, after colorimetric analysis (Olsen and Sommers 1982). Extraction methods involve treating soils with acids, bases, or both, followed by the determination of P in the extract before and after the oxidation of organic matter. The P_o content of the soil is the difference in the P content of the extract before and after oxidation, following colorimetric analysis (Olsen and Sommers 1982). The extraction method used most often is that of Mehta *et al.* (1954), which is a sequential treatment of soil with HCl and NaOH. Extraction with NaOH and EDTA has also been proposed (Bowman and Moir 1993).

The identification of specific P_o compounds in soil has traditionally been conducted using partition chromatography following extraction. For example, the inositol phosphates are extracted from soils with acid and alkali and are precipitated as insoluble Fe-salts from acid media or Ba-salts from alkaline media, prior to anion-exchange chromatography (Stevenson 1994). Phospholipids may be recovered from soil using sequential extraction with ethanol-benzene and methanol-chloroform (Baker 1975). These methods are time-consuming, and a different extraction procedure is required for each class of P_o compounds.

The introduction of ^{31}P nuclear magnetic resonance (NMR) spectroscopy as a soil science technique allows the qualitative and quantitative analysis of P_o. The theory of NMR spectroscopy is based on the fact that when a sample containing suitable nuclei is

placed in a magnetic field, the spin energy levels of the nucleus are split (Wilson 1991). The size of the splitting depends on the strength of the magnetic field H_0 (corresponding to the energy gap) and characteristics of the nucleus. Transitions between the new energy levels can be brought about by electromagnetic radiation ν_0 in the radiofrequency range. The strength of the magnetic field and frequency of irradiation are related by $2\pi\nu_0 = \gamma H_0$, where γ is the gyromagnetic ratio and is characteristic of the nucleus (Wilson 1991). Thus, different nuclei occur at characteristic radiofrequencies (the Larmour frequency) for a given field strength. In addition, the electrons around a nucleus shield the nucleus from the magnetic field so that nuclei of the same element with different electronic distributions resonate at different frequencies from the Larmour frequencies. This results in a range of 'chemical shifts', which can be used to detect different functional groups of an element (Wilson 1991). Not all nuclei undergo magnetic resonance. The number of energy states created in the presence of a magnetic field depends on the spin quantum number of the nucleus investigated. The most commonly studied nuclei in soils are ^{1}H, ^{13}C, ^{27}Al, ^{29}Si, ^{15}N, and ^{31}P.

In order to detect the signal from nuclei such as ^{31}P above the background noise, a large number of scans must be recorded and averaged so that the signal becomes proportionally larger than the background noise. As sweeping the spectrum takes many seconds, a more efficient way to collect spectral data is to pulse the sample

with a radiofrequency pulse, causing all the nuclei to resonate at the same time (Wilson 1991). Fourier transformation then converts the data into the form of a continuous wave spectrum. Between pulses, a sufficient length of time must be left for nuclei to relax back to their original equilibrium distribution (Wilson 1991). The length of time for which the pulse is left on during an NMR experiment is the pulse width, also expressed as a pulse angle. For maximum sensitivity, short pulse angles of 45° or less are normally used in solution studies of soil organic matter (Wilson 1991).

Many investigated nuclei are in close proximity to protons, the spins of which can be aligned with or against those of the nucleus under study. The process by which the interaction with protons is removed is called decoupling, and is essential to obtain a simple spectrum of the studied substance (Wilson 1991). Decoupling involves irradiating the protons in the sample, so that the protons undergo rapid transition, and the interactions with other nuclei are averaged out. The nuclei under study will also be affected by this irradiation, so that the signal intensity from the nuclei close to the protons will be greater than from nuclei remote from the protons. This is termed the nuclear Overhauser effect, and it can affect quantification (Wilson 1991). To reduce the enhancement effect, the decoupler may be turned off during the pulse delay and used only during the scan, in a process called inverse gated decoupling.

Both solutions and solids may be used for ^{31}P NMR spectroscopy. However, the natural P levels in soils are usually low. As ^{31}P NMR is relatively insensitive, requiring more than 100 ug P/ml for quantitative analysis (Adams and Byrne 1989), solution NMR is used for soil P, and extraction and concentration are required to produce clear spectra. Since the first use of ^{31}P NMR for soil P determination by Newman and Tate (1980), a number of extractants have been tried. The extractants tested include: 0.5 M NaOH (Newman and Tate 1980; Tate and Newman 1982; Ogner 1983; Hawkes *et al.* 1984; Zech *et al.* 1985; Preston *et al.* 1986; Zech *et al.* 1987; Hinedi *et al.* 1989; Gil-Sotres *et al.* 1990; Forster and Zech 1993); 0.5 M NaOH with a citrate-dithionite-bicarbonate pretreatment (Ingall *et al.* 1990); combined HCl, HF and $TiCl_4$ (Hinedi *et al.* 1989); sequential extraction with NaOH and acetylacetone (Condron *et al.* 1985); tetra-n-butyl ammonium hydroxide (Bu_4NOH) (Emsley and Niazi 1983); trichloroacetic acid and KOH (Hinedi *et al.* 1989); and Chelex, a cation exchange resin (Adams and Byrne 1989; Adams 1990). The soil P_o classes which ^{31}P NMR spectroscopy shows are: phosphonates, orthophosphate, phosphate monoesters, phosphate diesters, pyrophosphate, ATP and polyphosphates. There is close agreement on quantities of P estimated by both conventional laboratory methods and ^{31}P NMR spectroscopy (Tate and Newman 1982).

P Fractionation

Inorganic P bound to Ca, Fe or Al minerals in soil is usually estimated with various acid digests modified from the Chang and

Jackson (1957) method (Olsen and Sommers 1982; Cross and Schlesinger 1995). Phosphorus extracted by NaOH is thought to be the non-occluded phosphate bound to the surfaces of Al or Fe hydrous oxides; P removed by citrate-bicarbonate-dithionite extraction is the P occluded within the matrices of Fe and Al oxides and hydrous oxides; and P removed with HCl is the extracted calcium phosphate of the nonoccluded apatite fraction (Williams *et al.* 1980; Olsen and Sommers 1982). Another fractionation scheme, based on the function of P compound classes in soil rather than on chemical compounds, was developed by Hedley *et al.* (1982). This method is traditionally used to separate plant-available or labile forms of P from various refractory P pools, and was recently reviewed by Cross and Schlesinger (1995).

CHAPTER THREE

Study Area

The study site is located between Port Hardy and Port McNeill on northern Vancouver Island (Fig. 3-1), at 50°60' latitude and 127°35' longitude.

This is a wet area, with mild winters and cool summers. The annual rainfall is approximately 1700 mm, 65% of which falls between October and February. There is less rainfall in summer than winter, and in most years there is no soil moisture deficit (Lewis 1982). This area has a long frost-free period (175 days) and receives a small amount of snow from December to February. The

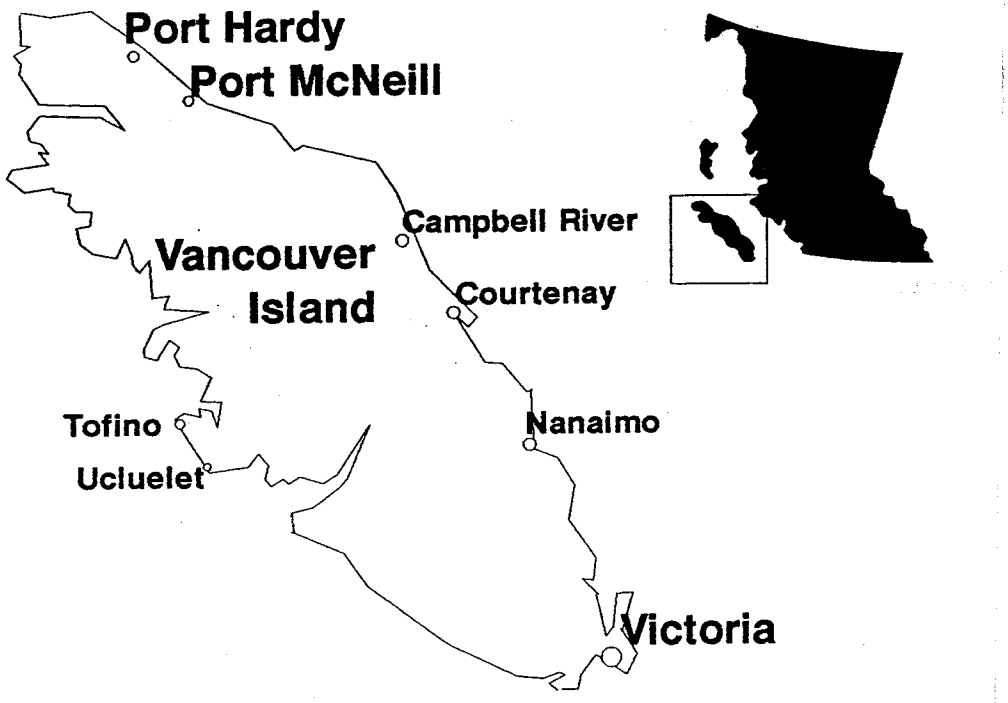


Figure 3-1: Map of Vancouver Island, with inset of British Columbia.

mean annual temperature is 7.0°C, with a mean daily temperature range from 3.0°C in January/February to 13.7°C in July/August. Theaverage hours of sunshine range from a high of 6.4 h/day in July to a low of 1.5 h/day in December, reflecting the frequency of fog in summer and frontal clouds in winter. All weather data are from the Port Hardy Airport weather station (Messier 1991, Keenan 1993).

The northern part of the study area sits in the Suquash basin, which is gently undulating, and seldom exceeds 300 m in elevation. Geologically, the surface material consists of deep unconsolidated morainal and fluvial sediments overlying three types of bedrock: gently dipping sedimentary rocks of the cretaceous Nanaimo formation, relatively soft volcanics of the Bonanza group, and a small area of harder Karmutsen Formation basalt (Lewis 1982). The southern portion of the northern area is located in the Nawhitti Lowland. It is underlain by the altered basaltic rock of the Karmutsen formation. Here, surficial materials tend to be discontinuous and are interrupted frequently by rock outcrops, in contrast with the relatively continuous cover of surficial materials of the Suquash Basin (Lewis 1982). This locality is considered to be one of the first places along the British Columbia coast to become ice-free after the most recent (Fraser) glaciation, with vegetation establishing about 14,000 years ago (Hebda 1983).

The study site is in the very wet maritime subzone of the Coastal Western Hemlock (CHW_{VM}) biogeoclimatic zone (Pojar *et al.*

1991) which occupies the lower and middle latitudes of Vancouver Island and the British Columbia coastal mainland. Lewis (1982) considered the ecosystem association to be the *Thuja plicata* - *Tsuga heterophylla* - *Abies amabilis* - *Gaultheria shallon* - *Rhytidiadelphus loreus* (the salal-moss S1) association. Due to the moist climate, wildfires are uncommon, and windstorms are the predominant source of disturbance. The forests in this area form two distinct types. The first, called CH, consists of old growth (more than 500 years old) stands of western red cedar (*Thuja plicata* Don.) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) (Fig. 3-2). This forest type has an open canopy which allows light to penetrate, and thus there is a dense understory of salal

Figure 3-2: A typical stand of the CH forest type.

(*Gaultheria shallon* Pursh.), and blueberry (*Vaccinium parvifolium* Howell and *V. alaskaense* Smith). The forest floor is occupied by the mosses *Rhytidiadelphus loreus* (Hedw.) Warnst., *Kindbergia oregana* (Sull.) Ochyra and *Hylocomium splendens* (Hedw.) B.S.C., with occasional ferns such as *Blechnum spicant* L. (Germain 1985; Prescott and Weetman 1994).

The forest floors of the CH forest type are lignohumimors and humimors (Klinka *et al.* 1981). Beneath these are moderately to somewhat imperfectly drained Duric or Orthic Ferro-Humic Podzols.

Following cutting and burning, the CH sites are quickly invaded by salal, which regenerates from rhizomes. Natural tree regeneration is slow and sparse, and consists mainly of western red cedar and western hemlock seedlings. Forests of the CH type are thought to not have been catastrophically disturbed for at least one thousand years, and are believed to represent the climatic climax association for this region (Lewis 1982).

The second forest type in this area, the HA, is characterized by closed stands of second growth western hemlock and amabilis fir (*Abies amabilis* Dougl.) (Fig. 3-3). These stands appear to be even-aged and originated following a wide-spread windstorm in 1906 (Lewis 1982). The understory is sparse, with small patches of blueberry (*Vaccinium alaskaense* and *V. parvifolium*), deer fern (*Blechnum spicant*), and the mosses *Kindbergia oregana*,

Figure 3-3: A typical stand of the HA forest type.

Rhytidiadelphus loreus and *Hylocomium splendens* (Germain 1985; Prescott and Weetman 1994).

The forest floors of the HA forest type are humimors (Klinka *et al.* 1981), overlying well-drained Ferro-Humic Podzolic soils. After cutting and burning, the HA sites are quickly invaded by a dense cover of fireweed (*Epilobium angustifolium* L.) and salal. Natural tree regeneration is rapid and dense, and consists mainly of western hemlock.

The transition between CH and HA is quite abrupt and there is no evidence of occupation of HA sites by cedar prior to the 1906 windstorm (Keenan 1993). In classifying the ecosystems of this region, Lewis (1982) could not distinguish between the CH and HA forest types on the basis of topography or mineral soil characteristics, and included them in the same ecosystem association. He further hypothesized that they were different stages of a successional sequence.

Sampling Design

Soil samples were collected July 14, 15, 31 and August 1, 2, 3, 1992. A nested design was used in this study, with three locations for each age or forest type, and three soil pits per location. The pit locations were randomly selected using a soil probe to determine suitability, and then pits were dug of no more than 0.1 m^3. Only Orthic Ferro-Humic Podzols lacking buried horizons or woody forest floor material were used.

The soils from each pit were sampled by horizon, based on visible characteristics such as colour and texture (Agriculture Canada Expert Committee on Soil Survey 1987). The horizons used were LF, H, Bhf and Bf, which is a typical horizon sequence for podzols in this region (Lewis 1976). The L and F horizons of the forest floor were not separated, because the L horizons were very thin. On newly burned sites, this horizon was an ash layer, which was sampled and labelled as LF. Ae horizons are rare in the podzols of northern Vancouver Island (Lewis 1976). In the few pits where they were observed, they were thin and discontinuous, and were avoided during sampling. The mean thickness and ranges of thickness for the LF, H and Bhf horizons sampled for each forest type and age postburn are shown in Table 3-1. The total thickness of the Bf horizon was not measured.

Table 3-1: The mean horizon thickness and ranges of thickness for the LF, H and Bhf horizons sampled for old growth CH and HA sites, and CH sites 0, 5 and 10 years after cutting and burning.

		Horizon Thick. (cm)				
		HA-OG	**CH-OG**	**CH-0**	**CH-5**	**CH-10**
LF	mean	5.4	5.6	3.7	5.1	2.8
	range	2-14	2-10	1.5-8	3-10	1-6
H	mean	7.2	9.0	4.6	5.2	4.3
	range	3-13	4-15	1.5-10	2-10	2-9
Bhf	mean	7.1	10.8	6.2	4.0	4.8
	range	2-14	2-25	2-10	2-8	1-8

Study Sites

The sites from which samples were collected were all located within Block 4 of Tree Farm Licence 25, (Fig. 3-4) which is operated by Western Forest Products Limited (WFP). The study sites were selected with the assistance of Paul Bavis of WFP. The locations of uncut CH and HA stands were chosen in part for their proximity to other research areas of the SCHIRP project, to facilitate the comparison of data. The selection of five-year and ten-year postburn sites was restricted to unfertilized CH areas, which limited the choices. The 0-year postburn sites were very limited, as only a few CH sites had been burned in the spring prior to sample collection.

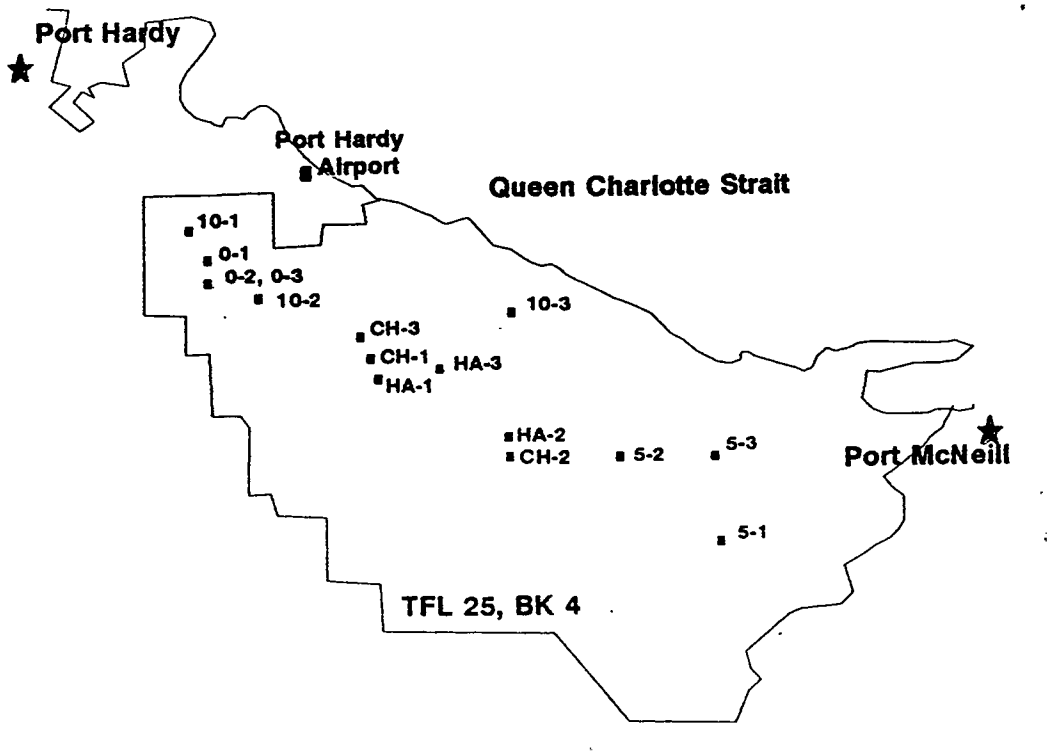


Figure 3-4: Location of sampling sites within Block 4, Tree Farm Licence 25. (Approx. scale is 1:190 000.)

A description of the sample locations follows.

10-1: WFP code 412/81, located at Rupert 470. This site was logged in 1981, burned in the fall of 1982, scarified in the winter of 1985, and planted in 1986. The elevation is 150 m, with a flat aspect, a slope of 0-65% and a rolling landform, and covers 67.9 ha. There are some rocky ridges and glacial surficial deposits. It was thought to have a heavy brush potential and poor natural regeneration potential.

10-2: WFP code 424/80, located at Rupert 200. This was logged in 1980, burned in the fall of 1980, and planted in 1981. The elevation is 150 m, with a SE aspect, a slope of 0-40% and a hummocky landform, morainal veneers, and covers 5.8 ha.

10-3: WFP code 451/80, located at Rupert 200. This was logged in 1980, burned in the fall of 1980, and planted in 1981. The elevation is 60 m, with a slope of 0-25% and a flat landform, on morainal blankets, and covers 23.1 ha.

5-1: WFP code 240/86, located at West 83A. This was logged in 1986, burned in the spring of 1987, and planted in 1988. There is 5% bedrock and glacial surficial deposits, with a slope of 0-60%, and covers 68.4 ha. It was thought to have a medium brush potential and a poor natural regeneration potential.

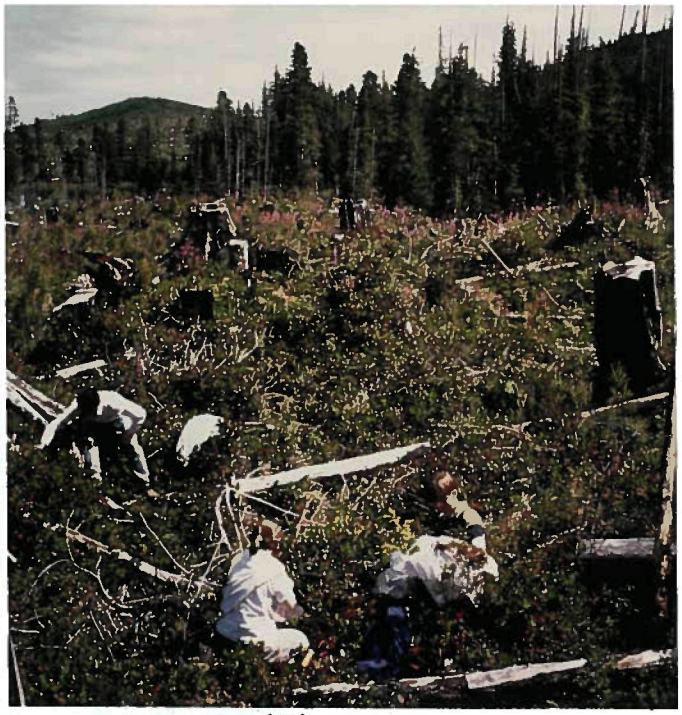
5-2: WFP code 263/86, located on Misty 930. This was logged in 1986, burned in the spring of 1987, and planted in 1988. It has 10% bedrock and glacial surficial deposits, and covers 94.2 ha. It was thought to have heavy brush potential and poor natural regeneration potential.

5-3: WFP code 264/86, located on Misty 900. This was logged in 1987, burned in the spring of 1987, and planted in 1988. It has glacial till, surficial deposits and covers 56.6 ha. It was thought to have a light brush potential and poor natural regeneration potential.

The 0-year sites were all logged in 1991 and burned in the spring of 1992. 0-1 was located at Rupert 470, 0-2 at Rupert 450 and 0-3 at Rupert 440.

Typical examples of 10-year, 5-year and 0-year sites can be seen in Figure 3-5.

(A)



(B)

(C)

Figure 3-5: Typical 10 year (A), 5 year (B) and 0 year (C) post-burn sites.

CHAPTER FOUR

A COMPARISON OF METHODS FOR TOTAL, ORGANIC AND AVAILABLE PHOSPHORUS

Introduction

Before comparing phosphorus in different types of forests or assessing the effects of burning on the forest P cycle, the concentrations of total, organic and available P must be reliably measured. Most soil phosphorus determinations have two phases: the preparation of a solution containing the desired soil P fraction, such as total (P_T), organic (P_O) or available (P_A); and the quantitative determination of P in the solution (Olsen and Sommers 1982). The second step usually involves colorimetric analysis, most often the molybdate blue method of Murphy and Riley (1962) (Olsen and Sommers 1982). For the first step, many different methods are currently in use to determine P_T, P_O and P_A. There does not appear to be an ideal method to determine any of the soil P fractions, and the results with any method appear to depend on soil type and environment (Anderson 1975).

Total P (P_T) analysis requires the conversion of insoluble materials to soluble form. The most commonly used methods are fusion with Na_2CO_3 and digestion with $HClO_4$ (Syers *et al.* 1967; Syers *et al.* 1968; Sommers and Nelson 1972; Dick and Tabatabai 1977; Olsen and Sommers 1982). The Na_2CO_3 fusion method gives quantitative recovery of all P forms in soils, but it is tedious and time consuming, and is not suitable for large numbers of samples (Dick and Tabatabai 1977). The $HClO_4$ digestion method is

preferred because of its simplicity and adaptability for routine analysis (Dick and Tabatabai 1977). However, this method requires careful handling because of the unsafe feature of boiling $HClO_4$, and its use has been discontinued in many areas, such as the University of British Columbia campus. Digestion with $HClO_4$ may also give low P recoveries in strongly weathered soils (Syers *et al.* 1967; Syers *et al.* 1968). Other methods, which retain the ease of the $HClO_4$ digestion method but which lack its unsafe aspects, have been introduced. These include: extraction with H_2SO_4 following ignition of the soil (Syers *et al.* 1967; Olila and Reddy 1995); the Thomas *et al.* (1967) method of hot H_2SO_4 followed by H_2O_2 (Roberts *et al.* 1985; Schoenau and Bettany 1987; Xiao *et al.* 1991; Compton 1994; Fyles and Cote 1994; Hanafi and Syers 1994); digestion with combined H_2SO_4, H_2O_2 and HF (Bowman 1988); digestion with *aqua regia* (HCl and HNO_3) (Crosland *et al.* 1995); and the Parkinson and Allen (1975) digest, which uses H_2SO_4 and H_2O_2 as oxidants, with the addition of Li_2SO_4 to elevate the digestion temperature and Se as a catalyst (Edmonds 1980; Rowland and Grimshaw 1985; Tiedemann and Klemmedson 1986; Prescott *et al.* 1993; Silver *et al.* 1994).

A direct method to determine the organic P (P_o) content of soil has not yet been devised (Olsen and Sommers 1982). Soil P_o is estimated by either of two indirect methods: ignition or extraction (Saunders and Williams 1955; Legg and Black 1955; Hance

and Anderson 1962; Dormaar and Webster 1963; Enwezor and Moore 1966; Williams and Walker 1967; Williams *et al.* 1970; Steward and Oades 1972; Ipinmidun 1973; Anderson 1975; Olsen and Sommers 1982; Condron *et al.* 1990b; Bowman and Moir 1993). For many soils, these methods give comparable values (Olsen and Sommers 1982).

Ignition methods utilize either low temperature (Legg and Black 1955) or high temperature (Saunders and Williams 1955) ashing to oxidize the soil organic matter prior to acid extraction. An unignited sample is concurrently extracted with acid, and the soil P_o is the difference between the P contents of the ignited and unignited extracts (Olsen and Sommers 1982). The ignition method can underestimate the P_o content of soils due to acid hydrolysis during treatment of unignited samples (Harrap 1963; Olsen and Sommers 1982) or by incomplete extraction of P released during ignition (Dormaar and Webster 1963; Williams *et al.* 1970; Anderson 1975; Olsen and Sommers 1982). Phosphorus may also be lost by volatilization during ignition (Dormaar and Webster 1964; Williams *et al.* 1970; Anderson 1975), although these losses are minimal at ignition temperatures under 800°C (Saunders and Williams 1955; Anderson 1975). After ignition, the solubility of P_I compounds may be increased, resulting in erroneously high P_o values (Legg and Black 1955; Dormaar and Webster 1963; Harrap 1963; Williams and Walker 1967; Williams *et al.* 1970; Anderson 1975; Olsen and Sommers 1982; Soltanpour *et al.* 1987; Condron *et al.* 1990b).

Extraction methods involve treating soils with acids, bases or

both, followed by the determination of P in the extract before and after the oxidation of organic matter. The P_o content of the soil is the difference in the P content of the extract before and after oxidation. The extraction method used most often is that of Mehta *et al.* (1954), which is a sequential treatment of soil with concentrated HCl, NaOH at room temperature and NaOH at 90°C (Olsen and Sommers 1982). This is a laborious procedure, and may result in underestimating the P_o content of a soil by the incomplete extraction of P_o or by hydrolysis of P_o during extraction (Williams *et al.* 1970; Anderson 1975; Olsen and Sommers 1982; Condron *et al.* 1990b; Bowman and Moir 1993). A new extraction procedure to determine P_o was recently introduced by Bowman and Moir (1993). This is a one-step extraction using a combination of Na_2EDTA and NaOH. The NaOH solubilizes the P_o associated with soil organic matter, while the EDTA chelates metal cations to increase the efficiency of the organic matter extraction.

The main extractants in use to determine available P (P_A) are grouped by the soil pH range at which they are most effective. For alkaline soils, the Olsen *et al.* (1954) extraction, which contains $NaHCO_3$, is most suitable, while for acid soils the Bray P1 (Bray and Kurtz 1945) and the Mehlich 3 (Mehlich 1984) methods are most commonly used. These are both dilute acid methods using fluoride as a complexing ion to release P (Curran 1984). In addition to NH_4F, Bray P1 contains HCl, while the Mehlich 3 extractant contains CH_3COOH, NH_4NO_3, HNO_3 and EDTA. The Mehlich 3 extractant was

developed as a multielement extractant, and can be used to quantitatively determine K, Ca, Mg, Cu, Zn and Mn, in addition to P, thus making it more useful than Bray in many laboratory situations. Comparisons of the Bray P1 and Mehlich 3 methods show that the P results are highly correlated for agricultural soils (Mehlich 1984; Wolf and Baker 1985; Michaelson *et al.* 1987; Tran *et al.* 1990; Cade 1989; Simard *et al.* 1994; Wendt 1995).

All of the soil tests described above were developed for agricultural soils, and some methods may be better suited to forest soils than others. The Bowman and Moir (1993) extraction for P_o in particular is very new and has not yet been adequately tested for use with forest soils. The objective of the research in this chapter was to compare the Saunders and Williams (1955) and Parkinson and Allen (1975) methods to determine P_T, the Saunders and Williams (1955) and Bowman and Moir (1993) methods to determine P_o and the Bray P1 and Mehlich methods to determine P_A, to determine the suitability of these procedures for use with the Orthic Ferro-Humic Podzolic soils of northern Vancouver Island.

Materials and Methods

For P_T, P_o and P_A, the P content of all solutions was read colorimetrically using the molybdate blue method (Murphy and Riley 1962) on a Lachat Flow Injection Analyzer (FIA). The soils used are those described and analyzed in Chapters 5, 6 and 7.

Total P

The two P_T methods used were the Parkinson and Allen (1975) digest and the first step of the Saunders and Williams (1955) ignition method for P_o (Olsen and Sommers 1982).

Parkinson and Allen

Soil samples were oven-dried overnight at 60°C prior to analysis. A 1 g sample was weighed into a 100 ml digestion tube, 5 ml of concentrated H_2SO_4 was added and the tube contents were mixed with a vortex mixer. A 1 ml aliquot of the digestion mix, containing 7.0 g Li_2SO_4, 0.21 g Se powder and 175 ml of 30% H_2O_2, was added. After the foaming reaction had ceased, three more 1 ml aliquots were added. The sample was then digested at 360°C on a block digester. After 1.5 h, the sample was cooled briefly and 0.5 ml of 30% H_2O_2 was added. After a further 30 min. of digestion, the sample was again briefly cooled and a second 0.5 ml aliquot of H_2O_2 was added. After a total digestion of 2.5 h, the samples were cooled and made to volume, and were analyzed colorimetrically.

Saunders and Williams

Soil samples were oven-dried overnight at 60°C prior to analysis. A 1 g sample was weighed into a porcelain crucible, and the crucible was placed in a cool muffle furnace. The temperature was raised to 550°C over a 2 hour period, and maintained at 550°C for 1 hour. After cooling, the ignited sample was transferred to a 100 ml centrifuge tube, and 50 ml of 0.5 M H_2SO_4 was added. The sample was shaken overnight, centrifuged, and then the extract was read colorimetrically.

Organic P

The two P_o methods used were the Saunders and Williams (1955) ignition method (Olsen and Sommers 1982) and the Bowman and Moir (1993) extraction method.

Saunders and Williams

In addition to the procedure described above for P_T, a 1 g sample of unignited soil was shaken overnight in 50 ml of 0.5 M H_2SO_4. After centrifugation, the samples were filtered with Whatman 41 filter paper to remove the floating organic matter. The extract was read colorimetrically, and the difference between the P contents in the ignited and unignited samples was calculated to determine the P_o content.

Bowman and Moir

A 5 g sample of air-dry soil was extracted in 100 mL of a 1:1 mix of 0.5 M NaOH and 0.1 M EDTA in a 125 mL erlenmeyer flask at room temperature overnight, with occasional stirring. After filtering through Whatman 41 filter paper with a Buchner funnel, a 2 mL subsample was digested with persulphate (Bowman 1989). The P in solution after digestion was determined using the Watanabe and Olsen (1965) procedure. This method was also used as an extraction procedure for ^{31}P NMR spectroscopy (Chapter 5).

Available P

The two P_A methods used were the Bray P1 method, as described by Olsen and Sommers (1982) and the Mehlich 3 method, as described by Mehlich (1984). For the Bray P1 method, 2 g of air-dried soil in 20 ml of extractant and a 5 min. extraction time were used,

while for the Mehlich 3 method, 5 g of air-dried soil in 50 ml of extractant and a 5 min. extraction time were used.

Results

The results obtained for the total P analyses are shown in Table 4-1. When all horizons were combined, the P_T results from

Table 4-1: Means and (standard deviations) of the results for total P analyses, in mg/kg. P&A is the Parkinson & Allen (1975) digest, while S&W is the Saunders & Williams (1955) ignition method. The correlations between the two methods for all horizons combined and for each horizon separately are shown at the bottom.

HORIZON	METHOD	HA-OG	CH-OG	CH-0	CH-5	CH-10
LF	P&A	714.0 (67.5)	585.8 (66.6)	713.9 (181.4)	675.8 (141.9)	485.3 (163.1)
	S&W	729.0 (75.6)	611.2 (78.6)	728.0 (211.0)	725.6 (134.7)	540.8 (121.8)
H	P&A	561.4 (98.3)	482.8 (152.1)	524.0 (219.9)	569.5 (150.6)	515.4 (150.9)
	S&W	546.9 (108.2)	511.7 (172.6)	558.0 (195.6)	618.7 (93.9)	528.6 (159.0)
Bhf	P&A	524.2 (110.5)	352.1 (89.6)	349.4 (199.5)	468.6 (170.6)	354.5 (66.1)
	S&W	459.8 (139.1)	330.3 (97.1)	341.2 (211.7)	445.1 (188.2)	353.3 (147.5)
Bf	P&A	361.0 (105.5)	289.2 (115.6)	230.8 (41.1)	235.5 (102.2)	211.8 (70.2)
	S&W	316.9 (69.7)	267.8 (115.6)	336.3 (237.3)	212.8 (70.0)	193.2 (70.2)

CORR. (r)	All	LF	H	Bhf	Bf
	0.917	0.869	0.917	0.907	0.561

the Parkinson and Allen digests were highly correlated with those of the Saunders and Williams ignition method. When the horizons were analyzed individually, the results for the LF, H and Bhf were highly correlated. The results for the Bf were also correlated, but not as highly as for the other horizons. Although the mean P_T values for all ages and horizons were very similar, the Saunders and Williams method produced higher P_T concentrations in the highly organic surface horizons, and more P_T was extracted from the mineral horizons when the Parkinson and Allen digest was used.

Table 4-2 displays the results of the organic P analysis. When all of the results were examined together, those obtained by the Bowman and Moir extraction method were highly correlated with the ones from the Saunders and Williams ignition method. Separately, the methods were correlated for each horizon, but not highly. Generally, the P_O concentrations obtained by ignition were higher than those from extraction for all but the LF and H horizons of the recent burn (CH-0), particularly in the Bf horizon.

The concentrations of available P are shown in Table 4-3. The Bray P1 extract was highly correlated with the Mehlich 3 extract for all horizons combined and for the LF, H and Bhf horizons when analyzed separately. There was no correlation between the results of the two methods in the Bf horizon. In the LF and H, the Mehlich 3 method consistently extracted more P_A. In the Bhf, the results are varied, while Bray P1 extracted more P_A in the Bf. Virtually no P_A was extracted from the Bf horizon with the Mehlich 3 extractant.

Table 4-2: Means and (standard deviations) of the results for organic P analyses, in mg/kg. B&M is the Bowman & Moir (1993) extraction, while S&W is the Saunders & Williams (1955) ignition method. The correlations between the two methods for all horizons combined and for each horizon separately are shown at the bottom.

HORIZON	METHOD	HA-OG	CH-OG	CH-0	CH-5	CH-10
LF	B&M	527.8 (162.0)	492.0 (98.8)	662.3 (272.4)	551.2 (177.6)	391.1 (124.2)
	S&W	580.2 (49.8)	471.2 (75.2)	358.2 (129.7)	578.7 (107.8)	420.3 (109.8)
H	B&M	283.8 (133.9)	308.7 (123.2)	360.4 (243.9)	401.0 (127.5)	306.2 (112.2)
	S&W	442.3 (90.1)	410.7 (156.0)	349.7 (93.4)	469.7 (101.1)	417.0 (133.9)
Bhf	B&M	227.8 (110.3)	145.1 (89.2)	174.2 (97.2)	220.2 (111.0)	198.7 (93.1)
	S&W	367.2 (119.9)	276.2 (86.0)	234.3 (148.6)	365.0 (168.1)	293.9 (134.6)
Bf	B&M	99.6 (35.7)	64.8 (36.4)	41.3 (23.0)	63.5 (36.8)	21.6 (24.8)
	S&W	188.8 (51.3)	157.7 (85.4)	205.9 (251.6)	109.7 (70.8)	65.4 (31.5)

CORR. (r)	All	LF	H	Bhf	Bf
	0.712	0.375	0.443	0.562	0.403

Table 4-3: Means and (standard deviations) of the results for available P analyses, in mg/kg. Bray is the Bray P1 extraction (Olsen and Sommers 1982), while Mehlich is the Mehlich 3 extraction (Mehlich 1984). The correlations between the two methods for all horizons combined and for each horizon separately are shown at the bottom.

HORIZON	METHOD	HA-OG	CH-OG	CH-0	CH-5	CH-10
LF	BRAY	38.2 (6.2)	30.5 (10.0)	89.0 (31.4)	27.2 (9.4)	17.3 (7.15)
	MEHLICH	57.6 (8.5)	54.9 (28.3)	110.5 (32.5)	56.3 (28.3)	33.9 (16.3)
H	BRAY	20.6 (6.8)	20.6 (9.7)	44.1 (24.9)	39.1 (21.4)	12.6 (5.1)
	MEHLICH	28.9 (13.7)	42.8 (29.4)	73.6 (31.4)	68.9 (45.9)	36.3 (18.2)
Bhf	BRAY	10.2 (7.0)	6.06 (2.92)	8.84 (8.12)	9.24 (6.54)	8.59 (1.78)
	MEHLICH	7.75 (15.2)	6.90 (10.21)	7.29 (12.47)	9.77 (17.4)	5.44 (5.89)
Bf	BRAY	4.80 (0.89)	6.31 (1.02)	6.43 (1.12)	6.06 (1.05)	6.60 (0.51)
	MEHLICH	0.41 (0.88)	0.03 (0.10)	0.36 (0.74)	0.0 (0.0)	0.21 (0.63)

CORR. (r)	All	LF	H	Bhf	Bf
	0.874	0.889	0.698	0.777	-0.106

Discussion

As was mentioned in the introduction, the traditional methods for P_T determination are Na_2CO_3 fusion and $HClO_4$ digestion. Fusion with Na_2CO_3 is thought to be the best method, recovering all forms of P from most soils (Syers *et al.* 1967; Dick and Tabatabai 1977), but it is difficult to use for routine analysis. Digestion with $HClO_4$ is believed to extract the majority of P_T from most soils (Sherrell and Saunders 1966; Syers *et al.* 1967; Syers *et al.* 1968), but its hazardous aspects have caused many laboratories to discontinue its use. The Saunders and Williams (1955) ignition method and the Parkinson and Allen (1975) digestion remove P_T concentrations which are comparable to those of $HClO_4$ (Syers *et al.* 1967; Sommers and Nelson 1972; Rowland and Grimshaw 1985) and these methods are simple enough for routine analysis. The Parkinson and Allen digest was initially developed for plant material, although Parkinson and Allen (1975) felt that it would also be suitable for soils. After extensive testing on a range of British soils, Rowland and Grimshaw (1985) demonstrated that it was a comparable method to $HClO_4$ digestion for the determination of P_T, even for samples high in apatite, which are known to be a problem in P_T analysis (Syers *et al.* 1967). The Saunders and Williams method was developed to determine P_O in soils, as will be discussed below. Although the ignition step is intended only to oxidize soil organic matter prior to extraction, it may also increase the solubility of many P_I compounds (Legg and Black 1955; Dormaar and Webster 1963;

Harrap 1963; Williams and Walker 1967; Williams *et al.* 1970; Anderson 1975; Olsen and Sommers 1982; Soltanpour *et al.* 1987; Condron *et al.* 1990b), allowing it to be used to determine P_T in some soils (Syers *et al.* 1967). For the orthic ferro-humic podzols of northern Vancouver Island, the high overall correlation suggests that either method would be suitable for P_T determinations. The lower P_T values in the Bf, and the lower r, indicate that the Parkinson and Allen method is better for mineral soils. The slightly higher P_T concentrations in the organic matter-rich surface horizons when the Saunders and Williams method is used may indicate volatilization losses during the Parkinson and Allen digest, or interferences during colorimetric analysis (Olsen and Sommers 1982).

Soil P_o is estimated by either ignition or extraction procedures (Olsen and Sommers 1982). Although it was used to determine P_T in the preceding section, the Saunders and Williams (1955) ignition method was developed for P_o analysis. To yield meaningful results, ignition must quantitatively mineralize the P_o in a sample without altering the acid solubility of the native P_I, the mineralized P_o should be completely extractable in acid, and no P must be lost through volatilization (Anderson 1975). Additionally, P_o must not be hydrolysed from the unignited sample during acid extraction (Harrap 1963). Extraction procedures, such as that of Bowman and Moir (1993) must ensure that all of the P_o is removed from a sample without hydrolysis of any of the P_o forms.

Ignition usually produces higher P_o concentrations than extraction (Dormaar and Webster 1963; Enwezor and Moore 1966; Ipinmidun 1973; Anderson 1975; Soltanpour *et al.* 1987; Condron *et al.* 1990b), as was observed in almost every horizon and age in this study. The difference between the results from ignition and extraction procedures increased with depth in the soil profile, suggesting that the ignition method is overestimating the soil P_o due to changes in the P_I during ashing, as was discussed in the section on P_T analysis. Any errors due to incomplete mineralization or volatilization losses are probably minimal.

Problems also exist with the Bowman and Moir (1993) extraction procedure. This method requires that P_I be determined in the extracts prior to digestion; the P_o content is calculated by subtracting the pre-digestion P_I content from the post-digestion P_I content. In this study, the P_I content of the undigested extracts could not be determined because the extracts were too darkly coloured for reliable colorimetric analysis. This is a common problem when soils with high organic matter contents are analyzed for P_o using extraction procedures (Olsen and Sommers 1982). The extracts from the Bowman and Moir procedure were analyzed by ^{31}P NMR spectroscopy (Chapters 4, 5 and 6), which revealed that 20% to 80% of the P in solution was orthophosphate. Consequently, not subtracting the P_I in the extract results in a serious overestimation of the soil P_o content, especially in the LF horizon of the recently burned (CH-O) sites and in the mineral horizons. Another problem inherent in extraction procedures for P_o is

hydrolysis of P_o compounds during extraction. The diversity of the P forms revealed by ^{31}P NMR spectroscopy suggests that hydrolysis is minimal with the Bowman and Moir extractant. Thus, if some way were found to read the P_I in the extract prior to digestion, this would be an excellent method for P_o determination in soils.

There was a high overall correlation between the P_A results obtained by the Bray P1 and Mehlich 3 methods, and good correlation in all horizons but the Bf. The Mehlich 3 concentrations were higher than those from the Bray P1 method in the LF and H horizons, while the Bray P1 results were higher in the Bf. Other researchers have reported high correlations between the two methods for agricultural soils (Mehlich 1984; Wolf and Baker 1985; Michaelson *et al.* 1987; Cade 1989; Tran *et al.* 1990; Wendt 1995) and although Wolf and Baker (1985) found that the two methods extracted similar P concentrations, the others report that the Mehlich 3 method extracted more P_A from soils than was extracted by the Bray P1 method. In this study, virtually no P_A was removed from the Bf horizon samples with the Mehlich 3 extractant, which suggests either that the Bray P1 method is overestimating the P_A in this horizon, or that the Mehlich 3 method is underestimating it. Tran *et al.* (1990) obtained similar results for a very acidic spodosol with a high P sorption capacity. They felt that the Mehlich 3 extractant was more reliable on these soils, as the higher NH_4F concentration in the Bray extractant was removing strongly fixed Al-P and thus overestimating the available P. Phosphorus-31 NMR spectroscopy (Chapters 4 and 5) shows that both organic and

inorganic P forms are present in most of the Bf horizons of these sites, and some of this P may be plant-available. Thus, although the Bray P1 method may be removing some of the fixed P, the Mehlich 3 method may not be extracting all of the available P, and the true P_A value probably lies somewhere between the results of the two procedures. A measure of P_A in this horizon may be immaterial, however, as feeder roots are rarely found as far down the soil profile as the Bf horizon.

Conclusions

For the Orthic Ferro-Humic Podzols of northern Vancouver Island, the Parkinson and Allen (1975) digest appears to be a good method for total P determinations, and to extract more completely the P_T from mineral soils than the Saunders and Williams (1955) method. For organic P analysis, there are problems with both the Saunders and Williams (1955) ignition method and the Bowman and Moir (1993) extraction procedure. If some way were found to read the solution P_I after extraction and prior to digestion, despite the dark colour of the solutions, then the Bowman and Moir method would be suitable for P_o analysis for these soils. Both the Bray P1 and the Mehlich 3 methods are suitable for measuring available P in the soils of this study. The Mehlich 3 procedure can be used for multielement analysis, so it may be preferred over the Bray P1 method in some circumstances.

CHAPTER FIVE

A COMPARISON OF SOIL EXTRACTION PROCEDURES FOR 31-P NMR SPECTROSCOPY

Introduction

Phosphorus-31 nuclear magnetic resonance (NMR) spectroscopy can be used to obtain both qualitative and quantitative estimates of the various forms of P in soils, including inorganic orthophosphate, polyphosphate, phosphonate, pyrophosphate, orthophosphate monoesters such as inositol phosphate, and orthophosphate diesters such as phospholipids, and it is analytically less complex than the detailed partition chromotography techniques otherwise required to identify specific organic P compounds. However, the natural P levels in soils are usually low. As ^{31}P NMR is relatively insensitive, requiring more than 100 *u*g P ml^{-1} for quantitative analysis (Adams and Byrne 1989), solution NMR is used for P, and extraction and concentration are required to produce clear spectra. An ideal extractant should remove virtually all of the P from a soil sample, without altering in any way the forms of P found in the soil.

Most ^{31}P NMR studies have employed a rapid extraction technique involving ultrasonic dispersion in 0.5 M NaOH, which usually extracts less than 50% of the total phosphorus (Newman and Tate 1980; Tate and Newman 1982; Ogner 1983; Hawkes *et al.* 1984; Zech *et al.* 1985; Zech *et al.* 1987; Hinedi *et al.* 1989; Gil-Sotres *et al.* 1990; Forster and Zech 1993). Others have used 0.5 M NaOH without

sonication (Preston *et al.* 1986) or with a citrate-dithionite-bicarbonate pretreatment (Ingall *et al.* 1990). These treatments also removed less than 50% of the total P. Hinedi *et al.* (1989) used water, ice cold $HClO_4$ and a combination of $HCl/HF/TiCl_4$, which only showed orthophosphate peaks on the NMR spectra. Condron *et al.* (1985) used a sequential extraction procedure of 0.1 M NaOH, 0.2 M aqueous acetylacetone and 0.5 M NaOH, washing between with 0.5 M HCl. Up to 80% of the total organic P as determined by ignition (Saunders and Williams 1955) was removed, mainly by the initial 0.1 M NaOH step of the extraction. Condron *et al.* (1990a) also used a sequential extraction procedure, with 0.5 M NaOH, 1 N HCl and 0.5 M NaOH, washing with water. This too removed about 80% of the total organic P. Emsley and Niazi (1983) tried tetra-*n*-butyl ammonium hydroxide (Bu_4NOH), hoping to utilize the salting-in effect of the large organic cation. However, this salting-in effect did not occur, and they concluded that Bu_4NOH was as effective, but no more so, as NaOH or KOH. Hinedi *et al.* (1989) tried a sequential treatment of trichloroacetic acid (TCA) and KOH, and found that it extracted 86-99% of the total P from sewage sludge.

One drawback to these methods is that, in addition to P, they extract other paramagnetic ions, such as Mn and Fe, which cause line broadening and distortion of ^{31}P NMR spectra (Hawkes *et al.* 1984; Hutson *et al.* 1992). Adams and Byrne (1989) and Adams (1990)

utilized Chelex, a cation exchange resin, as an extractant in an attempt to remove these interfering ions. Chelex ™ (Bio-Rad Laboratories) is a chelating cation exchange resin which shows a high preference for Fe and other polyvalent metal ions over cations such as Na or K. [The order of preference is: Fe>Al>>Ca>>>Na; Adams and Byrne (1989)]. In the Na form, Chelex is alkaline (pH 11-12) and so can also solubilize organic P from the soil sample. This method extracted approximately the same amount of total P as the NaOH method (Adams and Byrne 1989).

Recently, Bowman and Moir (1993) have proposed the use of a mixture of NaOH and EDTA as a one-step extractant to determine total soil organic P. The NaOH can solubilize the organic P, while the EDTA is able to chelate metal cations to increase the efficiency of P extraction. This method extracted as much as twice the amount of organic P as NaOH alone (Bowman and Moir 1993). It has not yet been tested as an extractant for ^{31}P NMR spectroscopy.

With any soil extraction procedure, there is a danger that soil P compounds will be chemically altered during or after extraction. Hydrolysis, especially of orthophosphate diester to monoester, is thought to be a problem with NaOH (Tate and Newman 1982; Hawkes *et al.* 1984) and with Chelex (Adams and Byrne 1989).

A detailed comparison of extraction methods has never been conducted on forest floor samples, which contain low levels of P in mainly organic form and relatively high levels of other paramagnetic ions. Results with any method for soil P determination are site specific (Anderson 1975). Therefore, one

objective of this research was to compare several soil extraction procedures, to determine the one most suitable for ^{31}P NMR analysis of forest floor samples from CH and HA forests of northern Vancouver Island. The second objective was to examine the method of Bowman and Moir (1993) to determine its effectiveness as an extractant for ^{31}P NMR spectroscopy.

Materials and Methods

Soil Samples

Five forest floor samples from the sites described in Chapter 3 were used for this extraction study. One was from under an old-growth stand of hemlock-amabilis fir (HA-OG), and one was from under an old-growth stand of cedar-hemlock (CH-OG). The other three were from cedar-hemlock sites 0 (CH-0), 5 (CH-5) and 10 (CH-10) years after logging and slash burning. These samples were all relatively high in total P, and had a range of other soil chemical properties (Table 5-1). These forest floor samples were air-dried and ground to pass through a 2 mm sieve.

Extractants

Four different extractants were used. These were:

1. 0.5 M NaOH + 0.1 M EDTA (1:1) (Bowman and Moir 1993)
2. 0.25 M NaOH
3. 1:6 soil:Chelex (weight basis) in deionized water (Adams and Byrne 1989)
4. 1:6 soil:Chelex (wt. basis) in 0.25 M NaOH

Table 5-1: Chemistry of the soils used for the extraction trials. Note: pH measured in $CaCl_2$; C measured with Leco; total N measured via modified Kjeldahl digest; available Ca, Mg, Fe and Al measured with Mehlich extraction; available P measured with Bray P1; total P, inorganic P and organic P 1 measured with Saunders & Williams ashing.

Sample	HA-OG	CH-OG	CH-0	CH-5	CH-10
pH	3.1	3.5	5.0	3.4	4.2
C (%)	49.9	37.2	23.2	46.7	49.6
LOI	3500	695	213	1742	1006
Total N (%)	1.159	0.851	0.879	0.807	0.995
C/N	43.1	43.7	26.4	57.9	49.8
Avail Ca (mg/kg)	1906.1	3300.2	7395.0	3639.1	7188.8
Avail Mg (mg/kg)	393.4	255.6	640.0	498.0	673.5
Avail Fe (mg/kg)	139.6	182.6	220.0	118.3	112.2
Avail Al (mg/kg)	380.7	649.1	720.0	364.9	193.9
Avail P (mg/kg)	40.04	51.5	68.4	23.47	21.16
Total P (mg/kg)	674.0	582.0	796.0	713.0	653.0
Inorg P (mg/kg)	129.0	162.0	396.0	140.0	124.0
Org P 1 (mg/kg)	545.0	420.0	400.0	513.0	589.0

For each extractant, 5 g of air-dry soil and 100 ml of liquid were used. The NaOH-EDTA and NaOH samples were extracted in 125 ml erlenmeyer flasks at room temperature overnight with occasional stirring. For both of the Chelex procedures, samples were

extracted in 250 ml plastic bottles overnight at room temperature on a reciprocal shaker. All samples were then filtered with Buchner funnels and Whatman 41 filter paper. A subsample of each was digested with persulphate (Bowman 1989) and was read using the Watanabe and Olsen (1965) method to determine the total amount of phosphorus which had been extracted. The remainder of each sample was freeze-dried.

Preparation of NMR Samples

Approximately 1 g of the freeze-dried extract was weighed into 50 ml plastic centrifuge tubes, with 2.5 ml of D_2O. Samples were vortexed for 2 min. A few of the Chelex + NaOH samples were prepared in duplicate, and to one of each pair was added 1 pellet (approx. 0.5 g) of NaOH prior to vortexing. All of the samples were left to stand for 2 h, and then were centrifuged. The supernatants were transferred into 10 ml NMR tubes and were refrigerated until used for NMR spectroscopy.

NMR Analysis

Phosphorus-31 NMR spectra were obtained at 101.27 MHz on a Bruker WM 250 high resolution NMR spectrometer using a 45° pulse with a 1.5 s delay and an acquisition time of 0.508 s. The P spectra were proton decoupled using an inverse-gated pulse sequence to overcome the nuclear Overhauser enhancement in order to achieve quantitative results. Accumulation times ranged from 24 to 48 h, and were dependent on the length of time necessary to achieve a strong signal-to-noise ratio. The assignment of peaks was based on Newman and Tate (1980) and Adams and Byrne (1989). Peak areas were

determined by integration.

Metals Analysis

To assess the effect of the chelators (EDTA and Chelex) on interfering paramagnetic ions, the concentrations of Fe, Mn, Cu and Zn in the solutions following extraction were measured by atomic absorption spectroscopy, prior to freeze-drying. Although Zn and Cu are not paramagnetic ions, as divalent cations they should be affected by EDTA and Chelex, and their levels in these forest floor samples were high enough for reliable measurement.

In addition, an adsorption trial was conducted. Iron at concentrations of 0, 20, 30, 40, 60 and 100 mg/l or Mn at 0, 15, 20, 30, 40 and 80 mg/l were added to samples containing either 30 ml of 0.05M EDTA or 5 g Chelex in 30 ml of water, with or without 1 g of air-dry soil. A blank containing 30 ml of water was used as a control. The soil sample used was CH-OG. NaOH was not used in this trial as it caused the metals to precipitate. The samples were placed in stoppered 100 ml centrifuge tubes. After shaking on a reciprocal shaker for 1 hour, the samples were filtered through Whatman 42 filter paper, and then metal concentrations were determined using atomic absorption spectroscopy.

Added P Compounds

To determine the effect of the extractants on various P compounds, 5 g samples of CH-OG had 0.05 g of the following compounds added prior to extraction by 0.25 M NaOH + 0.05 M EDTA or by 1:6 soil:Chelex (wt. basis) in 0.25 M NaOH: ATP (Adenosine 5'-Triphosphate, disodium salt, Grade II, Sigma A-3377);

glycerophosphate (disodium pentahydrate, Sigma G6504); or K-polyphosphate (formed by fusion of KH_2PO_4 as per Kulaev (1979)). These were then prepared for NMR analysis as was previously described.

Results

NMR Spectra

Figure 5-1, A-E, displays the NMR spectra generated from these extractant trials, while Table 5-2 shows a guide for the interpretation of the peaks. It should be noted that the lower pH in the Chelex + water extraction causes a peak shift, reversing the orthophosphate and the monoester peak positions relative to the other extraction procedures. This was also observed by O'Neill *et al.* (1980) and Adams and Byrne (1989). In some of the spectra for the extractant NaOH + EDTA the peaks for orthophosphate and monoester P overlap. When there was clear peak separation with this extractant, there was a valley between the peaks at 6 ppm, and this therefore was used as the dividing line where overlapping occurred. The sharpest peaks, with the best separation, were produced by the Chelex + NaOH extraction. These were further improved when extra NaOH was added to adjust the pH prior to NMR analysis. The broadest peaks were produced by the NaOH + EDTA extraction. These spectra also had the poorest separation of the orthophosphate and monoester peaks. The trends from sample to sample were quite consistent for each extraction method, but the

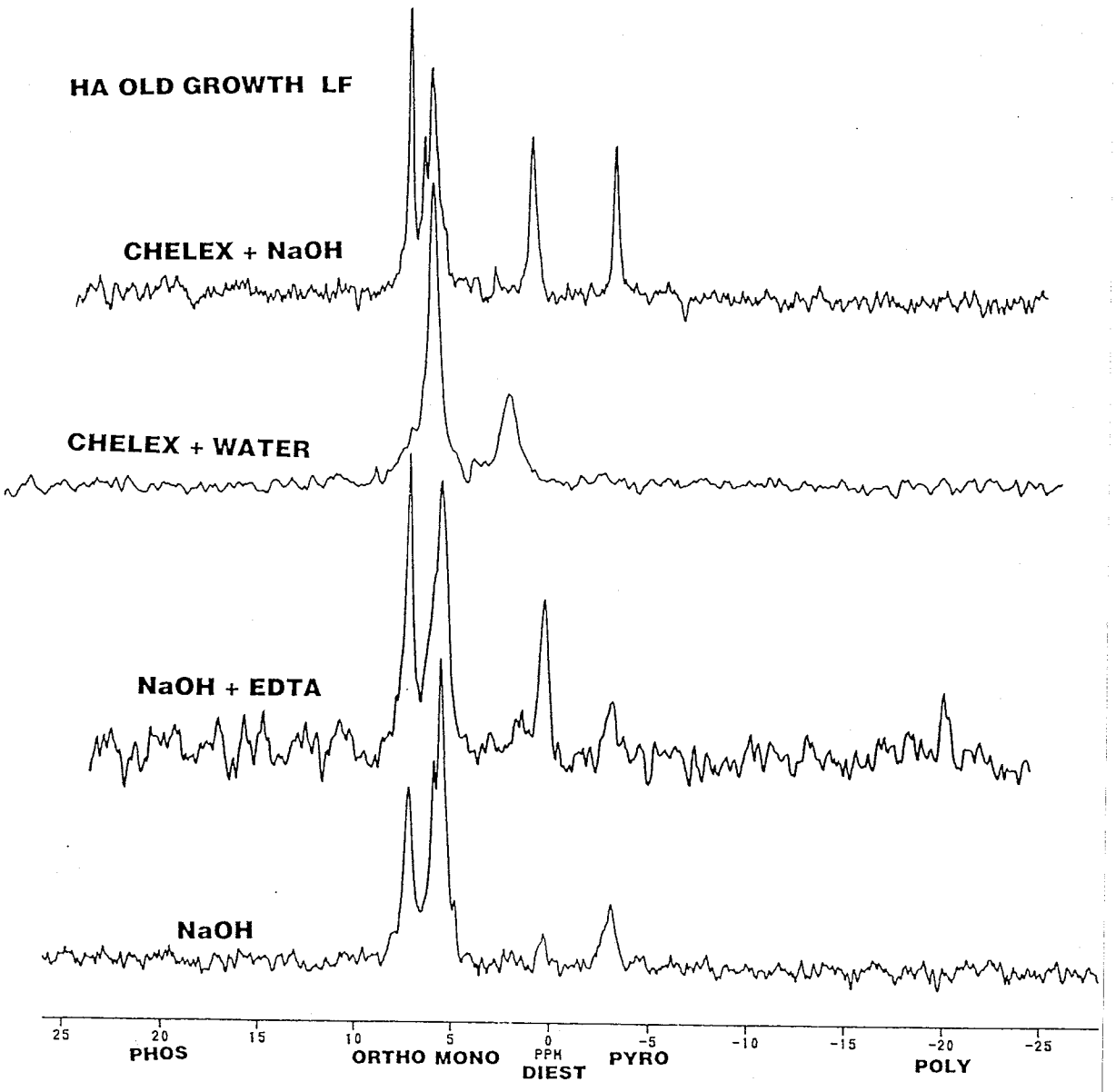


Figure 5-1A: Phosphorus-31 NMR spectra for HA old growth forest floor extracted with: Chelex + NaOH; Chelex + Water; NaOH + EDTA; and NaOH. Phos is phosphonate, ortho is orthophosphate, mono is monoester P, diest is diester P, pyro is pyrophosphate, and poly is polyphosphate.

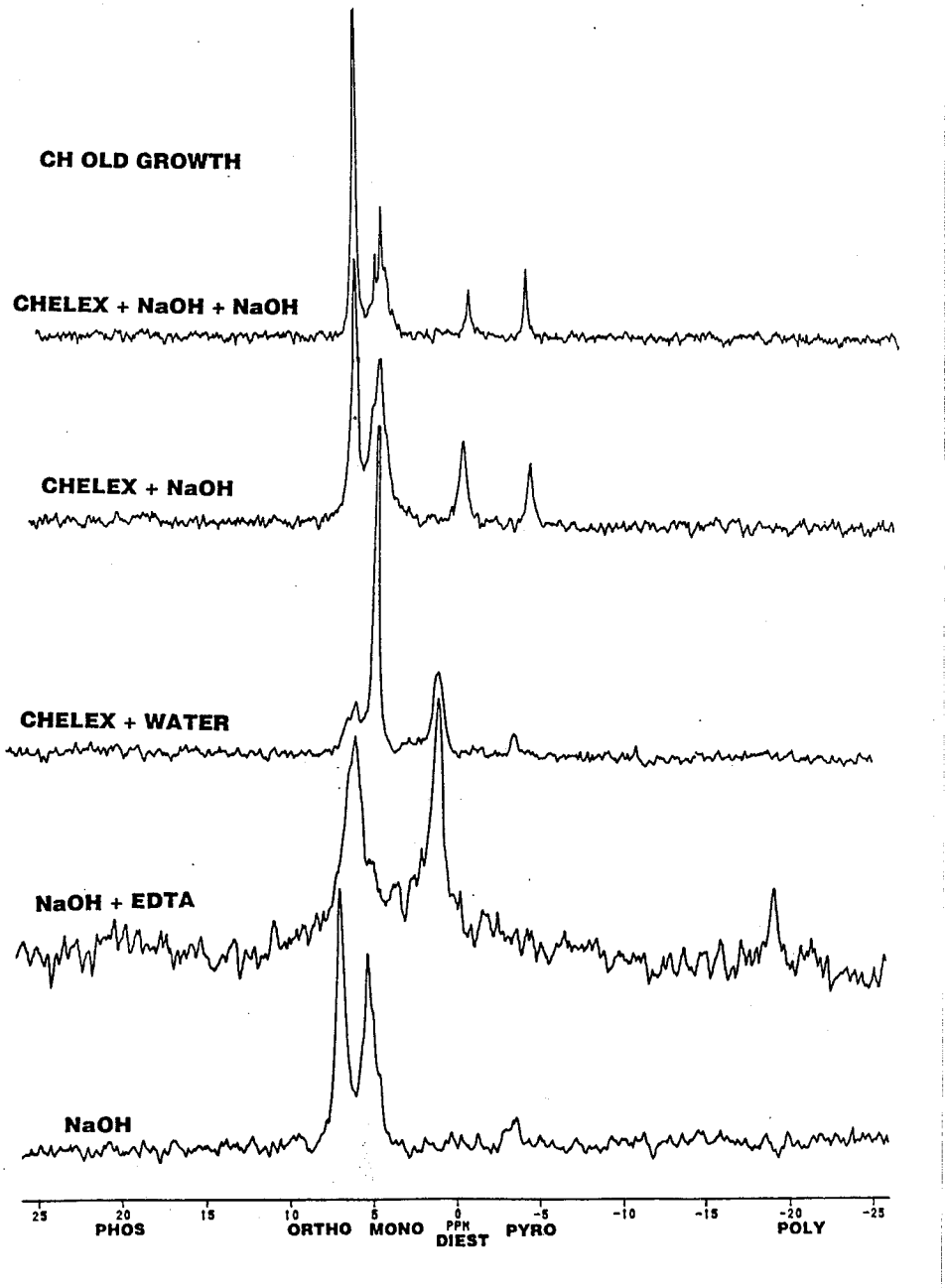


Figure 5-1B: Phosphorus-31 NMR spectra for CH old growth forest floor extracted with: Chelex + NaOH; Chelex + Water; NaOH + EDTA; and NaOH. Phos is phosphonate, ortho is orthophosphate, mono is monoester P, diest is diester P, pyro is pyrophosphate, and poly is polyphosphate.

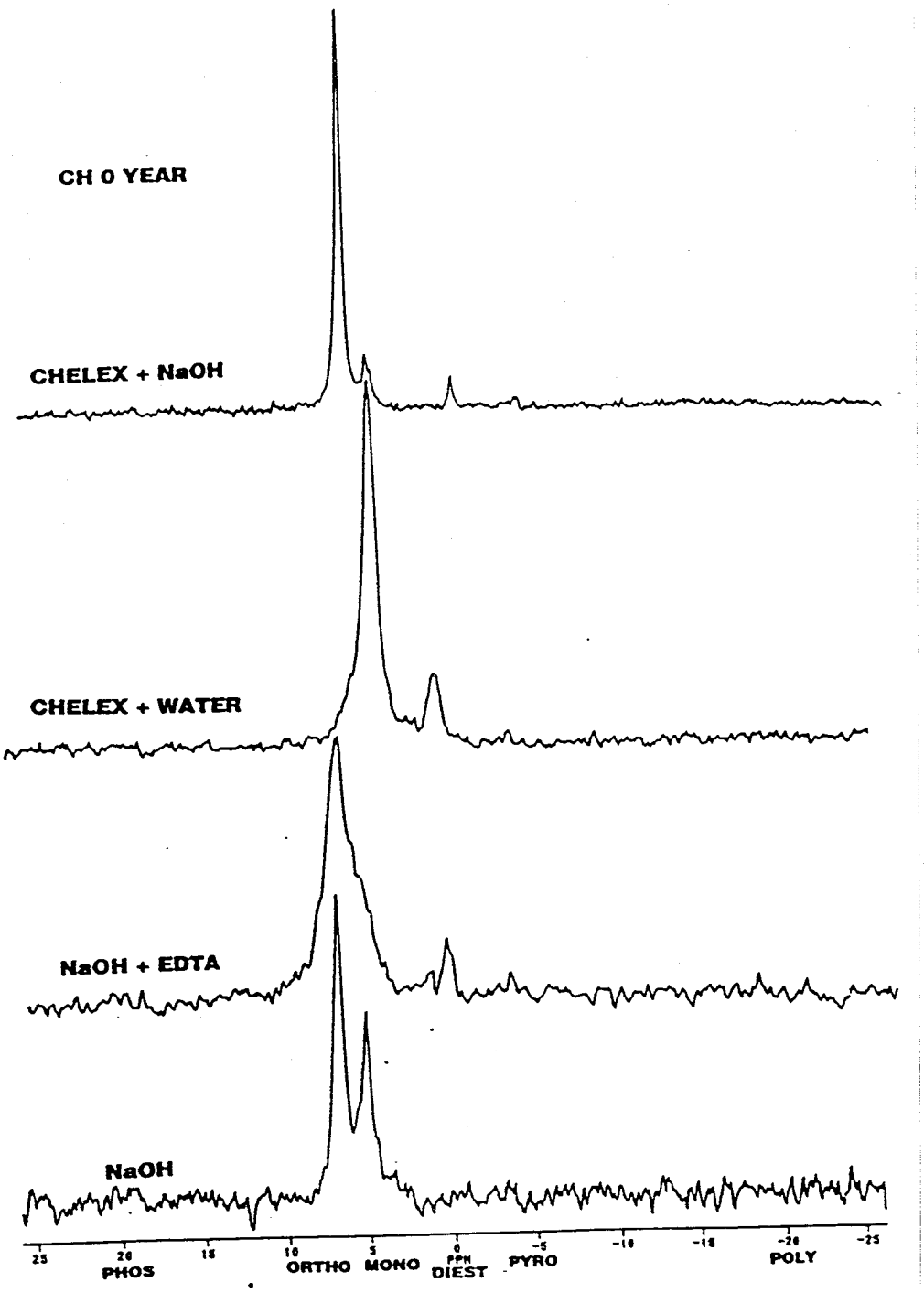


Figure 5-1C: Phosphorus-31 NMR spectra for CH 0-year forest floor extracted with: Chelex + NaOH; Chelex + Water; NaOH + EDTA; and NaOH. Phos is phosphonate, ortho is orthophosphate, mono is monoester P, diest is diester P, pyro is pyrophosphate, and poly is polyphosphate.

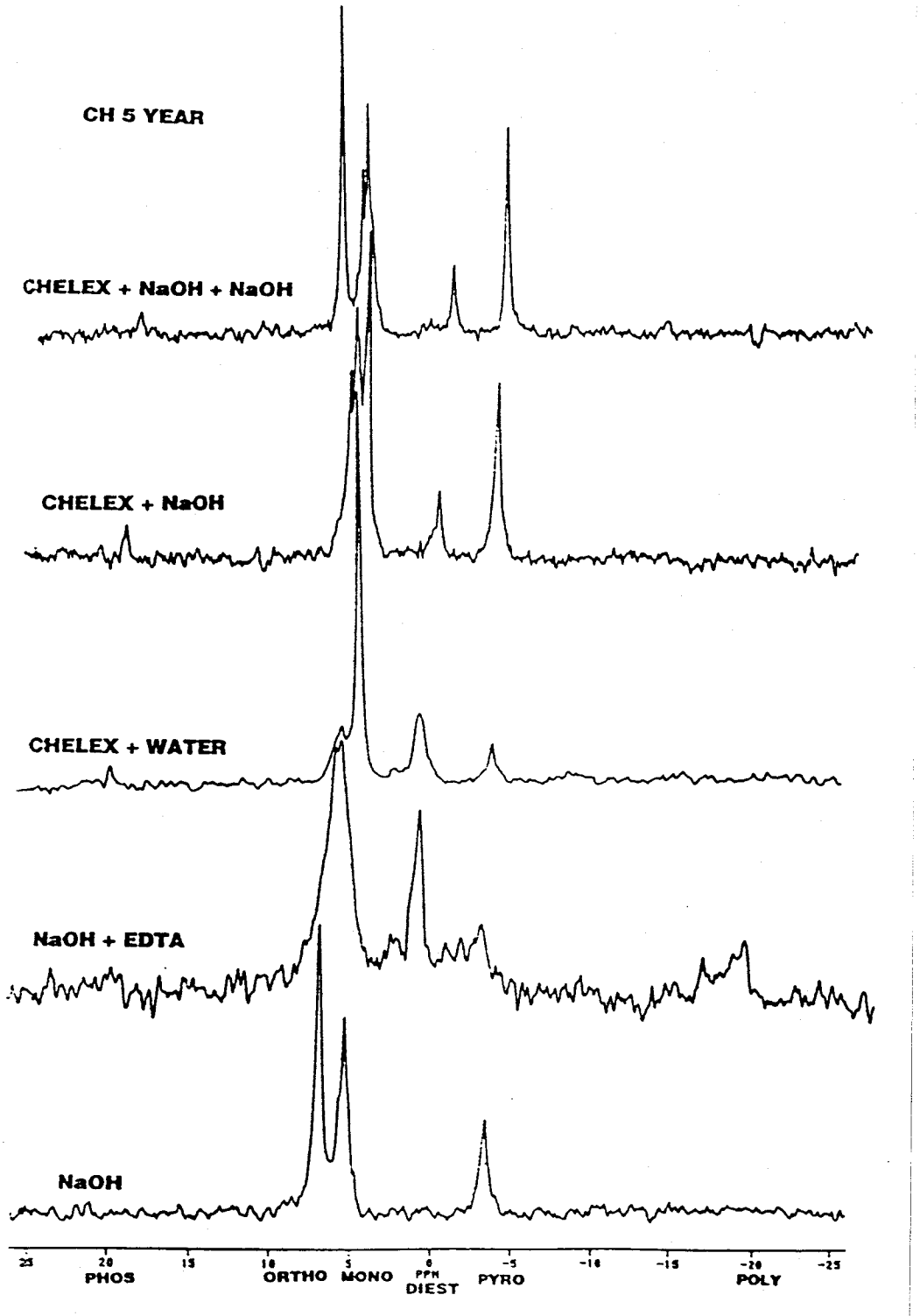


Figure 5-1D: Phosphorus-31 NMR spectra for CH 5-year forest floor extracted with: Chelex + NaOH; Chelex + Water; NaOH + EDTA; and NaOH. Phos is phosphonate, ortho is orthophosphate, mono is monoester P, diest is diester P, pyro is pyrophosphate and poly is polyphosphate.

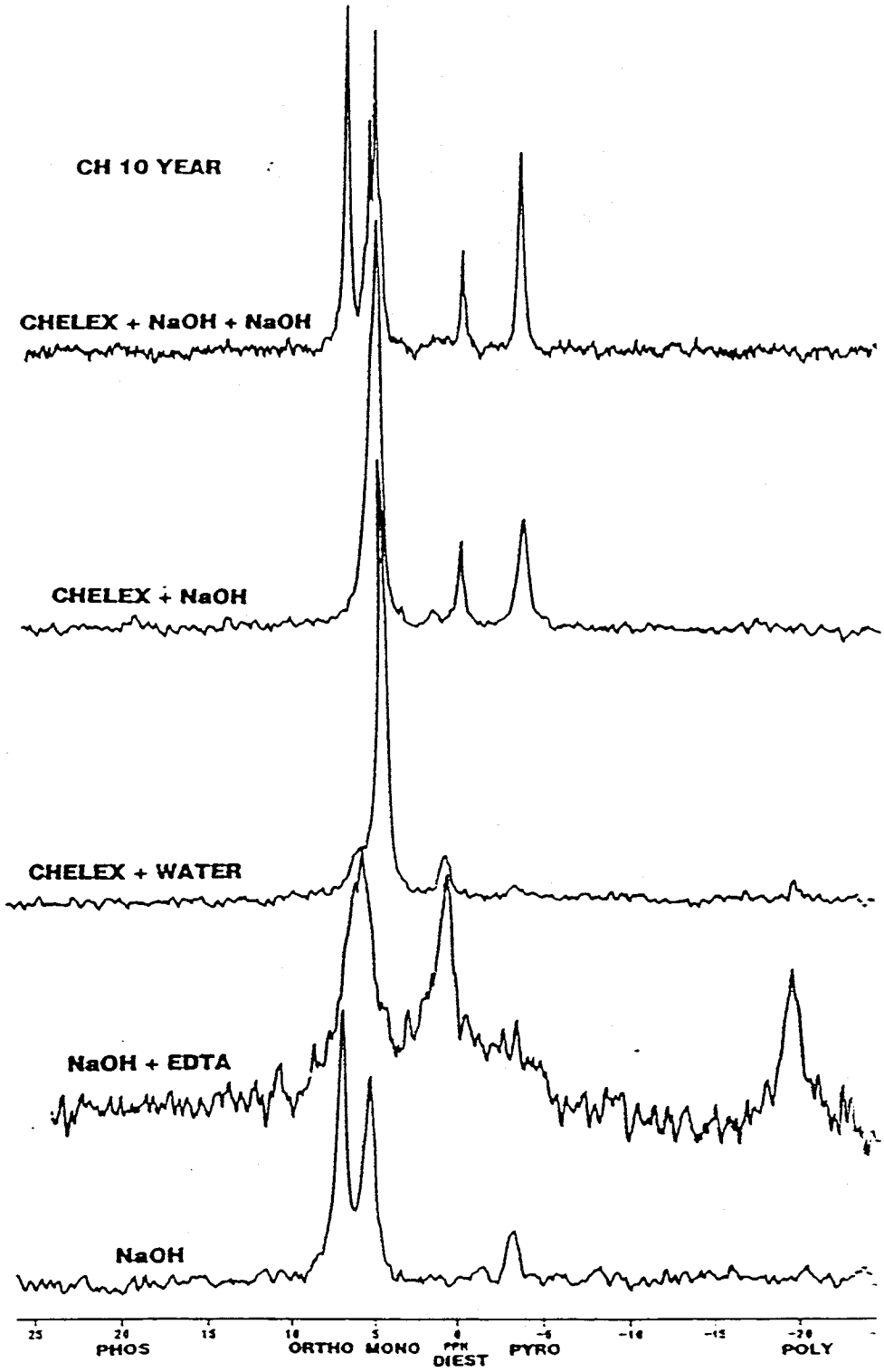


Figure 5-1E: Phosphorus-31 NMR spectra for CH 10-year forest floor extracted with: Chelex + NaOH; Chelex + Water; NaOH + EDTA; and NaOH. Phos is phosphonate, ortho is orthophosphate, mono is monoester P, diest is diester P, pyro is pyrophosphate and poly is polyphosphate.

TABLE 5-2: Interpretation of ^{31}P NMR spectra, showing the ppm range at which the various P compound classes are located.

PPM	COMPOUNDS
15-20	phosphonates
6-8	orthophosphate
3-6	phosphate monoesters -inositol phosphates -sugar phosphates -mononucleotides
1-(-1)	phosphate diesters -phospholipids -RNA, DNA
(-3)-(-6)	pyrophosphate
(-20)	polyphosphate ATP, ADP

results from each extractant were very different within each forest floor sample. NaOH + EDTA was the only method to show polyphosphate peaks for three of the samples, while diester peaks in the NaOH extracts were seen only with the HA-OG samples (Fig. 5-1A). Phosphonate was only unambiguously detected in sample CH-5 (Fig. 5-1D) using the three extractions involving Chelex. Table 5-3 shows the percentage of P found within each class of compounds, calculated from the spectra by integration. The NaOH + EDTA extraction seems to have produced the greatest range of compounds. It also extracted the most P of all the extractants: 63.3-98.5% of P_T, compared with 20.1-34.2% by NaOH, 7.7-11.4% by Chelex + water and 21.0-36.2% by Chelex + NaOH (Table 5-4). The NaOH + EDTA also extracted more diester P than monoester P (Table 5-5), producing higher diester/monoester ratios than any other method except the Chelex + water extraction. The NaOH extraction method extracted

Table 5-3: The percentage of total P in solution found in the various P compound classes, as calculated from ^{31}P NMR spectra by integration. Phos is phosphonate, orth is orthophosphate, mono is monoester P, dies is diester P, pyro is pyrophosphate and poly is polyphosphate.

Sample	Extractant	Phos	Orth	Mono	Dies	Pyro	Poly
HA-OG	Chelex + NaOH	0	26	44	15	15	0
	Chelex + H_2O	0	62	16	22	0	0
	NaOH + EDTA	0	21	49	15	7	7
	NaOH	0	36	51	4	9	0
CH-OG	Chelex + 2 NaOH	0	58	30	5	7	0
	Chelex + NaOH	0	36	40	14	10	0
	Chelex + H_2O	0	54	18	23	5	0
	NaOH + EDTA	0	17	33	39	0	11
	NaOH	0	51	43	0	6	0
CH-0	Chelex + NaOH	0	74	18	6	2	0
	Chelex + H_2O	0	73	13	13	1	0
	NaOH + EDTA	0	51	33	11	6	0
	NaOH	0	55	45	0	0	0
CH-5	Chelex + 2 NaOH	3	34	38	8	17	0
	Chelex + NaOH	2	31	39	9	19	0
	Chelex + H_2O	3	49	22	18	8	0
	NaOH + EDTA	0	23	40	18	7	12
	NaOH	0	49	33	0	18	0
CH-10	Chelex + 2 NaOH	0	34	42	8	16	0
	Chelex + NaOH	1	15	57	9	19	0
	Chelex + H_2O	0	68	14	10	4	4
	NaOH + EDTA	0	17	27	32	10	14
	NaOH	0	49	41	0	10	0

Table 5-4: P extracted by various methods. The percent of total P was calculated using the Total P values shown in Table 5-1.

Extractant		HA-OG	CH-OG	CH-0	CH-5	CH-10
NaOH+Chelex	(mg/kg)	210.1	210.5	166.8	182.6	183.7
	% Total P	31.2	36.2	21.0	25.6	28.1
H_2O+Chelex	(mg/kg)	77.4	66.2	82.8	55.0	71.6
	% Total P	11.4	11.4	10.4	7.71	11.0
NaOH+EDTA	(mg/kg)	526.4	424.8	643.5	451.6	643.1
	% Total P	78.1	73.0	80.8	63.3	98.5
NaOH	(mg/kg)	205.0	198.8	159.6	198.8	207.6
	% Total P	30.4	34.2	20.1	27.9	31.8

Table 5-5: Orthophosphate diester/monoester ratios, calculated from Table 5-3.

	HA-OG	CH-OG	CH-0	CH-5	CH-10
Chelex/2 NaOH	N/A	0.30	N/A	0.21	0.19
Chelex/NaOH	0.34	0.35	0.33	0.17	0.16
Chelex/H_2O	1.38	1.27	1.00	0.82	0.71
NaOH/EDTA	0.31	1.18	0.33	0.45	1.18
NaOH	0.08	0	0	0	0

the fewest types of P compounds, showing peaks for orthophosphate and monoesters in all samples, but peaks for diesters and pyrophosphate in only a few samples. There were no peaks for phosphonates or polyphosphates with this extraction procedure.

Metals Analysis

Table 5-6 displays the analysis of metals within each solution following extraction. The Fe concentrations extracted by NaOH alone and by H_2O + Chelex were comparable (240-1080 versus 120-870

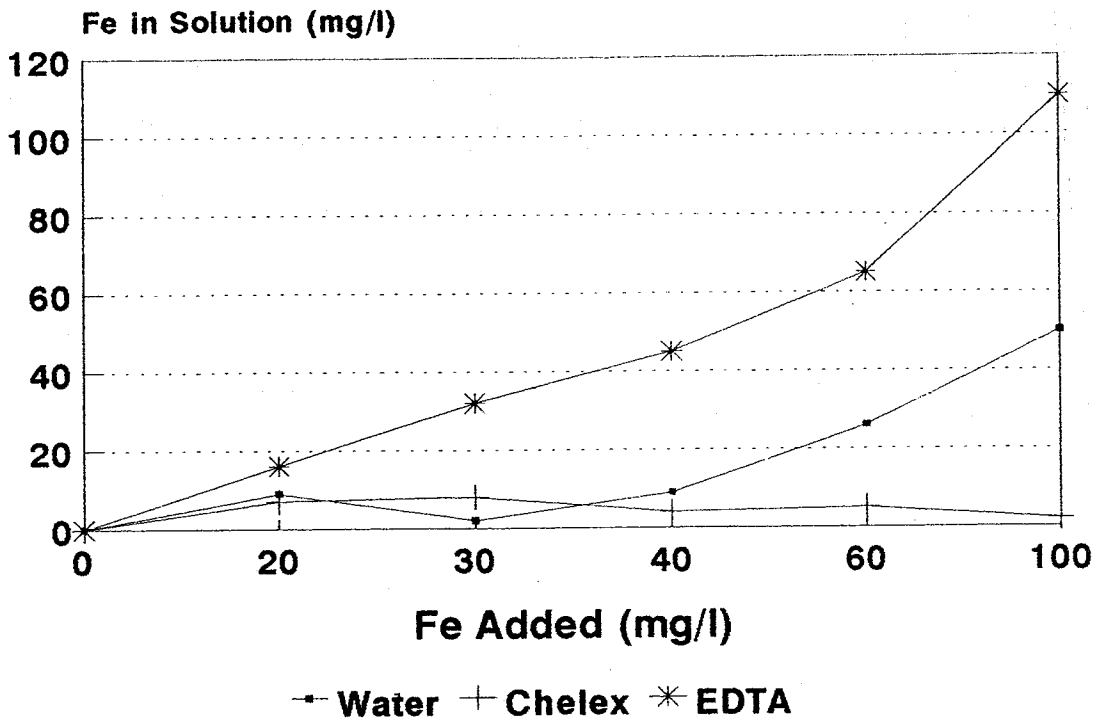
Table 5-6: Metals measured in each solution following extraction, in mg/kg.

Sample	Metal	NaOH	NaOH + EDTA	H_2O + Chelex	NaOH + Chelex
HA-OG	Fe	390	210	300	90
	Mn	60	240	0	0
	Cu	30	36	21	18
	Zn	12	21	0	0
CH-OG	Fe	1080	870	870	450
	Mn	150	720	60	0
	Cu	24	27	9	9
	Zn	0	15	0	0
CH-0	Fe	900	1890	780	450
	Mn	150	960	90	60
	Cu	24	33	24	3
	Zn	0	27	0	0
CH-5	Fe	240	120	120	0
	Mn	120	900	60	0
	Cu	36	24	21	12
	Zn	21	45	0	0
CH-10	Fe	300	180	300	120
	Mn	210	1530	60	0
	Cu	9	27	21	21
	Zn	9	51	12	0

ug/l). The Fe levels in the NaOH + EDTA solutions were intermediate, except for CH-0, the recent burn. NaOH + Chelex solutions contained the lowest levels of Fe. NaOH + EDTA extracted the greatest concentration of Mn, especially in the three postburn samples. The lowest Mn levels were found in the two Chelex extracts. The NaOH and NaOH + EDTA extracts contained similar levels of Cu; NaOH + Chelex had the least Cu. NaOH + EDTA extracts had the most Zn; the other methods generally contained very little if any Zn.

Figure 5-2 displays the results from the adsorption trial, using soil. For the samples without soil, the EDTA and water extracts contained the same levels of Fe or Mn that had been added to the samples, while the Chelex removed all of the Fe and Mn, reading 0 mg/l at all levels. In Figure 5-2(A), the EDTA kept all of the added Fe in solution, and also extracted additional Fe from the soil. The Chelex removed almost all of the added Fe from solution. With water alone, much of the Fe was adsorbed onto the soil surfaces, as the Fe in solution at all levels was lower than that which had been added to the sample. For Mn (Fig. 5-2(B)), the Chelex removed all of the added Mn from solution. The EDTA kept what had been added to the sample in solution, but did not appear to extract any additonal Mn from the soil. At the highest level of Mn addition (80 mg/l) there appeared to be some adsorption onto the soil. In water alone, all of the Mn added appeared to stay in solution.

A)



B)

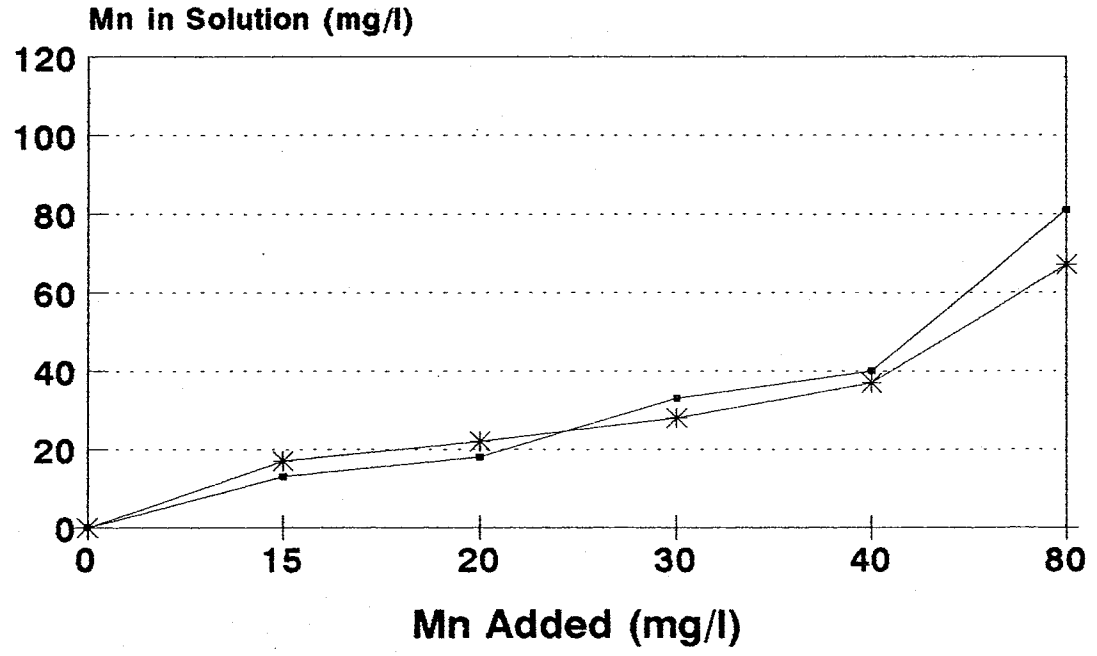


Figure 5-2: Metals in solution following the adsorption trial. The chelators used, Chelex and EDTA, were in water. CH-OG was the soil sample. The results with Fe are shown in (A), while those for Mn appear in (B).

P Addition

The results from the addition of P compounds to the NMR extracts are shown in Figure 5-3, A and B. The P concentrations in each extract and the proportion of P in each compound class are found in Table 5-7.

The Chelex + NaOH extraction produced much sharper peaks than those by NaOH + EDTA, but peaks are seen at the same positions in the spectra regardless of extractant (Fig. 5-3 A and B). The added P compounds dominate each spectrum, changing the spectra from those of the original extract. When added polyphosphate was extracted with NaOH + EDTA (Fig. 5-3A), there was a large, broad peak at -20 ppm, the polyphosphate position. Pyrophosphate, diester, monoester and orthophosphate peaks also appeared, but they were small and broad relative to the polyphosphate peak. In the Chelex + NaOH extract (Fig. 5-3B), there was a sharp polyphosphate peak, as well as sharp monoester and pyrophosphate peaks. The main difference between these two methods when polyphosphate was added was the amount of P extracted: 6500 mg/kg for NaOH + EDTA versus 3800 mg/kg by Chelex + NaOH (Table 5-7). NaOH + EDTA extracted more of the total P as orthophosphate, and less as monoester.

When ATP was added to the sample, orthophosphate, monoester and diester peaks appeared (Fig. 5-3, A and B). There were also three approximately equal peaks at -5, -10 and -20 ppm. These represent the alpha, beta and gamma phosphates in the ATP molecule. Both extraction methods yielded the same concentration of P in solution (Table 5-7) and approximately the same percentages of

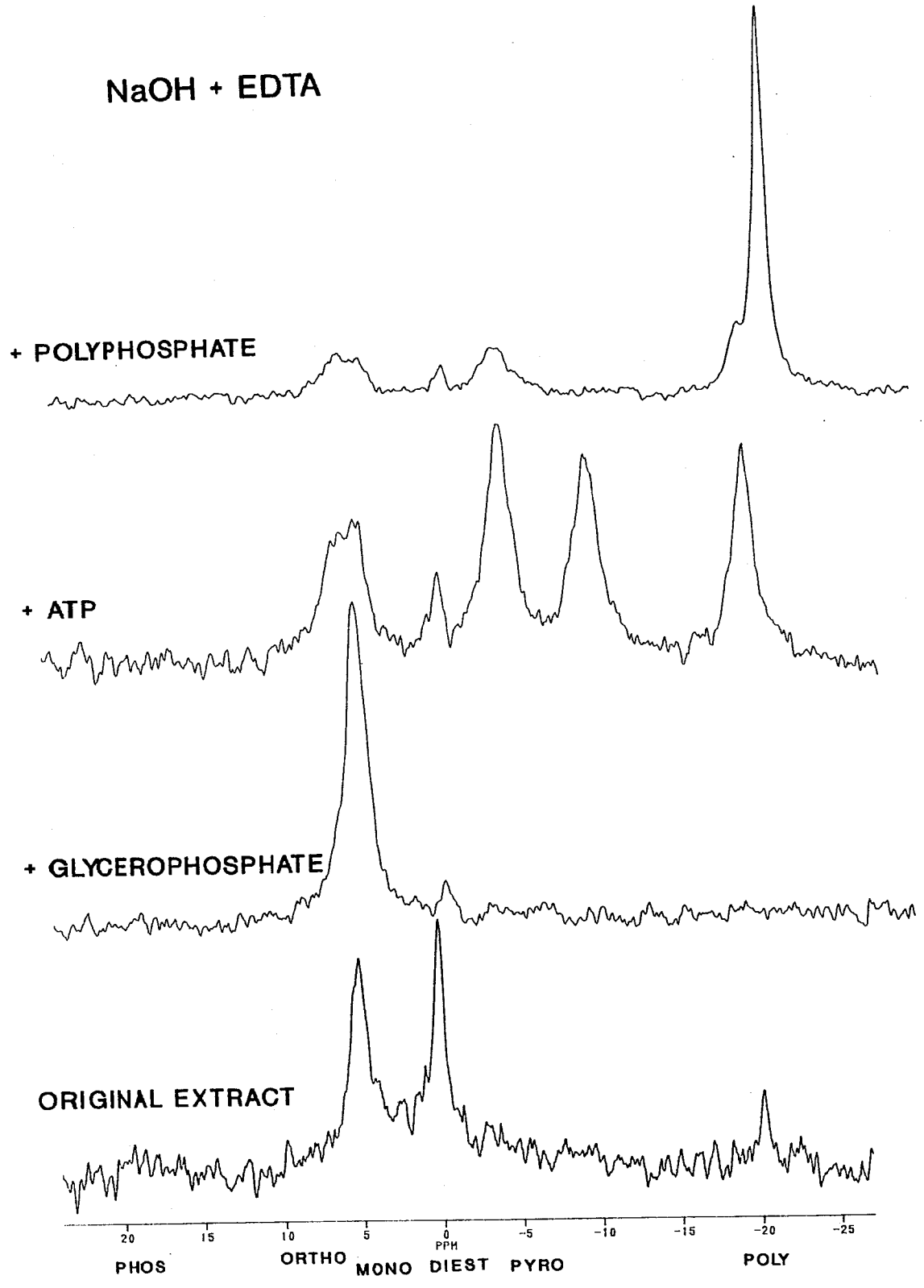


Figure 5-3A: Phosphorus-31 NMR spectra in soil and after the addition of polyphosphate, ATP and glycerophosphate, extracted with NaOH + EDTA.

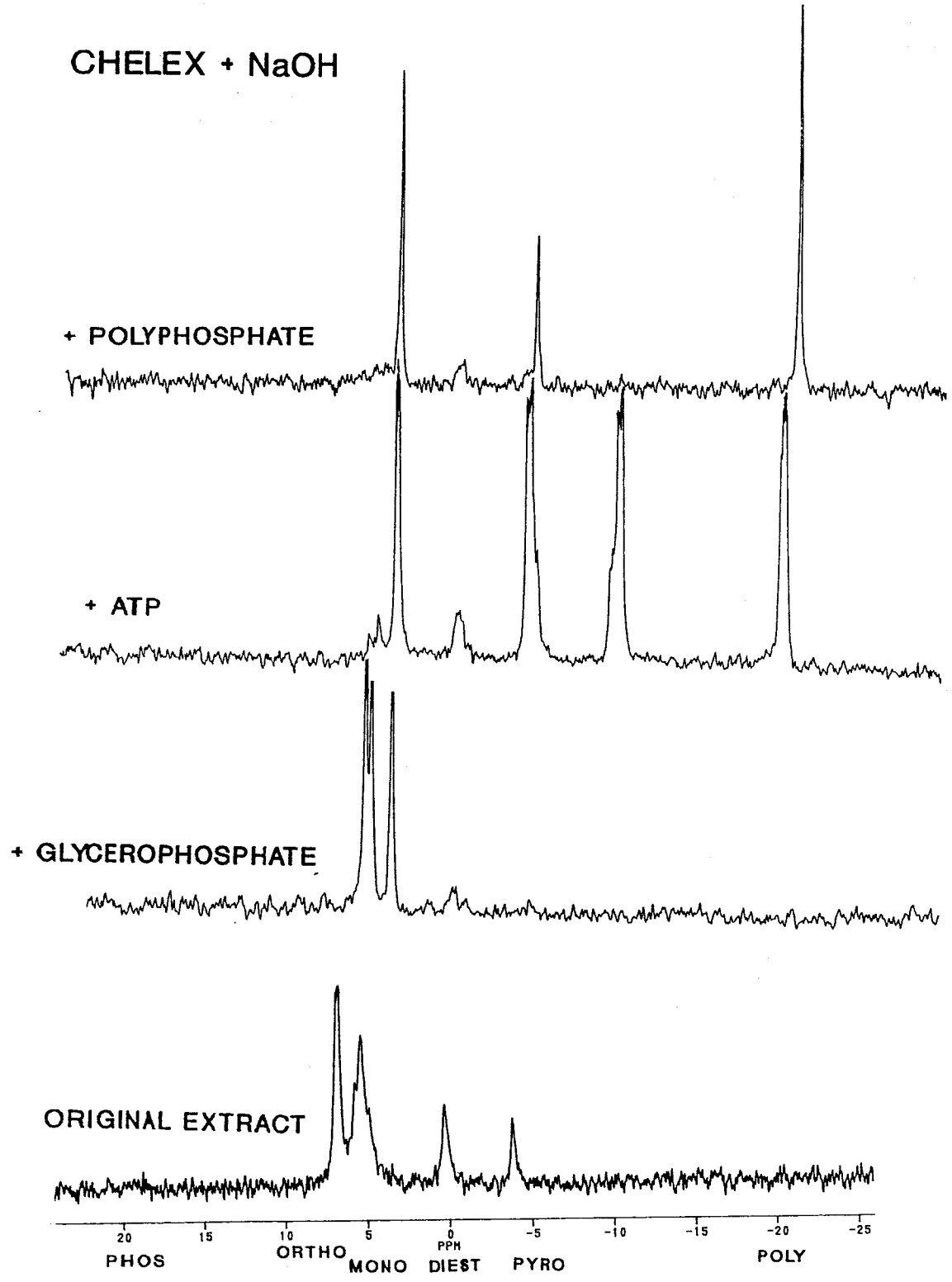


Figure 5-3B: Phosphorus-31 NMR spectra in soil and after the addition of polyphosphate, ATP and glycerophosphate, extracted with Chelex + NaOH.

Table 5-7: Total P content, and the proportions of P in the various soil P compound classes, after the addition of polyphosphate, ATP or glycerophosphate to soil, and after extraction with either NaOH + EDTA or Chelex + NaOH. For ATP, the peaks at -5, -10 and -20 are the alpha, beta and gamma phosphates in the ATP molecule.

Sample	P in Soil mg/kg	ortho phos. %	Mono-ester %	Di-ester %	Pyro-phos. %	(-10) peak %	poly phos %
NaOH+EDTA + polyphos.	6500	11.7	6.3	4.3	12.8	n/a	64.9
NaOH+Chelex + polyphos.	3800	0	40.3	4.9	16.1	n/a	38.7
NaOH+EDTA + ATP	3600	14.5	10.2	7.2	24.7 alpha	21.7 beta	21.7 gam.
NaOH+Chelex + ATP	3500	2.8	17.8	4.7	28.0 alpha	27.1 beta	19.6 gam.
NaOH+EDTA +glycerophos.	5900	26.4	63.9	9.7	0	n/a	0
NaOH+Chelex +glycerophos.	3000	3.8	89.7	6.5	0	n/a	0
NaOH+EDTA orig. extract	424.8	33.3	16.7	38.9	0	n/a	11.1
NaOH+Chelex orig. extract	210.5	36.1	40.3	13.9	9.7	n/a	0

total P in the various compound classes, although NaOH + EDTA had slightly more orthophosphate and less monoester P than did Chelex + NaOH.

Glycerophosphate, a monoester formed in soil after hydrolysis of glycerophosphatides (Hance and Anderson 1963), appeared at the monoester position with both extraction methods (Fig. 5-3, A and B). The NaOH + EDTA solution (Fig. 5-3A) contained nearly twice as much P as the Chelex + NaOH solution (Table 5-7), and had more P as orthophosphate and less as monoester.

Discussion

Of the reagents used in this study, NaOH + EDTA extracted the most P from each forest floor sample, with results comparable to those from sewage sludge extracted with TCA and KOH (Hinedi *et al.* 1989) or from sequential extraction of soil (Condron *et al.* 1990a). This agrees with the findings of Bowman and Moir (1993), that it is a good extractant of organic P. The amount of P extracted by NaOH (20-34%) is comparable to, or slightly lower than that reported in the literature (Newman and Tate 1980; Tate and Newman 1980; Hawkes *et al.* 1984; Ingall *et al.* 1990; Gil-Sotres *et al.* 1990; Forster and Zech 1993). The levels of P extracted by Chelex with both water and NaOH are lower than those obtained by Adams and Byrne (1989) and Adams (1990), which were comparable with NaOH levels, as was the amount extracted by Bu_4NOH (Emsley and Niazi 1983). There were also lower P levels in the Chelex + NaOH samples after P compounds such as polyphosphate and glycerophosphate were added prior to extraction.

The quality of the spectra produced by NaOH + EDTA was poor, however, relative to the other extractants used in this trial, with poor separation of the orthophosphate and monoester P peaks. This seems to be caused by the complexing by EDTA of paramagnetic ions other than P, particularly Fe and Mn. The one sample in which the peaks were clearly separated (HA-OG) contained the lowest concentrations of Fe and Mn. High concentrations of Mn seem to cause most of the peak overlap: spectra containing less than 200

ug/g of Mn have good separation between the orthophosphate and monoester P peaks. Peak broadening by Mn in ^{31}P NMR spectra has been observed elsewhere (Hutson *et al.* 1992). This is one drawback to the NaOH-EDTA method - it removed cations from P compounds to allow more P to be extracted, but did not remove the metals from solution as did Chelex. EDTA alone did not extract much Fe from the soil, however. More Fe was released when EDTA was combined with NaOH, probably due to the solubilization of organic matter (Stevenson 1994). The best spectra, in terms of peak separation and signal-to-noise ratio, were produced by the Chelex + NaOH extractions. These were further improved by adjusting the pH prior to analysis. However, overlapping of the orthophosphate and monoester P peaks was seen in all of the Chelex + H_2O spectra, and in the specta for the CH-10 sample extracted with Chelex + NaOH.

The quality of spectra reported in the literature are quite variable. NaOH often produces poorly resolved resonance in the orthophosphate monoester region, with monoesters appearing as shoulders on the orthophosphate peak (Newman and Tate 1980; Zech *et al.* 1987; Hinedi *et al.* 1989; Condron *et al.* 1990a; Gil-Sotres *et al.* 1990). This has also been reported in Chelex extracts (Adams and Byrne 1989). The TCA and KOH extractions (Hinedi *et al.* 1989) produced clear, sharp spectra, as did the sequential extraction procedure of Condron *et al.* (1985). However, Hinedi *et al.* 1989 examined sewage sludge, which may not be comparable to forest floor. It is difficult to judge the quality of Bu_4NOH as an

extractant, as spectra were not published, and results for orthophosphate and monoesters were reported as one peak (Emsley and Niazi 1983).

There were considerable differences in the diversity of compounds extracted by the different reagents in this study. NaOH + EDTA extracted the most, while NaOH extracted the least. This may in part be due to the amount of P extracted by each reagent, but it may also be due to the nature of the reagent. The diester/monoester ratios suggest that NaOH caused hydrolysis of orthophosphate diesters, which did not occur with NaOH + EDTA. Hydrolysis has been reported by other NMR researchers (Ogner 1983; Adams and Byrne 1989; Ingall *et al.* 1990), and Hance and Anderson (1963) reported that phospholipids were readily hydrolysed in alkaline solution. The diester/monoester ratios for NaOH are also comparable to reported values of 0.18-0.46 (Zech *et al.* 1985; Gil-Sotres *et al.* 1990; Forster and Zech 1993). The lack of diesters in NaOH samples in this particular study is probably due to the length of extraction - overnight in this case, as opposed to only a few minutes with sonication, used by other researchers. It should be noted that the lack of clear separation of the orthophosphate and monoester P peaks using NaOH + EDTA probably underestimates the proportion of P in either or both of these compound classes, and thus may widen the diester/monoester ratio.

The lower compound diversity in the Chelex extracts compared with NaOH + EDTA may be due to a loss of P compounds when the

Chelex is removed from the extracting solution. When P compounds such as polyphosphate and glycerophosphate were added to the soil prior to extraction, the NaOH + EDTA extracted more P than Chelex + NaOH. This would suggest that P is removed along with the Chelex during filtration, possibly via cation linkages. The K^{+} from the added K-polyphosphate may be exchanged with divalent cations such as Mn^{++} or Fe^{++}, which could then link the polyphosphate to the Chelex (Levesque and Schnitzer 1967).

Polyphosphates are rarely seen in reported NMR spectra (Tate and Newman 1980; Emsley and Niazi 1983; Zech *et al.* 1987; Adams and Byrne 1989), and are usually at very low levels. This is surprising as they are widely distributed in nature, especially in forest soils (Kulaev 1979; Martin *et al.* 1985). The high amounts extracted by NaOH + EDTA in this study may be due to the greater amount of total P extracted by this method, as they are not seen in the spectra of the other extractants. They may be hydrolysed by other reagents (Subbarao *et al.* 1977), they may be part of the more than 50% of the total P which was not extracted, or they may be lost during the extraction procedure. It is unlikely that they are artifacts, as the NaOH + EDTA extracting conditions are not conducive to polymerization (Kulaev 1979).

Conclusions

Phosphorus-31 NMR spectroscopy is a valuable tool for the direct identification of P compounds in soil extracts. The major

advantage is that it is analytically less complex than the detailed partition chromatography techniques otherwise required for identifying specific organic P compounds.

Extraction of P compounds from soil for ^{31}P NMR analysis is possible with a variety of reagents. The forms of P which dissolve depend on the reagent; the complex nature of soil organic P may make it impossible for a single extractant to dissolve all P compounds. It also may not be possible to produce high quality spectra without some alteration of P compounds by hydrolysis.

It appears from this study that the NaOH-EDTA extraction procedure for organic P (Bowman and Moir 1993) could be used as an extractant for ^{31}P NMR analysis. It extracted a higher concentration of P and a greater diversity of P compounds that any other extractant tested, with less apparent hydrolysis of compounds. However, it also maintained other paramagnetic ions in solution, causing line broadening and overlapping peaks, thus reducing the quality of spectra. Consequently, it would be most suitable for samples with high P levels and low levels of interfering ions, unless some way could be found to remove the EDTA-metal complexes after extraction without altering the P compounds in solution.

This study also demonstrated that the extractant used will greatly affect the results of ^{31}P NMR analysis of soil samples, making it difficult to compare results from studies using different extractants.

CHAPTER SIX

A COMPARISON OF PHOSPHORUS FORMS ON CH AND HA SITES

Introduction

As discussed in the general introduction, the forests examined on northern Vancouver Island exist as two phases. These are: the CH type, composed of western red cedar and western hemlock; and the HA type, composed of western hemlock and amabilis fir. After logging and planting, the trees on the CH sites suffer a growth check and become chlorotic, while the HA plantations grow well. Studies by Germain (1985) and Weetman *et al.* (1989a, b) indicated that trees on the CH sites were deficient in nitrogen and phosphorus and responded well to fertilization, suggesting a nutrient cycling problem.

Carbon and N forms on these sites have been examined in some detail (for example, see de Montigny 1992; Keenan 1993; Prescott and Weetman 1994; Chang 1995). Phosphorus has been less thoroughly studied. Some research suggests that there are higher concentrations of available and total P on the HA sites (Messier 1991; de Montigny 1992; Keenan 1993; Prescott *et al.* 1993; Prescott *et al.* 1994), but no detailed investigation of P has been conducted.

There appears to be less effective decomposition on the CH sites. All layers of the CH forest floor had smaller concentrations of total and extractable N, and mineralized less N

during aerobic incubations in the laboratory (Prescott *et al.* 1993). Also indicative of less complete decomposition was the slightly higher ratio of carbohydrate to lignin moieties in the CH forest, as revealed by ^{13}C NMR spectroscopy. Nitrogen alone does not appear to control the rate of litter decomposition (Prescott 1995); other nutrients such as P may play an important role. Differences in decomposition may result in different concentrations and forms of organic P between the CH and HA forest types. The objective of the research in this chapter was to examine the soil phosphorus of mature, uncut CH and HA stands. Concentrations of total, organic and available P were determined by extraction and digestion procedures, and fractionation and ^{31}P nuclear magnetic resonance (NMR) spectroscopy were used to characterize the P forms. Some aspects of the chemistry of these soils which could influence P forms and levels were also examined.

Materials and Methods

Sample Collection

The study sites and sampling design were described in Chapter 3. Sampling was conducted in late July and August, 1992, using three locations for each of the CH and HA forest types and three pits per location, for a total of nine samples per horizon per forest type. Samples from non-woody horizons were collected after pits were dug, using a metal trowel. They were placed in plastic bags in coolers, and later were frozen for transportation. In the

laboratory, the samples were thawed, air-dried at 25°C, and sieved to less than 2 mm. The forest floor samples were ground with a stainless steel coffee grinder prior to sieving. All samples were subsequently stored at room temperature in airtight plastic containers.

General Chemical Analysis

Gravimetric moisture content was determined by oven-drying at 105°C for 16 hours, using thawed samples prior to air-drying. Air-dried moisture contents were obtained the same way, but using dried and sieved material (Lavkulich 1981).

Soil pH was measured in both water and 0.01 M $CaCl_2$, using a 1:2 (w/v) soil:liquid ratio for mineral horizons and a 1:5 (w/v) ratio for forest floor material (Lavkulich 1981; McLean 1982).

To determine loss on ignition (LOI), oven-dried samples were burned in a muffle furnace for 1 hour at 375°C and for 16 hours at 550°C. The LOI was calculated from the weight difference between the oven-dried and the ashed sample.

Total C was estimated by the use of a Leco Induction Furnace (Bremner and Tabatabai 1971; Lavkulich 1981), using oven-dried material. Total N was measured on oven-dried samples by using a semimicro Kjeldahl procedure to convert the N to ammonium (Lavkulich 1981). The ammonium in solution was then determined colorimetrically with a Lachat Flow Injection Analyzer (FIA).

Calcium, Mg, Fe and Al were extracted from air-dried samples by the Mehlich method III (Mehlich 1984) and were read using atomic

absorption spectroscopy (AAS). Iron, Al and Mn were determined on oven-dried material using sodium pyrophosphate extraction, acid ammonium oxalate extraction and citrate bicarbonate dithionite extraction (Lavkulich 1981), followed by AAS.

P Methodology

Available P (P_A) was extracted from air-dried material by the Bray P1 method (Bray and Kurtz 1945; Olsen and Sommers 1982). Solution P was then measured colorimetrically (Watanabe and Olsen 1965) using the Lachat FIA. Total P (P_T) was determined on oven-dried material by the Parkinson and Allen (1975) digest, with colorimetric analysis on the Lachat FIA. The new extraction procedure of Bowman and Moir (1993) was utilized to extract P_O from air-dried samples, followed by persulphate digestion (Bowman 1989) and colorimetric analysis on a Technicon Autoanalyzer. A comparison of these methods to others for P_A, P_T, and P_O was discussed in Chapter 4.

The Chang and Jackson (1957) procedure, as described by Olsen and Sommers (1982) was utilized to fractionate P into NaOH (P_{NaOH}), citrate-bicarbonate (P_{CB}), citrate-bicarbonate-dithionite (P_{CBD}) and HCl (P_{HCl}) extractable forms.

NMR Spectroscopy

Two soil profiles for each of the CH and HA forest types were chosen for analysis by ^{31}P NMR spectroscopy. The samples selected were high in P_T, and had chemical characteristics close to the mean values for the forest type they represented. LF, H, Bhf and Bf

horizons were analyzed for each profile. Air-dried soils were extracted using 100 ml of a 1:1 mixture of 0.25 M NaOH and 0.05 M EDTA (Bowman and Moir 1993), as was described in detail in Chapter 5. The NMR sample preparation and analytical procedure was described in Chapter 5.

Statistical Analysis

Statistical analyses were conducted using the Systat program (Wilkinson 1990) to perform analysis of variance test at $p<0.05$, using a nested design (Hicks 1982), with location nested within each forest type. The model statement used was: VARIABLE = CONSTANT + TYPE + HORIZON + LOCATION{TYPE} + TYPE*HORIZON + HORIZON*LOCATION{TYPE}, with VARIABLE the measured parameters such as available P. The assumption with this model is that the three locations used for each forest type are not significantly different from one another. Thus the HORIZON*LOCATION{TYPE} term should not be significant. A significant result for the TYPE*HORIZON term indicates that the CH and HA forest types are significantly different for a particular horizon. Pearson pairwise correlations and Tukey's HSD tests were also conducted with the Systat program. Homogeneity of variance was determined by plotting residuals against estimates. Log and log (n+1) transformations were performed where necessary.

Results

The results from the analysis for field moisture, air dry moisture, pH in water and pH in $CaCl_2$ are shown in Table 6-1.

Table 6-2 displays the analysis of variance results for these data. The field moisture content was relatively uniform for the surface horizons, but dropped in the Bf. There were no significant differences between the CH and HA forest types. The air-dry moisture content was similar for all horizons, and there were no significant differences between the two forest types. Measuring the pH in water and in $CaCl_2$ produced similar trends, but the pH values were lower in $CaCl_2$ than in water. The highest pH with both methods was in the Bf horizon. The pH of the surface horizons of the CH forests was significantly higher than that of the HA in both water and $CaCl_2$. The pH in $CaCl_2$ of the H horizon of the CH was also significantly higher than that of the HA.

Table 6-1: The mean values and (standard deviations) of field moisture content, air-dry moisture content and pH in water and $CaCl_2$. A * indicates a statistically significant difference between the HA and CH forest types for a horizon at $p<0.05$. (n=9)

HOR.	FOR. TYPE	FIELD MOISTURE %	AIR-DRY MOISTURE %	pH IN WATER	pH IN $CaCl_2$
LF	HA	226 (120)	12.65 (0.84)	3.6(0.19)*	3.2(0.14)*
	CH	203 (122)	12.95 (1.18)	3.9(0.22)	3.6(0.27)
H	HA	230 (76)	13.39 (3.74)	3.4(0.11)	2.9(0.10)*
	CH	314 (149)	16.17 (6.06)	3.6(0.13)	3.1(0.19)
Bhf	HA	206 (109)	12.67 (10.17)	3.8(0.32)	3.2(0.32)
	CH	249 (152)	8.70 (4.69)	3.8(0.23)	3.2(0.17)
Bf	HA	97 (48)	13.53 (8.70)	4.6(0.31)	4.0(0.31)
	CH	84 (27)	10.44 (10.19)	4.5(0.32)	4.0(0.29)

The highest carbon concentrations were found in the organic horizons (Table 6-3). The HA forests had higher C concentrations in the Bhf and Bf horizons than the CH. However, the HORIZON*LOCATION{TYPE} term was also significant (Table 6-2), indicating that the locations used had significant differences in

Table 6-2: Analysis of variance table for field moisture, air-dry moisture, pH in water, pH in $CaCl_2$, total C, total N, C/N ratio, loss on ignition (LOI), and exchangeable Ca, Mg, Fe and Al. As a nested experimental design was used, the terms for the ANOVA were TYPE, HORIZON, LOCATION{TYPE}, which is location nested within forest type, TYPE*HORIZON, HORIZON*LOCATION{TYPE}, and multiple R^2. Shown here are the probabilities as calculated by the Systat statistical program. A * indicates statistical significance at $p<0.05$.

	TYPE	HORIZON	LOCATION {TYPE}	TYPE* HORIZON	HORIZON* LOCATION {TYPE}	MULT. R^2
Field Moisture	0.551	0.000	0.000	0.349	0.010	0.796
Air Dry Moisture	0.111	0.001	0.002	0.085	0.191	0.577
pH in Water	0.064	0.000	0.313	0.018*	0.052	0.833
pH in $CaCl_2$	0.003	0.000	0.005	0.003*	0.226	0.848
C	0.016	0.000	0.180	0.000*	0.006*	0.963
N	0.000	0.000	0.118	0.121	0.013	0.924
C/N	0.001	0.000	0.078	0.306	0.973	0.687
LOI	0.880	0.000	0.455	0.011*	0.055	0.946
Extr. Ca	0.666	0.000	0.003	0.890	0.243	0.795
Extr. Mg	0.001	0.000	0.000	0.135	0.013	0.871
Extr. Al	0.000	0.000	0.099	0.184	0.555	0.883
Extr. Fe	0.000	0.000	0.489	0.103	0.103	0.731

C, and thus did not fit the assumptions for the experimental design. Loss on ignition (LOI) was significantly higher in the Hhorizon of the CH forests than the HA, and was also higher, but not significantly so, in the LF horizons in the CH forests. In the Bhf and Bf horizons, LOI was generally higher on the HA sites. There were no significant differences between the HA and CH in total N concentration or C/N ratio (Tables 6-2 and 6-3). However, the HA forests generally had higher N concentrations in all horizons, and had a lower C/N ratio than the CH forests.

Table 6-3: The mean values and (standard deviations) of total carbon (%), total nitrogen (%), loss on ignition (LOI) and the C/N ratio. A * indicates a statistically significant difference between the HA and CH forest types for a horizon at $p<0.05$. (n=9)

HOR.	FOR. TYPE	C %	TOTAL N %	C/N	LOI
LF	HA	46.58 (5.10)	1.06 (0.18)	44.54 (4.80)	1975.4 (907.7)
	CH	48.05 (4.31)	0.92 (0.11)	52.56 (6.20)	2000.5 (612.2)
H	HA	40.85 (7.9)	1.01 (0.10)	40.53 (7.66)	1168.0* (921.3)
	CH	47.32 (6.2)	0.91 (0.16)	53.40 (11.59)	2014.4 (780.6)
Bhf	HA	20.03 (4.67)	0.77 (0.14)	26.09 (4.48)	142.1 (104.7)
	CH	14.32 (4.74)	0.54 (0.20)	29.78 (9.55)	87.8 (64.5)
Bf	HA	8.81 (2.55)	0.24 (0.06)	37.04 (4.99)	25.54 (6.41)
	CH	6.74 (1.21)	0.17 (0.04)	40.99 (8.25)	21.53 (4.53)

The total N concentrations were highest in the surface horizons and lowest in the Bf. The C/N ratio was lowest in the Bhf.

The concentrations of extractable Ca, Mg, Al and Fe are shown in Table 6-4, and the analysis of variance results may be found in Table 6-2. Generally, the CH contained more extractable Ca, while the HA contained more extractable Mg, Al and Fe. However, the variability was high and the differences were not significant.

Pyrophosphate-extracted Fe (Table 6-5) was generally higher in the HA than CH, but the results were not significant (Table 6-6).

Table 6-4: The mean values and (standard deviations) of extractable Ca, Mg, Fe and Al, all in mg/kg. A * indicates a statistically significant difference between the HA and CH forest types for a horizon at $p<0.05$. (n=9)

HOR.	FOR. TYPE	EXTR Ca (mg/kg)	EXTR Mg (mg/kg)	EXTR Al (mg/kg)	EXTR Fe (mg/kg)
LF	HA	1853.0 (307.3)	419.5 (78.6)	604.6 (155.4)	216.8 (73.7)
	CH	3354.3 (616.5)	372.7 (99.9)	274.4 (209.2)	112.4 (29.6)
H	HA	1660.9 (762.1)	532.9 (117.2)	956.5 (274.1)	309.0 (99.8)
	CH	2720.9 (1027.4)	441.3 (150.5)	355.8 (318.9)	127.3 (88.0)
Bhf	HA	945.0 (1045.2)	334.7 (207.5)	1336.9 (301.4)	442.5 (110.5)
	CH	1272.8 (1325.5)	194.6 (169.8)	1043.6 (406.4)	347.7 (166.8)
Bf	HA	39.4 (11.1)	55.9 (52.8)	2118.5 (515.4)	187.3 (117.0)
	CH	222.3 (286.1)	40.5 (39.9)	1917.7 (222.3)	156.3 (107.2)

Table 6-5: The mean values and (standard deviations) of pyrophosphate-extracted Fe, Al and Mn. A * indicates a statistically significant difference between the HA and CH forest types for a horizon at $p<0.05$. (n=9)

HOR.	FOR. TYPE	Fe-PYRO %	Al-PYRO %	Mn-PYRO %
LF	HA	0.041 (0.044)	0.089 (0.036)	0.030 (0.018)
	CH	0.010 (0.031)	0.051 (0.040)	0.063 (0.036)
H	HA	0.207 (0.219)	0.219 (0.157)	0.009 (0.012)
	CH	0.069 (0.184)	0.081 (0.157)	0.009 (0.012)
Bhf	HA	1.528 (0.662)	0.573 (0.149)	0.004 (0.003)
	CH	1.395 (0.934)	0.577 (0.234)	0.000 (0.000)
Bf	HA	1.442 (0.476)	1.559 (0.297)	0.002 (0.004)
	CH	0.910 (0.401)	1.188 (0.364)	0.000 (0.000)

Table 6-6: Analysis of variance table for pyrophosphate-extracted Fe, Al and Mn; citrate-dithionite-bicarbonate (CBD)-extracted Fe, Al and Mn; and acid ammonium oxalate (AAO)-extracted Fe and Al. As a nested experimental design was used, the terms for the ANOVA were TYPE, HORIZON, LOCATION{TYPE} (which is location nested within forest type), TYPE*HORIZON, HORIZON*LOCATION{TYPE}, and multiple R^2. Shown here are the probabilities as calculated by the Systat statistical program. A * indicates statistical significance at $p<0.05$.

	TYPE	HORIZON	LOCATION {TYPE}	TYPE* HORIZON	HORIZON* LOCATION {TYPE}	MULT. R^2
Fe AAO	0.206	0.000	0.200	0.104	0.292	0.634
Fe CBD	0.561	0.001	0.041	0.238	0.378	0.570
Fe Pyro	0.080	0.000	0.154	0.853	0.080	0.850
Al AAO	0.645	0.000	0.026	0.968	0.269	0.876
Al CBD	0.078	0.000	0.123	0.203	0.765	0.849
Al Pyro	0.014	0.000	0.552	0.331	0.831	0.909
Mn CBD	0.000	0.736	0.015	0.748	0.075	0.690
Mn Pyro	0.074	0.001	0.285	0.074	0.847	0.466

There was an increase in pyrophosphate-Fe and -Al with depth. Pyrophosphate-Al was higher in the HA forests in the LF, H and Bf horizons. Pyrophosphate-extracted Mn had high variability, and was highest in the forest floor. There were no significant differences in pyrophosphate-Mn between the CH and HA forests.

There were no significant differences between the HA and CH forests in citrate bicarbonate dithionite (CBD)-extracted Fe, Al or Mn (Table 6-7). The concentrations of Fe-CBD and Al-CBD were higher in the Bf horizon than the Bhf. The Mn-CBD results were highly variable. The percentages of Fe and Al extracted by acid ammonium oxalate (AAO) were also higher in the Bf than the Bhf (Table 6-7). The Bf horizons of the HA sites contained more Fe-AAO than the CH, but the difference was not significant at $p<0.05$

Table 6-7: The mean values and (standard deviations) for citrate-bicarbonate-dithionite (CBD)-extracted Fe, Al and Mn, and for acid ammonium oxalate (AAO)-extracted Fe and Al. The LF and H horizons were not extracted. A * indicates a statistically significant difference between the HA and CH forest types for a horizon at $p<0.05$. (n=9)

HOR.	FOR. TYPE	Fe-CBD %	Al-CBD %	Mn-CBD %	Fe-AAO %	Al-AAO %
Bhf	HA	1.802 (0.950)	0.525 (0.186)	0.009 (0.003)	1.235 (0.524)	0.560 (0.203)
	CH	2.013 (1.296)	0.477 (0.175)	0.001 (0.001)	1.283 (0.691)	0.519 (0.182)
Bf	HA	3.276 (0.437)	1.802 (0.442)	0.012 (0.013)	2.413 (0.342)	2.272 (0.576)
	CH	2.636 (1.111)	1.465 (0.385)	0.001 (0.001)	1.780 (0.479)	2.390 (1.097)

(Tables 6-6 and 6-7). There were no significant differences between CH and HA soils in Al-AAO.

Pyrophosphate, AAO and CBD each extract a different fraction of the soil Fe and Al. Pyrophosphate extracted the Fe and Al associated with organic matter. Acid ammonium oxalate (AAO) extracted both organic Al and Fe, and the amorphous Fe and Al oxides and hydroxides associated with allophane and imogolite. The CBD procedure extracted crystalline, amorphous and organic forms. A measure of the amorphous component was obtained by subtracting the pyrophosphate results from the AAO results. Subtracting the AAO concentrations from those obtained by CBD gave the crystalline Al and Fe. The results of these calculations are shown in Figure 6-1. There were no significant differences between the CH and HA forests in amorphous or crystalline Fe (Fig. 6-1A) or Al (Fig. 6-1B). There were, however, differences between the Bh and Bhf horizons. Iron associated with organic matter dominated both the Bhf and Bf horizons. There was no amorphous Fe in the Bhf, and relatively equal concentrations of crystalline and amorphous Fe in the Bf. Crystalline Al was not found in the Bhf or Bf horizons of either forest type. Only organic Al was present in the Bhf horizons.

The results from the analysis for P_A, P_T, P_O and the C/P ratio are displayed in Table 6-8. There were no significant differences between the two forest types for any of the horizons (Table 6-9). There was a decrease in P_A with depth in the soil profile, from a

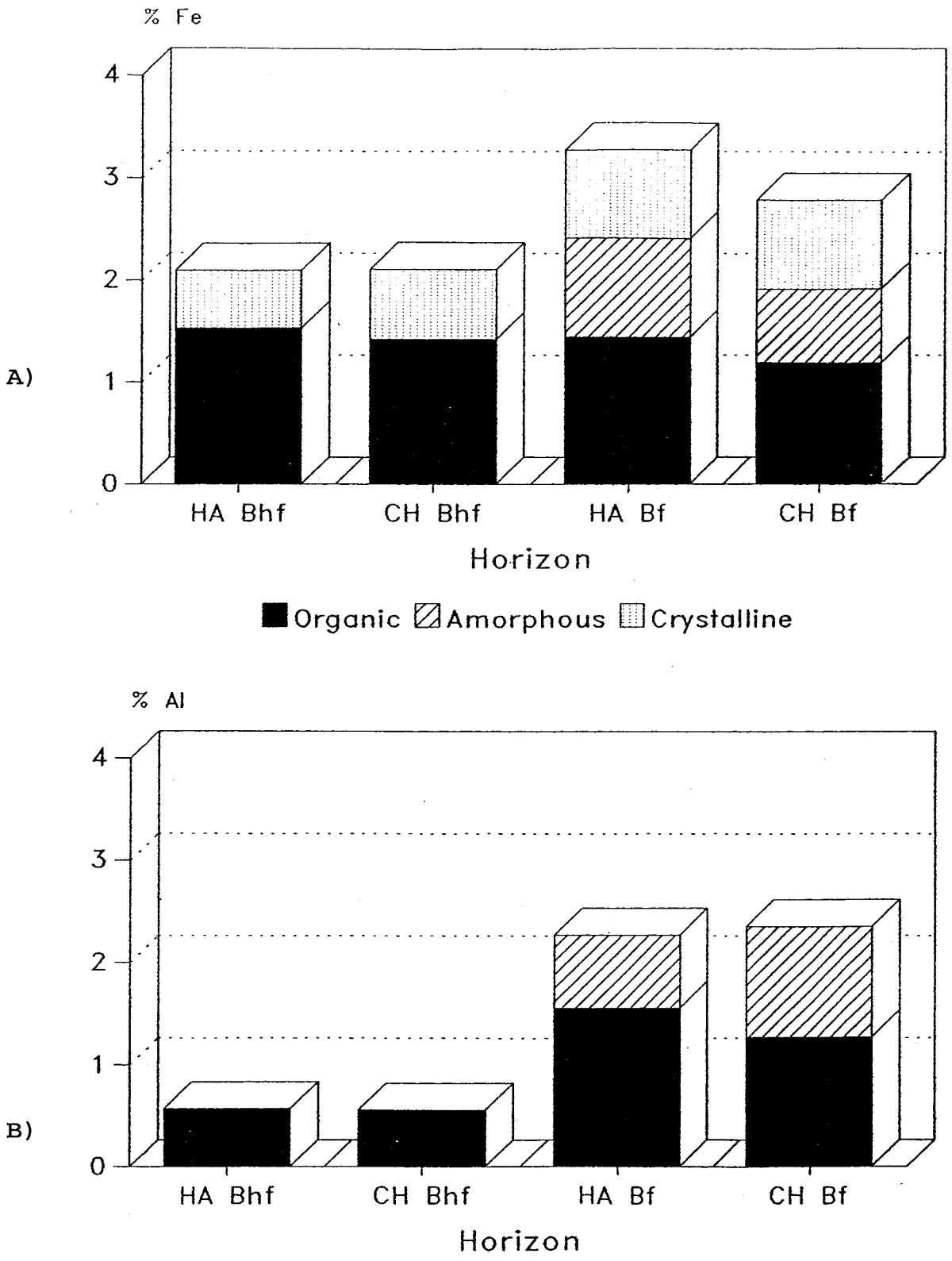


Figure 6-1: The mean percentages of organic, amorphous and crystalline iron (A) and aluminium (B) in the Bhf and Bf horizons of CH and HA forest types.

high of 38.2 mg/kg in the LF to 4.8 in the Bf. Both P_o and P_T were highest in the LF, and decreased with depth. In the LF and H horizons, the C/P was higher in the CH forests than the HA, but the difference was not significant. The C/P generally was higher in the surface horizons, and lower in the mineral soil.

The Chang and Jackson fractionations produced P_{HCl}, P_{NaOH}, and P_{CBD} (Table 6-10). No P was extracted by citrate bicarbonate (P_{CB}). The P_{HCl} concentrations were much lower than those for P_{NaOH} and P_{CBD}. There were no significant differences between the CH and HA

Table 6-8: The mean values and (standard deviations) of available P (Bray-extracted), total P (Parkinson & Allen digest), organic P (NaOH-EDTA extraction) and C/P ratio. A * indicates a statistically significant difference between the HA and CH forest types for a horizon at $p<0.05$. (n=9)

HOR.	FOR. TYPE	Avail. P (mg/kg)	Total P (mg/kg)	Org. P (mg/kg)	C/P
LF	HA	38.17 (6.19)	714.0 (67.45)	527.8 (162.0)	659 (102)
	CH	30.46 (10.01)	585.8 (66.59)	492.0 (98.8)	815 (135)
H	HA	20.55 (6.75)	561.4 (98.29)	283.8 (133.9)	736 (135)
	CH	20.62 (9.65)	482.8 (152.1)	308.7 (123.2)	1055 (389)
Bhf	HA	10.19 (7.02)	524.2 (110.5)	227.8 (110.3)	384 (67)
	CH	6.06 (2.92)	352.1 (89.55)	145.1 (89.21)	392 (152)
Bf	HA	4.80 (0.89)	361.0 (105.5)	99.59 (35.74)	256 (82)
	CH	6.31 (1.02)	289.2 (115.6)	64.78 (36.39)	252 (90)

forests. The P_{HCl} and P_{CBD} concentrations are highest in the Bf, while the P_{NaOH} is highest in the Bhf.

The percentage of P_T found as P_O and P_I, and the percent recovery of the P_T for each horizon and forest type are in Table 6-11. These are means calculated from each sample, and so are not completely additive. The P_I was calculated from the sum of P_{HCl}, P_{NaOH} and P_{CBD} extracted by the Chang and Jackson fractionation procedure. Inorganic P was not determined in the LF and H horizons, as a precipitate formed in highly organic samples. There was a decrease in P_O, and an increase in P_I, with soil depth. The

Table 6-9: Analysis of variance table for available P (Bray), organic P (NaOH-EDTA extraction), total P (Parkinson and Allen digest), P-HCl, P-NaOH and P-CBD (Chang & Jackson), and the C/P ratio. Because a nested experimental design was used, the terms for the ANOVA were TYPE, HORIZON, LOCATION{TYPE} (which is location nested within forest type), TYPE*HORIZON, HORIZON*LOCATION{TYPE}, and multiple R^2. Shown here are the probabilities as calculated by the Systat statistical program. A * indicates statistical significance at $p<0.05$.

	TYPE	HORIZON	LOCATION {TYPE}	TYPE* HORIZON	HORIZON* LOCATION {TYPE}	MULT. R^2
Avail P	0.171	0.000	0.028	0.242	0.198	0.872
Org P	0.293	0.000	0.763	0.641	0.564	0.779
Tot P	0.001	0.000	0.313	0.505	0.637	0.727
P-HCl	0.934	0.000	0.012	0.762	0.013	0.688
P-NaOH	0.580	0.000	0.000	0.116	0.000	0.858
P-CBD	0.012	0.000	0.814	0.339	0.589	0.823
C/P	0.021	0.000	0.931	0.056	0.891	0.808

Table 6-10: The mean values and (standard deviations) of P extracted by HCl, NaOH and citrate bicarbonate dithionite (CBD), during the Chang & Jackson fractionation procedure. The LF and H horizons were not extracted. A * indicates a statistically significant difference between the HA and CH forest types for a horizon at $p<0.05$. (n=9)

HOR.	FOR. TYPE	P-HCl (mg/kg)	P-NaOH (mg/kg)	P-CBD (mg/kg)
Bhf	HA	7 (0.5)	44 (15.4)	30 (9.8)
	CH	7 (0.5)	37 (28.0)	22 (17.0)
Bf	HA	16 (7.7)	23 (8.4)	96 (15.9)
	CH	19 (13.6)	25 (12.4)	74 (23.6)

Table 6-11: The means and (standard deviations) of total P found as organic and inorganic P, and the percentage of total P recovered. Organic P was determined with NaOH-EDTA extraction and total P by Parkinson & Allen digest. Inorganic P is the sum of the fractions determine by Chang & Jackson fractionation. N/A indicates not analyzed. (n=9)

HOR.	FOR. TYPE	ORG. P	INORG. P	% RECOVERY
LF	HA	73.4 (20.0)	N/A	73.4 (20.0)
	CH	83.3 (12.7)	N/A	83.3 (12.7)
H	HA	51.4 (23.3)	N/A	51.4 (23.2)
	CH	65.5 (28.3)	N/A	65.5 (28.3)
Bhf	HA	42.7 (17.4)	17 (6.1)	59 (15.7)
	CH	39.1 (17.9)	19 (10.7)	58 (22.1)
Bf	HA	27.5 (3.4)	39 (7.8)	67 (8.7)
	CH	23.9 (12.0)	44 (15.6)	68 (17.3)

percentage recovery ranged from 51.4% to 83.3%, but the variability was high.

Table 6-12 displays the correlation matrix. Extractable Al, and Al extracted by CBD, AAO and pyrophosphate all had high positive correlations with one another. Acid ammonium oxalate (AAO)-Al was also positively correlated with P_{HCl} and pH in water and $CaCl_2$, and was negatively correlated with extractable Fe. Aluminium extracted by CBD was positively correlated with P_{CBD} and both pH methods. There was a positive relationship between Fe extracted with CBD and AAO, and between extractable Al and P_{CBD}. Extractable Fe had a negative correlation with pH determined by either method. Loss on ignition was positively correlated with extractable Mg, C, total N and available P. There was a positive relationship between the values for the two methods for pH. Positive correlations were also seen with extractable Mg to Ca, and with Mn extracted by pyrophosphate to CBD. Total N correlated positively with C, extractable Mg, and P_o. There was a negative relationship between C and pH in $CaCl_2$, and a positive one with P_T to P_o.

The ^{31}P NMR spectra for the two HA soil profiles are in Figure 6-2 (A and B); those for the CH profiles are in Figure 6-3 (A and B). The percentage of P found within each class of compounds, calculated from the spectra by integration, is in Table 6-13. A guide for the interpretation of NMR spectra can be found in Chapter 5 (Table 5-2).

Table 6-12: Correlation matrix. Al- and Fe-AAO were extracted by acid ammonium oxalate, Al-, Fe-, and Mn-CBD by citrate bicarbonate dithionite. Al-, Fe-, and Mn-pyro by pyrophosphate. Avail P was extracted by Bray. Extr-Al, -Ca, -Fe, and -Mg were extracted by Mehlich. H20A is air dry moisture content; H2OF is field moisture content. Org P is by NaOH-EDTA extraction and Tot P is by Parkinson & Allen digest.

	Al-AAO	Al-CBD	Al-Pyro	AvailP	C	C/N	C/P	ExtrAl	ExtrCa	ExtrFe	ExtrMg	Fe-AAO	Fe-CBD	Fe-Pyro
Al-AAO	1.000													
Al-CBD	0.918	1.000												
Al-Pyro	0.885	0.945	1.000											
AvailP	-0.320	-0.397	-0.302	1.000										
C	-0.586	-0.589	-0.486	0.492	1.000									
C/N	0.690	0.616	0.568	-0.377	-0.453	1.000								
C/P	-0.118	-0.093	-0.073	-0.116	0.210	0.104	1.000							
ExtrAl	0.796	0.858	0.817	-0.237	-0.396	0.538	-0.120	1.000						
ExtrCa	-0.506	-0.575	-0.499	0.317	0.509	-0.499	0.007	-0.548	1.000					
ExtrFe	-0.713	-0.687	-0.661	0.148	0.661	-0.360	0.297	-0.499	0.221	1.000				
ExtrMg	-0.559	-0.594	-0.511	0.620	0.722	-0.603	0.033	-0.511	0.820	0.330	1.000			
Fe-AAO	0.434	0.613	0.509	-0.591	-0.534	0.449	0.106	0.573	-0.617	-0.117	-0.644	1.000		
Fe-CBD	0.373	0.558	0.411	-0.610	-0.473	0.452	0.206	0.431	-0.579	-0.067	-0.608	0.890	1.000	
Fe-Pyro	-0.321	-0.182	-0.159	-0.249	0.187	-0.053	0.439	-0.142	-0.212	0.667	-0.118	0.474	0.501	1.000
H20Air	0.059	0.095	0.137	0.119	0.151	-0.143	0.003	0.175	0.406	-0.206	0.385	-0.077	-0.123	-0.237
H2OF	-0.519	-0.540	-0.522	0.275	0.302	-0.676	-0.017	-0.517	0.472	0.196	0.495	-0.586	-0.495	-0.182
LOI	-0.504	-0.549	-0.452	0.824	0.794	-0.473	0.100	-0.414	0.628	0.413	0.822	-0.610	-0.577	-0.036
Mn-CBD	0.039	0.083	0.148	-0.015	0.003	-0.181	-0.188	0.127	-0.068	-0.107	0.126	0.164	0.047	0.101
Mn-Pyro	-0.179	-0.158	-0.140	0.108	0.158	-0.482	-0.051	-0.138	0.086	0.118	0.345	-0.060	-0.055	0.145
OrgP	-0.478	-0.520	-0.440	0.430	0.678	-0.523	-0.080	-0.298	0.381	0.451	0.552	-0.527	-0.573	-0.070
pHCaCl2	0.729	0.748	0.675	-0.399	-0.703	0.459	-0.335	0.645	-0.318	-0.752	-0.422	0.535	0.437	-0.276
pHH20	0.733	0.730	0.671	-0.357	-0.637	0.395	-0.392	0.677	-0.292	-0.760	-0.375	0.463	0.304	-0.382
P-HCl	0.824	0.644	0.589	-0.186	-0.394	0.590	-0.090	0.616	-0.309	-0.607	-0.378	0.209	0.164	-0.421
P-NaOH	-0.532	-0.519	-0.519	0.224	0.097	-0.474	-0.127	-0.509	0.194	0.316	0.274	-0.247	-0.260	0.016
P-CBD	0.666	0.768	0.670	-0.323	-0.570	0.508	-0.286	0.713	-0.391	-0.559	-0.465	0.637	0.490	-0.096
TotN	-0.684	-0.694	-0.604	0.581	0.934	-0.689	0.104	-0.527	0.64	0.603	0.852	-0.647	-0.584	0.092
TotP	-0.293	-0.354	-0.247	0.416	0.658	-0.393	-0.186	-0.131	0.087	0.524	0.362	-0.341	-0.401	0.184

	H20Air	H2OF	LOI	Mn-CBD	Mn-Pyro	OrgP	pHCaCl2	pHH20	P-HCl	P-NaOH	P-CBD	TotN	TotP
H20Air	1.000												
H2OF	0.187	1.000											
LOI	0.247	0.344	1.000										
Mn-CBD	0.193	-0.117	-0.052	1.000									
Mn-Pyro	0.183	0.218	0.111	0.715	1.000								
OrgP	0.080	0.451	0.494	0.097	0.111	1.000							
pHCaCl2	0.255	-0.449	-0.562	0.286	-0.058	-0.494	1.000						
pHH20	0.267	-0.394	-0.531	0.312	-0.031	-0.365	0.953	1.000					
P-HCl	0.216	-0.384	-0.318	0.113	-0.133	-0.323	0.634	0.661	1.000				
P-NaOH	-0.166	0.433	0.142	-0.067	0.237	0.182	-0.368	-0.321	-0.465	1.000			
P-CBD	0.130	-0.471	-0.427	0.199	-0.105	-0.427	0.692	0.673	0.507	-0.451	1.000		
TotN	0.251	0.531	0.840	0.052	0.382	0.706	-0.689	-0.622	-0.472	0.263	-0.621	1.000	
TotP	-0.041	0.215	0.477	0.202	0.262	0.738	-0.467	-0.337	-0.171	0.115	-0.332	0.631	1.000

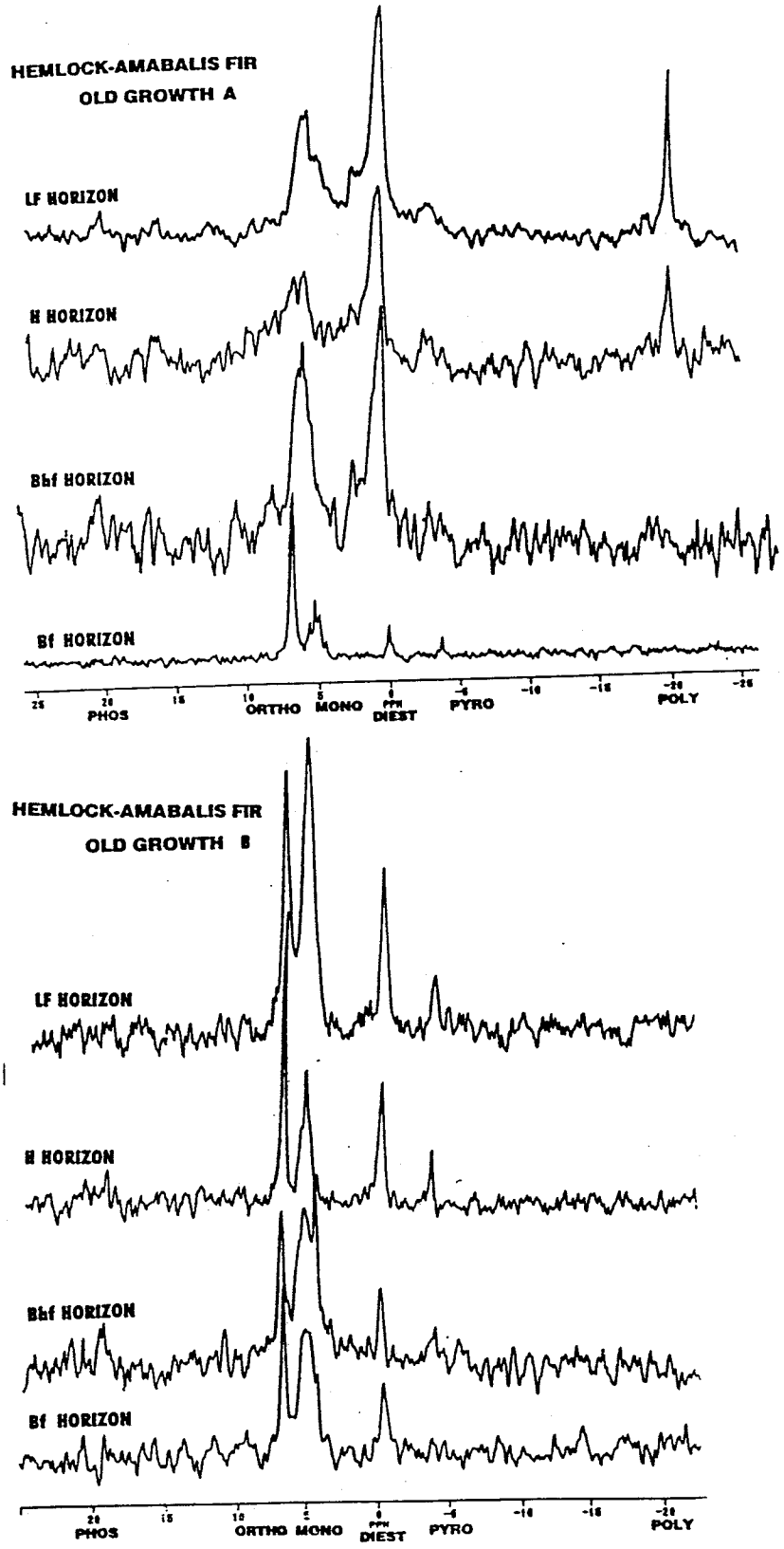


Figure 6-2: ^{31}P NMR spectra for two soil profiles from mature HA sites, extracted with NaOH-EDTA. Phos is phosphonate, ortho is orthophosphate, mono is monoester phosphate, diest is diester phosphate, pyro is pyrophosphate and poly is polyphosphate.

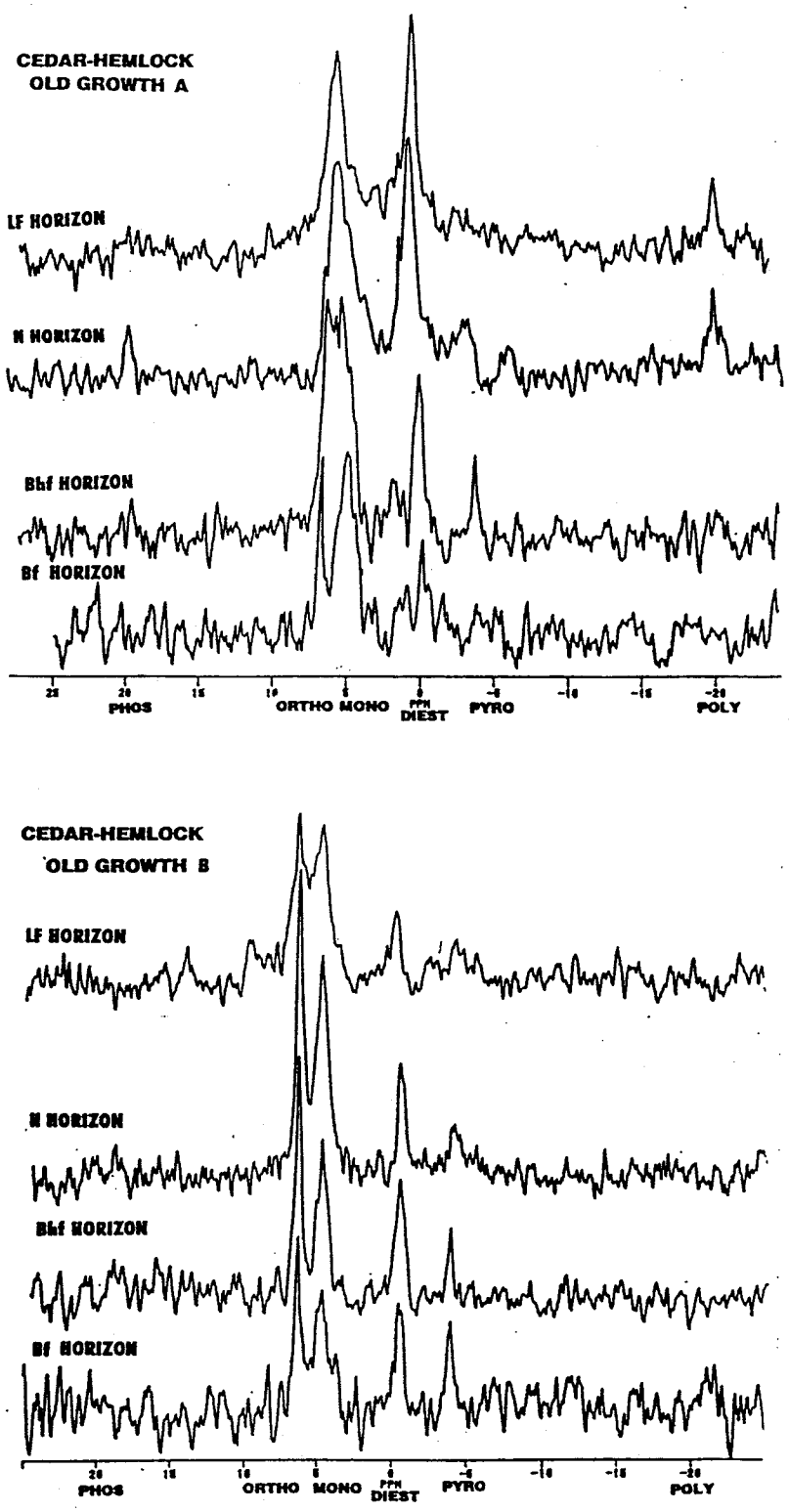


Figure 6-3: ^{31}P NMR spectra for two soil profiles from mature CH sites, extracted with NaOH-EDTA. Phos is phosphonate, ortho is orthophosphate, mono is monoester phosphate, diest is diester phosphate, pyro is pyrophosphate and poly is polyphosphate.

Table 6-13: The percentage of total P in solution found within each P form revealed by ^{31}P NMR spectroscopy. Phos is phosphonate, orth is orthophosphate, mono is monoester phosphate, dies is diester phosphate, pyro is pyrophosphate and poly is polyphosphate.

For. Type	Hor.	Phos	Orth	Mono	Dies	Pyro	Poly
HA A	LF	3	10	24	39	8	16
	H	0	20	20	44	0	16
	Bhf	4	17	29	50	0	0
	Bf	0	60	25	10	5	0
HA B	LF	0	33	45	17	5	0
	H	9	30	30	26	5	0
	Bhf	0	22	54	15	0	0
	Bf	0	31	51	18	0	0
CH A	LF	0	17	33	39	0	11
	H	5	7	38	36	5	7
	Bhf	4	38	36	17	5	0
	Bf	0	23	61	16	0	0
CH B	LF	0	31	38	18	13	0
	H	0	39	32	20	9	0
	Bhf	0	35	34	24	7	0
	Bf	0	29	32	24	15	0

In HA profile A (Fig. 6-2A; Table 6-13), the predominant P class in the LF, H and Bhf horizons was diester, and orthophosphate dominated in the Bf. The percentages of orthophosphate and monoester P were similar in the LF, H, and Bhf. There were small pyrophosphate peaks in the LF and Bf horizons, and very small phosphonate peaks in the LF and Bhf. Sharp polyphosphate peaks appeared in the LF and H. The orthophosphate and monoester peaks

were separated completely in only the Bf horizon. One problem with the extractant used in this study for ^{31}P NMR spectroscopy is that the orthophosphate and monoester peaks overlap in some spectra. As a valley between peaks occurs at 6 ppm when there is clear separation, this was chosen as the dividing line where overlapping occurred. This is discussed in more detail in Chapter 5.

Profile B for the HA (Fig. 6-2B, Table 6-13) was quite different from profile A. The orthophosphate and monoester peaks were distinct in all horizons. Monoester P dominated in all but the H horizon, where it was proportionally comparable to orthophosphate. Small pyrophosphate peaks occurred in the LF and H, and very small phosphonate peaks were found in the H and Bhf. There was no detectable polyphosphate in this profile.

The two CH soil profiles (Figs. 6-3A and 6-3B) were also quite different from one another. The orthophosphate and monoester peaks were well separated in only the Bf horizon in profile A, but were distinct in all horizons of profile B. In A, (Fig. 6-3A; Table 6-13), the percentages of monoester and diester P were comparable in the LF and H, and were nearly double that of orthophosphate P in these horizons. Orthophosphate and monoester were the dominant P classes in the Bhf; monoesters dominated in the Bf. There were small phosphonate and pyrophosphate peaks in the H and Bhf horizons, and polyphosphate peaks in the LF and H horizons. In CH profile B (Fig. 6-3B; Table 6-13), most of the P was found as orthophosphate or as monoesters, at comparable percentages.

Diester and pyrophosphate peaks also occurred, but there was no phosphonate or polyphosphate in any horizon.

Discussion

The pH results are comparable to other measurements in CH and HA forests (eg: de Montigny 1992; Keenan 1993; Prescott *et al.* 1994). The pH was significantly higher in the LF and H horizons of the CH forests than in the HA. Higher pH in soils under cedar than under other conifers has been noted in the literature (Stone 1975; Keenan 1993), and is thought to be due to the nature of cedar foliage. The high negative correlation of C to pH in $CaCl_2$ indicates that the acidity of these soils is due to organic matter. Schnitzer (1977) has suggested that much of the total acidity of cool, temperate acid soils is due to oxygen-containing functional groups associated with organic matter, and ^{13}C NMR spectra show that COOH groups are present in the non-woody organic horizons of the CH and HA forests (deMontigny *et al.* 1993). The increased pH in the Bf horizons is typical of podzols in this region (Lewis 1976) and reflects in part the lower organic matter content of these horizons.

Carbon and loss on ignition (LOI) both decrease with depth in the soil profile. There is a high positive correlation between LOI and C. Loss on ignition is a measure of soil organic matter content, although it may include C from carbonates, water and hydroxyl groups from clays, and other volatiles in the soil (Kalra

and Maynard 1991). The C values are comparable to those obtained by de Montigny (1992), Keenan (1993) and Prescott *et al.* (1994). The increased C in the LF and H horizons of CH forests supports the theory that decomposition is less effective on these sites (de Montigny *et al.* 1993; Prescott *et al.* 1995). Carbon-13 NMR spectroscopy revealed a higher ratio of carbohydrate to lignin C in CH samples, and higher levels of lipids and total and labile polysaccharides (de Montigny 1992; de Montigny *et al.* 1993). The change in the Bhf and Bf horizons, with the C content significantly higher in the HA forests, suggests increased mixing of organic and mineral material. The HA forests are known to maintain a higher abundance and biomass of soil fauna than do CH forests (Battigelli *et al.* 1994), although the abundance of fauna which would mix organic and mineral soils, such as earthworms, is low in both forest types. This higher C concentration may also be due to increased illuviation of organic compounds through the soil profile.

The high positive correlation of total N to C and to LOI indicates that much of the nitrogen on these sites is associated with organic matter. In general, there is more total N in the HA for all horizons, but the differences were not significant. These results agree with those found by others in the SCHIRP project (Germain 1985; de Montigny 1992; Keenan 1993; Prescott *et al.* 1993; Prescott *et al.* 1995). Cedar had consistently lower foliar N concentrations and a significantly higher rate of N resorption than

western hemlock or amabilis fir (Keenan 1993) which in turn was expressed as lower N concentrations in the soil organic matter of CH forests. The C/N ratio was wider in the CH than HA forests for all horizons, but the difference was not significant. Keenan (1993) also reported a drop in the C/N ratio in the Bhf horizon, relative to the other horizons. The percentage of carbon dropped by nearly half in the Bhf horizons of both CH and HA forests, but the change in nitrogen is not nearly as precipitous. The C levels dropped again in the Bf, but the N concentrations do also, which raises the C/N ratio. Jenkinson (1988) attributes the drop in the C/N ratio when moving from organic to mineral horizons to the presence of fixed NH_4 in clays. There appears to be more NH_4 fixation at the interface between the organic and mineral layers in the podzols of the SCHIRP site than lower in the profile at the Bf horizon.

Extractable Ca and Mg were measured by a different extraction procedure from the ammonium acetate procedure used by de Montigny (1992) and Keenan (1993). de Montigny (1992) found significantly more Ca in the F horizon of CH than HA, but no significant differences for Mg. Keenan (1993) found increased Ca in the mineral horizons of some, but not all, of the CH sites which he examined, and the differences were not statistically significant. He did not measure Mg. Cedar is known to be a Ca accumulator species (Krajina 1969; Ballard and Carter 1986) relative to associated tree species. Consequently, the higher Ca on the CH

sites is due to the incorporation of cedar foliage into the soil organic matter. The high positive correlations of extractable Mg to LOI and total N suggest that it is also a component of soil organic matter.

Iron and Al were quantified thoroughly because of their known role in P retention and cycling in podzolic soils (Sanborn 1987; Yuan and Lavkulich 1994). The only Fe and Al results reported by SCHIRP researchers were those of Keenan (1993), who found significantly higher pyrophosphate Fe in the Bhf of HA soils than CH, and no significant differences in pyrophosphate Al. His reported values are comparable to those found in this study. The predominance of organic-associated Fe and Al reflects the characteristic illuviation of organic matter and organo-metallic complexes in podzolic soils (Oades 1989). The presence of crystalline Fe but not Al is typical of podzols on Vancouver Island (Lewis 1976; Sanborn 1987). The amorphous Fe and Al in the Bf horizons reflects the volcanic parent material of this region. The positive correlation of AAO-Al to pH and C indicates that the partitioning of Al between organically complexed and allophanic forms is controlled by pH and soil organic matter content, an observation also made by Sanborn (1987). The higher organic Al in the HA Bf than in the CH Bf may reflect a number of factors: tree species differences in Al content; differences in the organic ligands transporting the Al to the Bf horizon (Keenan 1993); or faunal activity, which is higher in the HA forests, and which may

alter the illuviation patterns (Sanborn 1987). The finding of Keenan (1993) of significantly more pyrophosphate-Fe on the HA sites, but no difference in the Al, whereas this study found the reverse is probably due to the high spatial variability in these forests.

The correlation of all of the Al extraction procedures with one another suggests that these methods are less selective for particular Al fractions than they are for Fe fractions (Sanborn 1987), and also reflects the more limited number of Al forms relative to Fe forms in these soils.

The Mn results were highly variable, as noted by de Montigny (1992), and were highest in the LF and H horizons.

There were no significant differences between the CH and HA forest types for any of the P measurements. The HA forests were generally higher in available P (P_A), total P (P_T) and organic P (P_O). The P_A results are comparable to those of de Montigny (1992), but are much higher than those of Keenan (1993) for the Bhf, and Prescott *et al.* (1995) for the LF. This probably reflects methodological differences, such as length of extraction. Prescott *et al.* (1995) found significantly lower concentrations of P_A in the L and H layers of CH forests; de Montigny (1992) in the H of CH forests. The P_T results are comparable to those of Keenan (1993) and Prescott *et al.* (1995), although Prescott *et al.* (1995) found significantly more P_T in the L horizon of the HA forests. Organic P was not measured by other SCHIRP researchers.

The lack of significant differences in any kind of phosphorus between the CH and HA forests for any horizon suggests that the two forest types are not inherently different in P contents and cycling. However, differences may be masked by the high variability in all measured P concentrations, and as shown by the ^{31}P NMR spectroscopy. This variability might have been reduced by more intensive sampling.

The cycling of P in these forests occurs mainly within the internal (biological) cycle (Dighton and Boddy 1988). The external (geological) cycle is only of any importance in the Bf horizon. In the LF, virtually all of the soil phosphorus is found as P_o. The P_A is highest in this horizon, indicating a high level of labile, easily leached P. This horizon is most influenced by the P forms and contents of litterfall. The HA has slightly higher concentrations of P_o and P_T, which may be explained by the greater resorption of P by cedar than by western hemlock or amabilis fir (Keenan 1993). The diversity of P forms, as shown by ^{31}P-NMR also reflects the influence of litter (Compton 1994). Diester P in plant tissues occurs as phospholipids in cell walls, and as nucleic acids. Plant monoesters consist of inositol phosphates, mononucleotides and sugar phosphates, which are metabolic intermediaries (Bieleski 1973). ADP and ATP are involved in energy transfer, but are not seen in ^{31}P-NMR spectra of extracted soil samples, probably because they are found in such low levels in these soils. It is unlikely that they were destroyed during the

extraction process, because added ATP was readily recovered during the extraction trial (Chapter 6). Some higher plants may store excess P as polyphosphates (Bieleski 1973). These compounds are also found in soil organisms, which also produce P compounds not found in plants. Pyrophosphate is believed to be involved in biological P cycling in the soil, and may be present as an organic ester which is hydrolysed during extraction for NMR analysis (Condron *et al.* 1985). Fungi and other microbes have been shown to store excess P in polyphosphates (Bieleski 1973), while ectomycorrhizal fungi are thought to transport P within their hyphae as polyphosphate (MacFall *et al.* 1992). The polyphosphates of the LF and H horizons are most likely from the mycorrhizae associated with trees on these sites.

Phosphonates are formed by a variety of soil microbes (Hilderbrand 1983), and are thought to accumulate under acid conditions, where bacteria containing phosphonatase enzymes are low in number (Hawkes *et al.* 1984). Thus, they are characteristic of cool moist acidic soils, such as those in CH and HA forests. The presence of pyrophosphate is also thought to be a marker for restricted biological decomposition (Preston *et al.* 1986). Acidic conditions generally suppress bacteria and actinomycetes, so that decomposition is primarily fungal (Harris 1988). Fungal mats are common in the F layers of these forests (de Montigny 1992; personal observation). Fungal decomposition is slower than bacterial (Dighton and Boddy 1988), and may be responsible for the wide

variety of P forms seen in the LF and H horizons of these sites. The relatively high C/N and C/P ratios are also indicative of slower decomposition. Although John *et al.* (1965) did not find a direct relationship between C and P_o when examining P_o in a range of BC soils (agricultural), C is correlated with P_o in these forests, which also suggests reduced decomposition.

The lower P_A concentrations in the H horizon, relative to the LF, are due to increased leaching of labile P compounds from the H horizon. The increased humification of this horizon relative to the LF is shown by the lower P_o concentration and the reduced percent recovery. Differences in the methods used to determine P_o and P_T also contribute to the lower recovery rate: the higher temperature of the P_T digest releases P from aromatic groups and humic substances, which would not occur in the lower temperature P_o extraction (Kristensen 1990). In the H horizon, the P forms shown by ^{31}P-NMR spectroscopy are similar to those seen in the LF. There was more phosphonate and pyrophosphate in this horizon in some of the profiles, showing that P in this horizon is more influenced by soil microbes, and less by the kind of litterfall. The diesters in this horizon are probably lipids, which de Montigny (1992) also found in the forest floor of these forests using ^{13}C-NMR spectroscopy. Lipids are usually considered to be quite labile in soil (Stevenson 1986); their accumulation is indicative of slow decomposition, as is the high C/P ratio. Decomposition is usually lower in the H than the LF, as the more labile materials are no

longer available. However, more nutrients are mineralized per C atom in the H than in the LF (Hart *et al.* 1994).

In the Bhf horizon, the external P cycle begins to influence P forms. The Chang and Jackson fractionation shows the presence of inorganic P forms: P_{NaOH}, P_{CBD} and P_{HCl}. P_{NaOH} is thought to be the non-occluded phosphate bound to the surfaces of Al or Fe hydrous oxides (Olsen and Sommers 1982). The P_{CBD} fraction is comprised of P occluded within the matrices of Fe and Al oxides and hydrous oxides, while the P_{HCl} is thought to be the extracted calcium phosphates of the non-occluded apatite fraction (Williams *et al.* 1980; Olsen and Sommers 1982). In the Bhf horizon of these forests, P_{NaOH} is higher than P_{CBD} and P_{HCl}, suggesting that P_I is non-occluded, most likely with organically associated Fe, as suggested by the positive correlation. The diversity of P forms is reduced relative to the forest floor, with orthophosphate and monoester phosphate predominating. The accumulation of monoesters in the Bhf and Bf is due to the adsorption of inositol phosphate onto surface hydroxyls of soil colloids by ligand exchange mechanisms (Ognalaga *et al.* 1994), which hampers their biodegradation. The diesters and other monoesters do not appear to adsorb to soil colloids, and thus are lost by leaching and degradation. Some diester phosphates are retained in the Bhf and Bf of the CH and HA forests, perhaps by Al or Fe bridges to humic substances (Gerke 1992). Pyrophosphate is also present in some Bhf and Bf horizons. The high affinity of pyrophosphate for organically-bound Fe and Al has led to its use as

an extractant. Therefore, it is possible that, in these horizons, pyrophosphate is linked to Al- and/or Fe-organic matter complexes.

In the Bf horizon, P_{CBD} is the largest inorganic P fraction, and it correlates strongly to amorphous Al and Fe. This suggests that much of the P in this horizon is occluded, and is sequestered in amorphous sesquioxides. This is typical of the podzolic Bf horizon (Sanborn 1987; Yuan and Lavkulich 1994). It would appear from the increased P_{HCl} fractions in the Bf relative to the Bhf that apatite is present in this horizon. This is somewhat unusual in that apatite should weather under acidic conditions. Lindsay (1979) reports that brushite ($CaHPO_4.2H_2O$) and monetite ($CaHPO_4$) are readily formed under acid conditions. Thus the P_{HCl} fraction attributed by most authors to apatite may be either brushite or monetite. In addition, the strong correlation of P_{HCl} to AAO-extracted Al suggests that P sorbed to amorphous Al may have been extracted instead of, or in addition to, calcium phosphate. Orthophosphate and monoester phosphate are the main P forms revealed by ^{31}P-NMR spectroscopy. Fares *et al.* (1974) have suggested that P_o does not actually occur in B horizons, but that the P_o determined by chemical procedures is instead inorganic P bridged to humic substances. The results from ^{31}P-NMR spectroscopy show that this is likely for some of the P, as orthophosphate is the predominant P form in the Bf, but organic P compounds are also present in these horizons.

The ^{31}P-NMR results reveal that P forms are quite specific to

a particular microsite, as no obvious trends for the CH or HA forest types were observed. In retrospect, better results might have been obtained by using composite samples. Although a different extractant was used in this study (see Chapter 6), the results are comparable to others examining P forms in forest soils with ^{31}P-NMR spectroscopy. The low pH of forest soils has been shown to favour a wider variety of P species than are seen in agricultural soils (Preston *et al.* 1986), with the persistence and accumulation of relatively labile compounds such as diesters (Tate and Newman 1982; Condron *et al.* 1990a; Gil-Sotres *et al.* 1990).

Conclusions

Although this study sampled only Orthic Ferro-Humic Podzols, the results from the general chemical analyses are very similar to those obtained by other SCHIRP researchers, who did not restrict their sampling to only Orthic Ferro-Humic Podzols, sampling all of the soil types present on these sites. No significant differences in phosphorus forms or concentrations were found between the CH and HA forest types, suggesting that P-related nutrient cycling problems after logging and slash burning are not due to inherent site differences in soil P. However, the high variability in all measured P forms may have masked statistical differences in P between the forest types. More intensive sampling may be required to reveal differences P on these sites, or these soils may not be inherently different in soil P concentrations or levels.

The diversity of P forms as revealed by ^{31}P-NMR spectroscopy was typical of cool, moist acidic forests, as was the persistence of the very labile diester phosphates throughout the soil profile, albeit at very low levels in the mineral horizons. This, together with the relatively high C/P ratio, indicates that decomposition is slow. Most of the P in the LF is in organic forms typical of litterfall. In the H horizon there is more humification, and P forms associated with soil organisms are seen. In the Bhf, inorganic phosphorus is predominantly non-occluded. Organic P is present, mainly as monoester phosphates which are probably adsorbed on soil colloids. In the Bf, most of the P is occluded in amorphous sesquioxides, and there are low levels of organic P, mainly as monoester phosphates.

CHAPTER SEVEN

A COMPARISON OF PHOSPHORUS FORMS ON CH SITES AFTER BURNING

Introduction

The lack of significant differences in phosphorus forms or concentrations between the CH and HA forest types (Chapter 6) suggests that the P-related growth check observed in trees on the CH sites five to eight years after cutting and replanting is not due to inherent differences in P between the CH and HA forest types. To achieve more rapid regeneration on the CH and HA sites and to allow planter access, slash-burning is used to reduce slash accumulations and to control the heavy cover of the ericaceous shrub salal, especially in the CH forests (Prescott and Weetman 1994). Burning is known to affect forest soils, and the general effects of fire have been summarized in several reviews (Ahlgren and Ahlgren 1960; Kozlowski and Ahlgren 1974; Feller 1982). During a fire, the nutrients incorporated in vegetation, litter and soil can potentially be volatilized during pyrolysis or combustion, mineralized during oxidation, or lost by ash convection (Grier 1975). After a fire is out, nutrients may be redistributed by wind and water erosion or by leaching of the ash layer and soil. Some frequently observed nutrient effects include: increases in soil pH; increases in the availability of P, Ca and Mg; and decreases in total N and S (Ahlgren and Ahlgren 1960; Kozlowski and Ahlgren 1974; Feller 1982; Ellis and Graley 1983; Feller *et al.* 1983;

Khanna and Raison 1986; Macadam 1987; Tomkins *et al.* 1991; Brockley *et al.* 1992; Mangas *et al.* 1992; Rice 1993; Romanya *et al.* 1994). These nutrient changes generally occur in the forest floor and surface soil; the changes at depth in the soil profile are less and occur more slowly as nutrients are leached down (Feller 1982; Brockley *et al.* 1992). The magnitude of these effects will depend on fire severity, site and soil characteristics, and fire intensity and duration (Brockley *et al.* 1992).

In forests, P is tightly conserved. The P cycle is virtually closed, with most plant P recycled by microbial breakdown of litter and organic matter. Fire is one of the few sources of P loss in forests, during prescribed burning or wildfires. Many researchers have reported large increases in available P in surface horizons immediately after fire, but these are short term increases that can produce long term losses which may reduce forest productivity (DeBano and Klopatek 1988; Saa *et al.* 1993; Romanya *et al.* 1994). Most studies of burning have investigated changes in available P; little is known about the effects of fire on other P forms, or the changes in P levels and forms which may occur with time after burning.

The objective of the research in this chapter was to compare the soil phosphorus and related soil chemistry of cut CH stands 10 years, 5 years and immediately after burning to old growth CH stands. Concentrations of total, organic and available P were determined by extraction and digestion procedures, while

fractionation and ^{31}P nuclear magnetic resonance (NMR) spectroscopy were used to characterize the P forms. Some aspects of the chemistry of these soils which could influence P forms and levels were also examined.

Materials and Methods

Sample Collection

The study sites and sampling design were described in Chapter 3, and the procedures for sample collection and preparation were described in Chapter 6. The CH old growth (OG) samples used in this chapter are the same ones which were used in Chapter 6. The 0-year sites were sampled within one month of burning, with little rainfall between the times of burning and sampling. The 5-year and 10-year sites were sampled 5 and 10 years postburn, respectively. The LF, H, Bhf and Bf horizons were collected from each pit, except from the 0-year sites, where an ash layer was collected for the LF horizon. Three locations per age were sampled, with 3 pits per location, for a total of 9 samples per horizon per age. The samples were collected in late July and early August, 1992.

General Chemical Analysis

The analytical procedures used were described in Chapter 6.

P Methodology

The analytical procedures used were described in Chapter 6. A comparison of the methods used in this chapter to other soil P methods is found in Chapter 4.

NMR Spectroscopy

Two soil profiles for each of the old growth and 0-, 5- and 10-year postburn sites were chosen for analysis by ^{31}P NMR spectroscopy. The criteria for sample selection are listed in Chapter 6. The extraction, sample preparation and analytical procedures for ^{31}P NMR spectroscopy are found in Chapter 5.

Statistical Analysis

Statistical analyses were conducted using the Systat program (Wilkinson 1990) to perform analysis of variance test at $p<0.05$, using a nested design (Hicks 1982), with location nested within each forest type, as was described in Chapter 4. The model statement used was: VARIABLE = CONSTANT + AGE + HORIZON + LOCATION{AGE} + TYPE*HORIZON + HORIZON*LOCATION{AGE}. Pearson pairwise correlations and Tukey's HSD tests were also conducted with the Systat program. Homogeneity of variance was tested by plotting residuals against estimates. Log and log (n+1) transformations were performed where necessary.

Results

The results from the analysis for field moisture, air-dry moisture, pH in water and pH in $CaCl_2$ are shown in Table 7-1. Table 7-2 displays the analysis of variance results for these data. The field moisture content was significantly lower in the LF of all of the postburn sites relative to the old growth sites. As noted in Chapter 6, the field moisture content was relatively uniform in

Table 7-1: Mean values and (standard deviations) for field moisture content, air-dry moisture content and pH in water and $CaCl_2$. Different letters indicate statistical significance among the ages for a horizon at $p<0.05$. OG is old growth (n=9)

HOR.	AGE	FIELD MOISTURE %	AIR DRY MOISTURE %	pH IN WATER	pH IN $CaCl_2$
LF	OG	203 (122) a	12.95 (1.18) a	3.90 (0.22) b	3.60 (0.27) b
	0-YR	67 (45) b	12.57 (2.58) a	4.96 (0.45) a	4.58 (0.40) a
	5-YR	106 (132) b	12.95 (0.77) a	4.27 (0.32) b	3.81 (0.35) b
	10-YR	80 (94) b	11.97 (1.24) a	4.16 (0.42) b	3.66 (0.39) b
H	OG	314 (149) a	16.17 (6.06) a	3.59 (0.13) b	3.12 (0.19) b
	0-YR	275 (85) a	24.17 (12.46) a	4.36 (0.61) a	3.76 (0.65) a
	5-YR	249 (87) a	17.04 (6.30) a	3.96 (0.32) a	3.32 (0.31) ab
	10-YR	188 (116) a	17.62 (10.29) a	3.90 (0.36) ab	3.41 (0.23) ab
Bhf	OG	249 (152) a	8.70 (4.69) a	3.75 (0.23) a	3.23 (0.17) a
	0-YR	276 (93) a	9.25 (5.03) a	4.23 (0.49) a	3.67 (0.41) a
	5-YR	183 (99) a	10.90 (4.06) a	4.09 (0.24) a	3.36 (0.30) a
	10-YR	207 (211) a	8.64 (5.05) a	3.79 (0.24) a	3.26 (0.16) a
Bf	OG	84 (27) a	10.44 (10.19) a	4.45 (0.32) a	3.96 (0.29) a
	0-YR	86 (35) a	15.56 (7.09) a	4.81 (0.30) ab	4.31 (0.23) a
	5-YR	120 (99) a	16.35 (8.78) a	4.93 (0.20) ab	4.28 (0.35) a
	10-YR	53 (10) a	9.39 (3.96) a	4.94 (0.21) b	4.61 (0.21) a

Table 7-2: Analysis of variance (ANOVA) table for field moisture, air-dry moisture, pH in water, pH in $CaCl_2$, total C, total N, C/N ratio, loss on ignition (LOI), and extractable Ca, Mg, Fe and Al, showing the probabilities as calculated by the Systat statistical program. As a nested experimental design was used, the terms for the ANOVA were AGE, HORIZON, LOCATION{AGE},which is location nested within forest type, AGE*HORIZON, HORIZON*LOCATION{AGE}, and multiple R^2. A * indicates statistical significance at $p<0.05$.

	AGE	HORIZON	LOCATION {AGE}	AGE* HORIZON	HORIZON* LOCATION {AGE}	MULT. R^2
Air-Dry Moisture	0.015	0.000	0.349	0.152	0.645	0.513
Field Moisture	0.000	0.000	0.000	0.012*	0.007*	0.781
pH in Water	0.000	0.000	0.000	0.002*	0.977	0.769
pH in $CaCl_2$	0.000	0.000	0.000	0.000*	0.959	0.805
C	0.034	0.000	0.218	0.192	0.362	0.905
N	0.023	0.000	0.000	0.696	0.145	0.879
C/N	0.075	0.010	0.073	0.511	0.893	0.376
LOI	0.000	0.000	0.003	0.055	0.119	0.924
Extr. Ca	0.010	0.000	0.001	0.097	0.324	0.827
Extr. Mg	0.000	0.000	0.000	0.007*	0.001*	0.884
Extr. Al	0.000	0.000	0.338	0.652	0.987	0.482
Extr. Fe	0.238	0.000	0.864	0.400	0.411	0.523

the H and Bhf horizons, but dropped in the Bf. There were no significant differences in moisture content among the sites in these horizons. The air-dry moisture content was relatively uniform for all horizons, with no significant differences among the sites.

There were significant differences in pH among the sites, with similar trends when pH was measured in water or $CaCl_2$. At the 0-year sites, the pH of the LF was significantly higher than that of the other ages. The pH was also higher in the LF of the 5- and 10-year postburn sites than in the old growth samples, but the difference was not significant. This same pattern was seen in the H horizon, with increased pH values in all postburn samples relative to the old growth, but with only the values of the 0-year samples significantly different. There were no significant differences in pH in the Bhf, but the values were still higher in the postburn soils than in those from the old growth. In the Bf, the pattern changed. The highest pH values were found 10 years after burning, and these were significantly different from those of the old growth when pH was measured in water.

There were no significant differences among the ages in any horizon for total carbon, total nitrogen or the C/N ratio (Tables 7-2, 7-3). Generally, the C content was lower in the 0-year LF than in the LF of the other ages. The C concentration was lower in all of the postburn samples than in the old growth samples in the LF and H horizons, but was higher in the postburn samples in the Bhf. In the Bf, the lowest C concentration was found in the 10-year postburn sites. Total N concentration was lowest on the 0-year sites in the LF horizon, but for the H, Bhf and Bf horizons it was lowest in the 10-year postburn samples. The C/N ratio was widest in the old growth stand in the LF and H horizons. In the Bhf and Bf horizons, it was widest on the 10-year postburn sites.

Table 7-3: The mean values and (standard deviations) for total carbon (%), total nitrogen (%), loss on ignition (LOI) and the C/N ratio. Different letters indicate statistically significant differences among the ages for a horizon at $p<0.05$. OG is old growth. (n=9)

HOR	AGE	C %	TOTAL N %	C/N	LOI
LF	OG	48.05 (4.31) a	0.92 (0.11) a	52.56 (6.20) a	2001 (612) a
	0-YR	35.50 (11.48) a	0.79 (0.17) a	46.34 (16.28) a	694 (588) a
	5-YR	43.85 (5.49) a	0.96 (0.15) a	46.38 (7.69) a	1381 (567) a
	10-YR	39.05 (8.30) a	0.82 (0.20) a	48.46 (5.49) a	774 (597) a
H	OG	47.32 (6.20) a	0.91 (0.16) a	53.40 (11.59) a	2014 (780) a
	0-YR	41.55 (9.70) a	0.93 (0.14) a	45.95 (15.11) a	1512 (1845) a
	5-YR	40.98 (13.90) a	0.90 (0.22) a	47.16 (19.44) a	1549 (851) a
	10-YR	41.46 (4.69) a	0.84 (0.21) a	51.00 (10.05) a	823 (540) a
Bhf	OG	14.32 (4.74) a	0.54 (0.20) a	29.78 (9.55) a	88 (65) a
	0-YR	15.07 (7.01) a	0.51 (0.35) a	34.17 (9.53) a	88 (79) a
	5-YR	19.41 (13.43) a	0.57 (0.22) a	34.87 (21.40) a	114 (79) a
	10-YR	16.67 (6.31) a	0.41 (0.17) a	53.29 (50.78) a	99 (75) a
Bf	OG	6.74 (1.21) a	0.17 (0.04) a	40.99 (8.25) a	22 (5) a
	0-YR	6.19 (2.71) a	0.14 (0.07) a	44.19 (10.47) a	21 (9) a
	5-YR	5.14 (2.56) a	0.13 (0.08) a	40.62 (10.30) a	18 (9) a
	10-YR	4.08 (2.15) a	0.08 (0.04) a	53.69 (22.37) a	13 (3) a

Table 7-4: The mean values and (standard deviations) for extractable Ca, Mg, Fe and Al, all in mg/kg. Different letters indicate statistically significant differences among the ages for a horizon at $p<0.05$. OG is old growth. (n=9)

HOR.	AGE	EXTR Ca (mg/kg)	EXTR Mg (mg/kg)	EXTR Al (mg/kg)	EXTR Fe (mg/kg)
LF	OG	3354.3 (616.5) a	372.7 (99.9) a	274.4 (209.2) a	112.4 (29.6) a
	0-YR	4366.4 (2145.5) a	662.4 (64.5) b	621.0 (318.7) a	200.4 (64.9) a
	5-YR	4631.8 (1342.8) a	586.7 (70.2) b	455.9 (274.8) a	159.3 (61.4) a
	10-YR	3331.7 (1746.1) a	574.4 (101.1) b	690.9 (439.8) a	239.7 (106.1) a
H	OG	2720.9 (1027.4) a	441.3 (150.5) a	355.8 (318.9) a	127.3 (88.0) a
	0-YR	2814.7 (1448.3) a	652.8 (85.9) b	742.1 (265.4) a	189.7 (91.0) a
	5-YR	2550.0 (934.6) a	552.8 (129.2) ab	696.4 (473.4) a	275.1 (381.7) a
	10-YR	2189.5 (1236.9) a	599.4 (137.2) b	813.2 (358.4) a	267.6 (106.1) a
Bhf	OG	1272.8 (1325.5) a	194.6 (169.8) a	1043.6 (406.4) a	347.7 (166.8) a
	0-YR	1303.6 (1243.8) a	301.2 (188.5) a	1198.6 (306.7) a	421.3 (81.3) a
	5-YR	847.7 (1253.4) a	266.7 (171.3) a	1162.8 (455.7) a	366.0 (139.1) a
	10-YR	348.2 (377.9) a	242.6 (308.0) a	1552.4 (328.8) a	387.5 (135.6) a
Bf	OG	222.3 (286.1) a	40.5 (39.9) a	1917.7 (222.3) a	156.3 (107.2) a
	0-YR	159.1 (87.0) a	50.2 (28.8) a	2102.5 (219.4) a	150.0 (60.7) a
	5-YR	88.5 (132.0) a	26.4 (23.3) a	2236.6 (229.8) a	144.2 (65.4) a
	10-YR	57.4 (64.5) a	16.0 (5.3) a	2130.8 (111.2) a	101.4 (15.0) a

There were no significant differences among the ages for loss on ignition (LOI) in any horizon. Generally, LOI was lower than the old growth on the 0- and 10-year postburn sites for the LF horizon, and on the 10-year sites in the H horizon, and decreased with depth through the soil profile.

The concentrations of extractable Ca were similar for all of the ages in the LF and H horizons (Tables 7-2, 7-4), and the variability was high. In the Bhf and Bf, extractable Ca was lower in the 5- and 10-year postburn sites, but the differences were not significant. There were significant differences among the sites for extractable Mg in the LF and H horizons, but there was also a significant location effect (Table 7-2). More extractable Mg was found on the postburn sites than on the old growth sites in the LF, H and Bhf horizons. There were no significant differences in extractable Al or Fe among the sites for any horizon. Generally, the postburn sites contained more extractable Al for all horizons, and more extractable Fe in the LF, H and Bhf horizons. In the Bf, the lowest extractable Fe levels were found in the 10-year postburn samples.

There were no significant differences in pyrophosphate-extracted Fe among the sites in the LF and H horizons (Tables 7-5, 7-6). In the Bhf, the 10-year postburn sites were significantly lower in pyrophosphate-Fe than the recently burned sites. Pyrophosphate-Fe was also significantly lower than in soils from the other ages in the Bf horizons of the 10-year sites. There were no significant differences in pyrophosphate-Al in the LF, H and Bhf

horizons. In the Bf, the 10-year sites were significantly lower in pyrophosphate-Al. However, there was also a significant location effect (Table 7-6). There were significant differences in pyrophosphate-extracted Mn in the LF, with the highest concentration in the recent burn and the lowest in the 10-year postburn and old growth sites. There were no significant

Table 7-5: The mean values and (standard deviations) for pyrophosphate-extracted Fe, Al and Mn. Different letters indicate statistically significant differences among the ages for a horizon at $p<0.05$. OG is old growth. (n=9)

HOR.	AGE	Fe-PYRO %	Al-PYRO %	Mn-PYRO %
LF	OG	0.010 (0.031) a	0.051 (0.040) a	0.063 (0.036)a
	0-YR	0.121 (0.155) a	0.128 (0.086) a	0.170 (0.178)b
	5-YR	0.013 (0.029) a	0.071 (0.038) a	0.126(0.113)ab
	10-YR	0.111 (0.113) a	0.176 (0.128) a	0.035 (0.034)a
H	OG	0.069 (0.184) a	0.081 (0.157) a	0.009 (0.012)a
	0-YR	0.121 (0.162) a	0.127 (0.077) a	0.044 (0.056)a
	5-YR	0.123 (0.189) a	0.135 (0.147) a	0.013 (0.010)a
	10-YR	0.167 (0.202) a	0.169 (0.124) a	0.014 (0.012)a
Bhf	OG	1.395 (0.934)ab	0.577 (0.234) a	0.000 (0.000)a
	0-YR	1.527 (0.642) a	0.470 (0.253) a	0.011 (0.023)a
	5-YR	1.174 (0.633)ab	0.374 (0.166) a	0.001 (0.002)a
	10-YR	0.772 (0.511) b	0.410 (0.162) a	0.002 (0.004)a
Bf	OG	0.910 (0.401) a	1.188 (0.364) a	0.000 (0.000)a
	0-YR	0.887 (0.528) a	1.119 (0.481) a	0.004 (0.009)a
	5-YR	0.886 (0.698) a	1.030 (0.533) a	0.001 (0.002)a
	10-YR	0.294 (0.118) b	0.637 (0.170) b	0.000 (0.001)a

differences in the other horizons, and the values were low.

There were no significant differences among the sites in any horizon for citrate bicarbonate dithionite (CBD)-extracted Fe, Al or Mn (Table 7-7), or for Al and Fe extracted by acid ammonium oxalate (AAO) (Table 7-7). In the Bhf horizon, the concentrations of Fe-CBD were highest in the recent burn, and lowest on the 10-year postburn sites. In the Bf, the 5-year sites had the most Fe-CBD, while the 10-year sites contained the least. The Al-CBD concentrations were similar among the sites in the Bhf horizon,

Table 7-6: Analysis of variance (ANOVA) table for pyrophosphate-extracted Fe, Al and Mn; citrate-dithionite-bicarbonate (CBD)-extracted Fe, Al and Mn; and acid ammonium oxalate (AAO)-extracted Fe and Al, showing the probabilities as calculated by the Systat statistical program. As a nested experimental design was used, the terms for the ANOVA were TYPE, HORIZON, LOCATION{TYPE} (which is location nested within forest type), TYPE*HORIZON, HORIZON*LOCATION{TYPE}, and multiple R^2. A * indicates significance at $p<0.05$.

	AGE	HORIZON	LOCATION {AGE}	AGE* HORIZON	HORIZON* LOCATION {AGE}	MULT. R^2
Fe AAO	0.197	0.000	0.038	0.489	0.357	0.612
Fe CBD	0.103	0.000	0.008	0.370	0.689	0.595
Fe Pyro	0.006	0.000	0.015	0.006*	0.140	0.800
Al AAO	0.763	0.000	0.005	0.659	0.086	0.878
Al CBD	0.141	0.000	0.001	0.268	0.208	0.863
Al Pyro	0.071	0.000	0.003	0.000*	0.023*	0.841
Mn CBD	0.157	0.627	0.190	0.879	0.936	0.311
Mn Pyro	0.003	0.000	0.706	0.013*	8.582	0.616

and were lowest in the 10-year sites for the Bf horizon. Acid ammonium oxalate (AAO)-extracted Fe was lowest on the 10-year sites in the Bhf. In the Bf, the concentrations were very similar for all ages. The values of Al-AAO were also very similar for all sites in the Bhf and Bf.

Amorphous Fe was found only in the 5-year postburn sites in the Bhf (Figure 7-1A). The Fe of this horizon was predominantly in organic form, and the 10-year postburn sites contain less organic Fe than the other sites. In the Bf, Fe is found in amorphous, crystalline and organic forms. The 10-year postburn sites contain

Table 7-7: The mean values and (standard deviations) for citrate-bicarbonate-dithionite (CBD)-extracted Fe, Al and Mn, and for acid ammonium oxalate (AAO)-extracted Fe and Al. The LF and H horizons were not extracted. Different letters indicate statistically significant differences among the ages for a horizon at $p<0.05$. OG is old growth. (n=9)

HOR	AGE	Fe-CBD %	Al-CBD %	Mn-CBD %	Fe-AAO %	Al-AAO %
Bhf	OG	2.013 (1.296)a	0.477 (0.175)a	0.001 (0.001)a	1.283 (0.691)a	0.519 (0.182)a
	0-YR	2.304 (1.124)a	0.469 (0.217)a	0.014 (0.027)a	1.289 (0.450)a	0.446 (0.164)a
	5-YR	2.126 (1.127)a	0.488 (0.204)a	0.005 (0.006)a	1.285 (0.624)a	0.483 (0.235)a
	10-YR	1.362 (0.662)a	0.451 (0.195)a	0.003 (0.004)a	0.791 (0.536)a	0.439 (0.199)a
Bf	OG	2.636 (1.111)a	1.465 (0.385)a	0.001 (0.001)a	1.780 (0.479)a	2.390 (1.097)a
	0-YR	3.456 (1.195)a	1.535 (0.324)a	0.016 (0.034)a	1.914 (0.560)a	2.312 (0.702)a
	5-YR	3.825 (1.492)a	1.593 (0.583)a	0.004 (0.004)a	2.033 (0.571)a	2.326 (0.777)a
	10-YR	3.197 (0.723)a	1.231 (0.186)a	0.011 (0.023)a	1.813 (0.296)a	2.663 (0.427)a

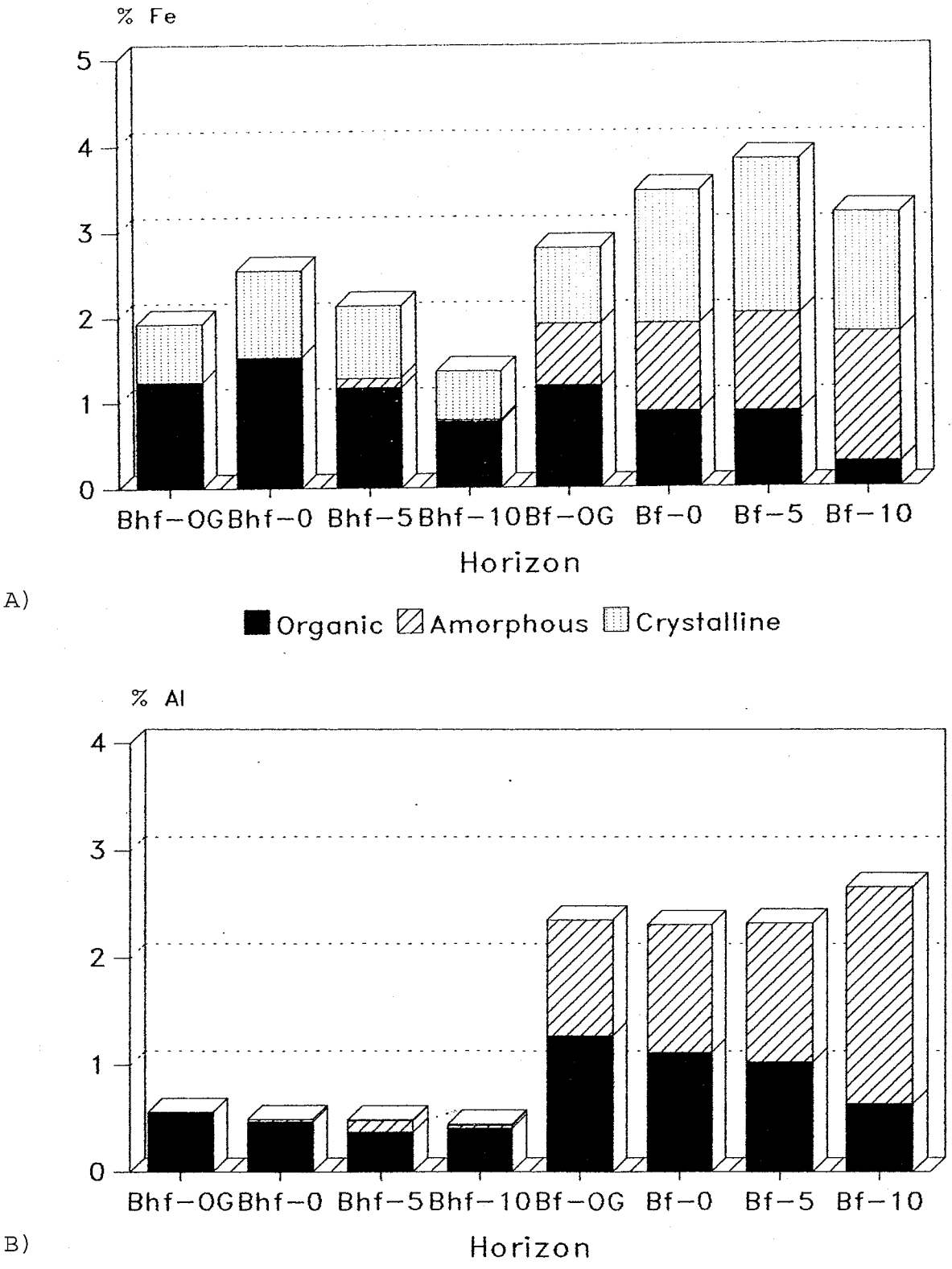


Figure 7-1: The mean percentages of organic, amorphous and crystalline iron (A) and aluminium (B) in the Bhf and Bf horizons of CH old growth (OG) and 0, 5 and 10 years postburn sites.

less organic Fe, and more amorphous Fe. The old growth sites contain the least crystalline Fe. These differences, however, were not significant. Aluminium was almost entirely in organic form in the Bhf horizon of all sites (Figure 7-1B). In the Bf, the 10-year postburn samples contained significantly more amorphous Al. Crystalline Al was not present in these soils.

The results from the analysis for P_A, P_T, P_O and the C/P ratio are displayed in Tables 7-8 and 7-9. Available P (P_A) was significantly higher on the recently burned sites in the LF horizon, and on the 0- and 5-year sites in the H horizon. There were no significant differences in P_A in the Bhf and Bf. There were no differences among the sites in P_O in the LF, H and Bhf horizons. However, in the Bf, P_O was significantly lower on the 10-year sites. There were no significant differences for any horizon among the sites for P_T or the C/P ratio, and the variability was high.

The Chang and Jackson fractionation procedure produced P_{HCl}, P_{NaOH} and P_{CBD}. As was noted in Chapter 6, P_{NaOH} is thought to be the non-occluded phosphate bound to the surfaces of Al or Fe hydrous oxides (Olsen and Sommers 1982). The P_{CBD} fraction is comprised of P occluded within the matrices of Fe and Al oxides and hydrous oxides, while the P_{HCl} is thought to be the extracted calcium phosphates of the non-occluded apatite fraction (Williams *et al.* 1980; Olsen and Sommers 1982). There were no significant differences among the sites for P_{HCl} or P_{CBD} in either the Bhf or Bf

Table 7-8: The mean values and (standard deviations) for available P, total P, organic P and C/P ratio. Different letters indicate statistically significant differences among the ages for a horizon at $p<0.05$. OG is old growth. (n=9)

HOR	AGE	Avail. P (mg/kg)	Total P (mg/kg)	Org. P (mg/kg)	C/P
LF	OG	30.46 (10.01) b	585.8 (66.6) a	492.0 (98.8) a	815 (135) a
	0-YR	88.97 (31.25) a	713.9 (181.4) a	622.3 (272.4) a	539 (242) a
	5-YR	27.16 (9.38) b	675.8 (141.9) a	551.2 (177.6) a	673 (162) a
	10-YR	17.25 (7.15) b	485.3 (163.1) a	391.1 (124.2) a	878 (295) a
H	OG	20.62 (9.65) b	482.8 (152.1) a	308.7 (123.2) a	1055 (389) a
	0-YR	44.11 (24.89) a	524.0 (219.9) a	360.4 (243.9) a	958 (536) a
	5-YR	39.05 (21.37) a	569.5 (150.6) a	401.0 (127.5) a	826 (368) a
	10-YR	12.62 (5.08) b	515.4 (150.9) a	306.2 (112.2) a	866 (258) a
Bhf	OG	6.06 (2.92) a	352.1 (89.6) a	145.1 (89.2) a	392 (152) a
	0-YR	8.84 (8.12) a	349.4 (199.5) a	174.2 (97.7) a	450 (111) a
	5-YR	9.24 (6.54) a	468.6 (170.6) a	220.2 (111.0) a	343 (112) a
	10-YR	8.59 (1.78) a	354.5 (66.1) a	198.7 (93.1) a	468 (151) a
Bf	OG	6.31 (1.02) a	289.2 (115.6) a	64.8 (36.4) a	252 (90) a
	0-YR	6.43 (1.12) a	230.8 (41.1) a	41.3 (23.0) a	268 (104) a
	5-YR	6.09 (1.05) a	235.5 (102.2) a	63.5 (36.8) a	221 (65) a
	10-YR	6.60 (0.51) a	211.8 (70.2) a	21.6 (24.8) b	193 (71) a

Table 7-9: Analysis of variance (ANOVA) table for available P, organic P, total P, P-HCl, P-NaOH and P-CBD (Chang & Jackson), and the C/P ratio, showing the probabilities as calculated by the SYSTAT statistical program. Because a nested experimental design was used, the terms for the ANOVA were AGE, HORIZON, LOCATION{AGE} (which is location nested within forest type), AGE*HORIZON, HORIZON*LOCATION{AGE}, and multiple R^2. A * indicates significance at $p<0.05$.

	AGE	HORIZON	LOCATION {AGE}	AGE* HORIZON	HORIZON* LOCATION {AGE}	MULT. R^2
Avail P	0.000	0.000	0.128	0.000*	0.572	0.863
Org P	0.002	0.000	0.318	0.004*	0.324	0.785
Tot P	0.011	0.000	0.000	0.093	0.984	0.724
P-HCl	0.481	0.000	0.175	0.496	0.175	0.601
P-NaOH	0.011	0.000	0.135	0.027*	0.000*	0.685
P-CBD	0.049	0.000	0.008	0.228	0.032	0.732
C/P	0.095	0.000	0.035	0.065	0.922	0.783

horizons. P_{NaOH} was significantly higher in the Bhf horizon of the recently burned sites than the old growth or 10-year sites, and was lowest in the 10-year sites, but there was also a location effect. There were no significant differences in the Bf horizon.

The percentage of total P (P_T) found as organic P (P_O) and inorganic P (P_I), and the percent recovery of the P_T for each horizon and age can be found in Table 7-11. As these means were calculated from each sample, they are not completely additive. There were no significant differences among the ages in any horizon for P_O or percent recovery. Significantly more of the P_T was found as P_I in the Bhf of the recent burn than on the 5- and 10-year

Table 7-10: The mean values and (standard deviations) for P extracted by HCl, NaOH and citrate bicarbonate dithionite (CBD), during the Chang & Jackson fractionation procedure. The LF and H horizons were not extracted. Different letters indicate statistically significant differences among the ages for a horizon. OG is old growth. (n=9)

HOR.	AGE	P-HCl (mg/kg)	P-NaOH (mg/kg)	P-CBD (mg/kg)
Bhf	OG	7 (0.5) a	37 (28.0) b	22 (17.0) a
	0 YR	8 (1.3) a	58 (34.8) a	34 (20.5) a
	5 YR	7 (1.0) a	42 (13.6) ab	26 (19.3) a
	10 YR	7 (0.7) a	27 (16.9) b	15 (13.9) a
Bf	OG	19 (13.6) a	25 (12.4) a	74 (23.6) a
	0 YR	24 (12.1) a	23 (12.0) a	59 (24.8) a
	5 YR	22 (24.0) a	17 (10.8) a	68 (35.8) a
	10 YR	30 (14.5) a	18 (6.8) a	50 (14.7) a

postburn sites. There were no significant differences in P_I in the Bf. Table 7-12 displays the correlation matrix for the results of this chapter. Extractable Al, and Al extracted by CBD, AAO and pyrophosphate all had high correlations with one another. Acid ammonium oxalate (AAO)-Al was also positively correlated with P_{HCl} and pH in water and in $CaCl_2$, and was negatively correlated with extractable Fe, total N and the C/P ratio. Aluminium extracted by CBD was positively correlated with P_{HCl}, Fe-AAO, Fe-CBD and both pH methods. Pyrophosphate-Al correlated positively with P_{CBD} and negatively with extractable Mg and total N. There was a positive relationship between Fe extracted by CBD and AAO, and with pyrophosphate-extracted Mn to Mn-CBD and organic P. Extractable Al

Table 7-11: The means and (standard deviations) of total P found as organic and inorganic P, and the percentage of total P recovered. Inorganic P is the sum of the fractions determined by Chang & Jackson fractionation. N/E indicates not extracted. Different letters indicate statistically significant differences between ages for a horizon at $p<0.05$.

HOR.	AGE	ORG. P	INORG. P	% RECOVERY
LF	OG	83.3 (12.7) a	N/E	83.3 (12.7) a
	0 YR	85.6 (16.9) a	N/E	85.6 (16.9) a
	5 YR	80.0 (10.8) a	N/E	80.0 (10.8) a
	10 YR	83.5 (17.9) a	N/E	83.5 (17.9) a
H	OG	65.5 (28.3) a	N/E	65.5 (28.3) a
	0 YR	65.7 (16.7) a	N/E	65.7 (16.7) a
	5 YR	73.4 (28.6) a	N/E	73.4 (28.6) a
	10 YR	62.7 (25.1) a	N/E	62.7 (25.1) a
Bhf	OG	39.1 (17.9) a	19 (10.7) ab	58.0 (22.1) a
	0 YR	50.4 (12.9) a	33 (14.6) a	83.2 (18.7) a
	5 YR	49.1 (20.1) a	17 (6.1) b	65.9 (21.5) a
	10 YR	57.4 (24.8) a	13 (7.5) b	70.8 (25.7) a
Bf	OG	23.9 (12.0) a	44 (15.6) a	68.1 (17.3) a
	0 YR	17.8 (10.2) a	46 (9.7) a	64.2 (18.7) a
	5 YR	28.1 (12.5) a	48 (14.8) a	65.9 (21.5) a
	10 YR	10.2 (10.9) a	50 (15.6) a	60.3 (14.5) a

had a negative correlation with extractable Fe and Mg, and with C. Loss on ignition (LOI) was positively correlated with total N, C and the C/P ratio. There was a positive relationship between the two pH methods. Positive correlations were also seen for:

Table 7-12: Correlation matrix. Al- and Fe-AAO were extracted by acid ammonium oxalate, Al-, Fe-, and Mn-CBD by citrate bicarbonate dithionite. Al-, Fe-, and Mn-pyro by pyrophosphate. Avail P is extracted by Bray. Extr-Al, -Ca, Fe, and -Mg were extracted by Mehlich. H20A is air-dry moisture content; H2OF is field moisture content. Org P is by NaOH-EDTA extraction and Tot P is by Parkinson & Allen digest.

	Al-AAO	Al-CBD	Al-Pyro	AvailP	C	C/N	C/P	ExtrAl	ExtrCa	ExtrFe	ExtrMg	Fe-AAO	Fe-CBD	Fe-Pyro
Al-AAO	1.000													
Al-CBD	0.867	1.000												
Al-Pyro	0.711	0.852	1.000											
AvailP	-0.252	-0.312	-0.428	1.000										
C	-0.610	-0.544	-0.702	0.506	1.000									
C/N	0.216	0.165	-0.117	0.139	0.274	1.000								
C/P	-0.596	-0.494	-0.547	0.286	0.797	0.379	1.000							
ExtrAl	0.799	0.768	0.797	-0.475	-0.861	-0.089	-0.644	1.000						
ExtrCa	-0.438	-0.455	-0.582	0.511	0.662	-0.005	0.365	-0.720	1.000					
ExtrFe	-0.756	-0.675	-0.040	-0.151	-0.227	-0.231	-0.187	0.093	-0.082	1.000				
ExtrMg	-0.572	-0.561	-0.679	0.533	0.792	0.055	0.600	-0.734	0.703	0.037	1.000			
Fe-AAO	0.515	0.631	0.430	-0.405	-0.521	-0.018	-0.472	0.462	-0.412	-0.351	-0.497	1.000		
Fe-CBD	0.443	0.641	0.371	-0.382	-0.436	-0.007	-0.366	0.363	-0.375	-0.294	-0.365	0.882	1.000	
Fe-Pyro	-0.312	-0.076	0.563	-0.400	-0.576	-0.309	-0.448	0.444	-0.464	0.429	-0.474	0.363	0.340	1.001
H20Air	0.281	0.331	-0.136	0.175	0.383	0.136	0.409	-0.188	0.164	-0.260	0.278	0.287	0.275	-0.244
H2OF	-0.455	-0.364	-0.174	-0.015	0.307	-0.037	0.310	-0.262	-0.013	0.033	0.119	-0.295	-0.274	0.064
LOI	-0.524	-0.494	-0.540	0.371	0.808	0.271	0.715	-0.685	0.385	-0.366	0.521	-0.389	-0.337	-0.505
Mn-CBD	-0.056	-0.001	-0.060	0.366	0.117	0.070	-0.041	-0.069	0.215	-0.016	0.212	0.164	0.213	0.124
Mn-Pyro	-0.181	-0.141	-0.295	0.466	0.353	-0.004	0.053	-0.383	0.509	-0.135	0.380	-0.008	0.005	-0.26
OrgP	-0.609	-0.587	-0.570	0.622	0.659	0.003	0.276	-0.662	0.735	0.025	0.707	-0.632	-0.538	-0.444
pHCaCl2	0.725	0.583	0.286	0.152	-0.390	-0.114	-0.536	0.386	0.102	-0.221	-0.201	0.437	0.392	-0.064
pHH20	0.683	0.594	0.338	0.121	-0.423	-0.210	-0.559	0.413	0.069	-0.171	-0.186	0.439	0.417	0.014
P-HCl	0.797	0.609	0.510	-0.087	-0.404	0.137	-0.438	0.551	-0.274	-0.542	-0.367	0.376	0.322	-0.256
P-NaOH	-0.513	-0.475	-0.345	0.456	0.411	-0.179	0.184	-0.523	0.466	0.389	0.435	-0.259	-0.269	0.23
P-CBD	0.592	0.72	0.614	-0.214	-0.370	0.012	-0.317	0.408	-0.161	-0.459	-0.265	0.516	0.546	0.062
TotN	-0.627	-0.568	-0.672	0.459	0.881	-0.079	0.569	-0.842	0.723	-0.047	0.827	-0.431	-0.359	-0.414
TotP	-0.333	-0.303	-0.451	0.549	0.638	-0.095	0.130	-0.611	0.671	0.022	0.647	-0.274	-0.284	-0.294

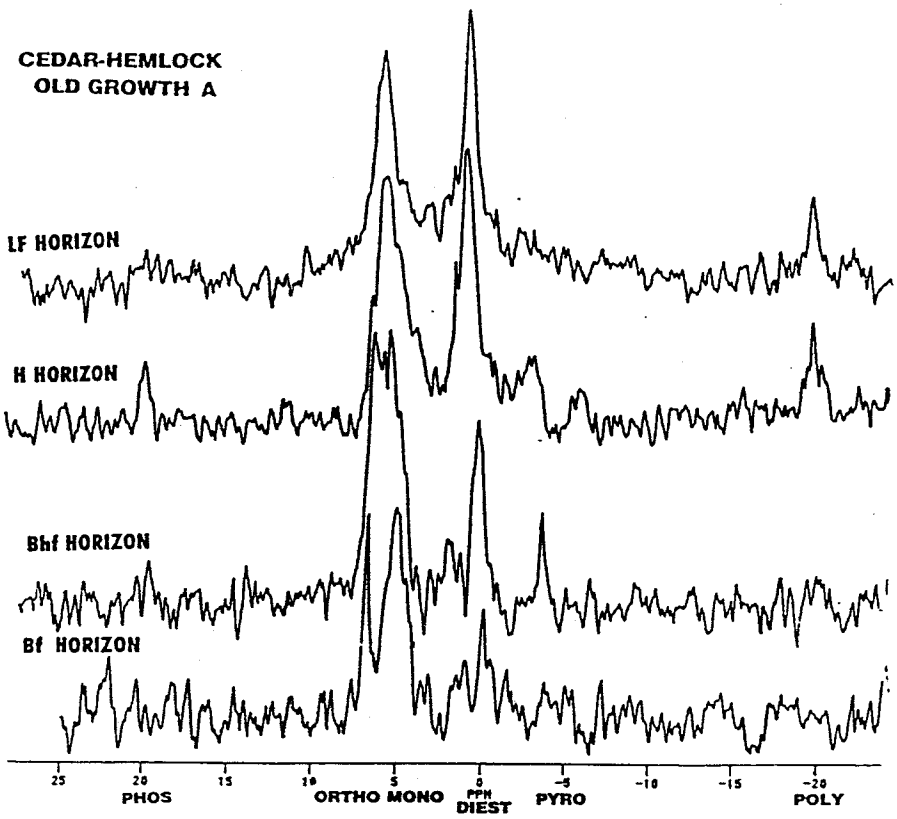
	H20Air	H2OF	LOI	Mn-CBD	Mn-Pyro	OrgP	pHCaCl2	pHH20	P-HCl	P-NaOH	P-CBD	TotN	TotP
H20Air	1.000												
H2OF	0.287	1.000											
LOI	0.419	0.333	1.000										
Mn-CBD	0.263	0.026	0.131	1.000									
Mn-Pyro	0.009	-0.148	0.191	0.740	1.000								
OrgP	0.062	0.041	0.403	0.085	0.649	1.000							
pHCaCl2	-0.040	-0.510	-0.384	0.212	0.275	0.029	1.000						
pHH20	0.080	-0.430	-0.433	0.320	0.217	0.006	0.936	1.000					
P-HCl	0.377	-0.344	-0.346	-0.024	-0.088	-0.410	0.590	0.538	1.000				
P-NaOH	-0.123	0.394	0.392	0.310	0.540	0.458	-0.191	-0.149	-0.398	1.000			
P-CBD	0.284	-0.221	-0.299	0.007	-0.089	-0.401	0.441	0.477	0.467	-0.249	1.000		
TotN	0.287	0.344	0.642	0.152	0.385	0.740	-0.328	-0.310	-0.412	0.478	-0.325	1.000	
TotP	0.118	0.151	0.370	0.191	0.535	0.864	-0.003	0.007	-0.160	0.477	-0.241	0.792	1.000

available P with organic P and total P; organic P with total N, total P, Ca, Mg and C; and total P with total N and Ca. Carbon correlated positively with C/P, total N and Ca. Extractable Ca was positively correlated with extractable Mg and total N.

The ^{31}P NMR spectra for the two old growth profiles are found in Figure 7-2 (A and B). The percentage of P found within each class of compounds, calculated from the spectra by integration, are shown in Table 7-13 for the old growth and recently burned profiles, and in Table 7-14 for the 5- and 10-year sites. Figure 7-3 shows the spectra from the recently burned sites, while Figure 7-4 displays spectra from sites 5 years after burning, and Figure 7-5 from sites 10 years after burning. A guide for the interpretation of NMR spectra can be found in Chapter 5 (Table 5-2).

As discussed in Chapter 6, the spectra for the two mature CH forests were quite different from one another. The orthophosphate and monoester peaks were separated well in only the Bf horizon in profile A, but were distinct in all horizons of profile B. This problem with peak separation is discussed in Chapters 5 and 6. In A (Fig. 7-2A; Table 7-14) the proportionss of monoester and diester P were comparable in the LF and H, and were nearly double those of orthophosphate P in these horizons. Orthophosphate and monoester P were the dominant P classes in the Bhf; monoesters dominated in the Bf. There were small phosphonate peaks in the LF and H horizons. In profile B (Fig. 7-2B; Table 7-13), most of the P was found as orthophosphate or as monoesters, at comparable

A)



B)

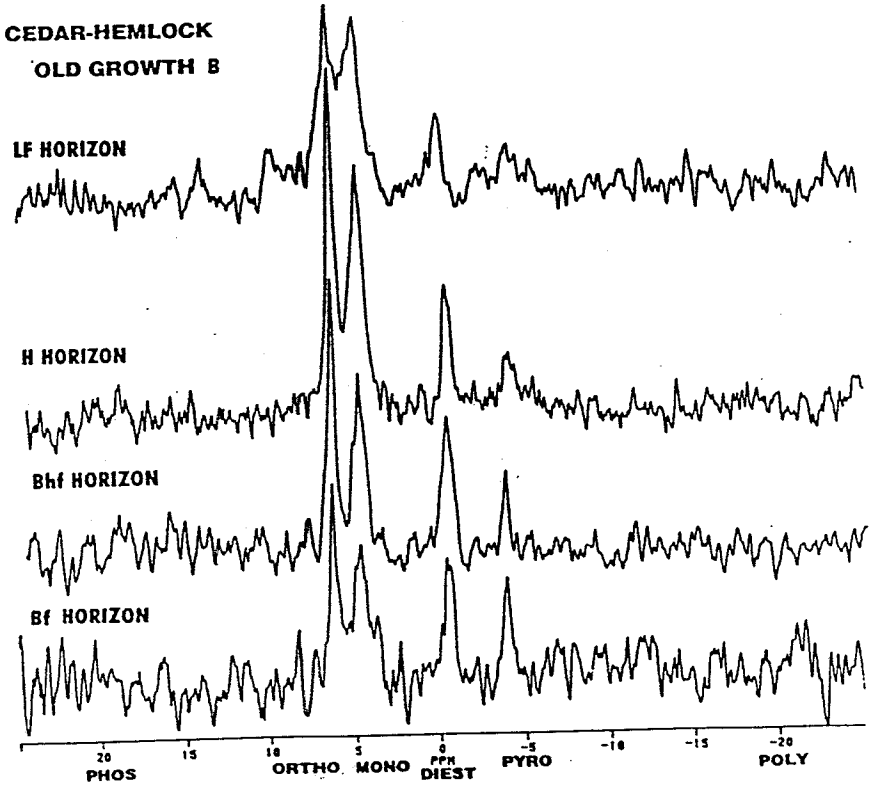


Figure 7-2: ^{31}P NMR spectra for two soil profiles from mature CH sites, extracted with NaOH-EDTA.

Figure 7-13: The proportion of P found within each P form, as calculated from the ^{31}P-NMR specta by integration, for the old growth (OG) and 0-year postburn profiles. Phos is phosphonate, orth is orthophosphate, mono is monoester P, diest is diester P, pyro is pyrophosphate and poly is polyphosphate.

AGE	HOR	Phos %	Orth %	Mono %	Dies %	Pyro %	Poly %
OG A	LF	0	17	33	39	0	11
	H	5	7	38	36	5	7
	Bhf	4	38	36	17	5	0
	Bf	0	23	61	16	0	0
OG B	LF	0	31	38	18	13	0
	H	0	39	32	20	9	0
	Bhf	0	35	34	24	7	0
	Bf	0	29	32	24	15	0
0-YR A	LF	0	51	32	11	6	0
	H	0	12	52	23	12	0
	Bhf	0	25	43	24	8	0
	Bf	0	85	15	0	0	0
0-YR B	LF	0	24	41	29	6	0
	H	5	23	34	33	0	5
	Bhf	10	14	47	24	5	0
	Bf	0	13	62	25	0	0

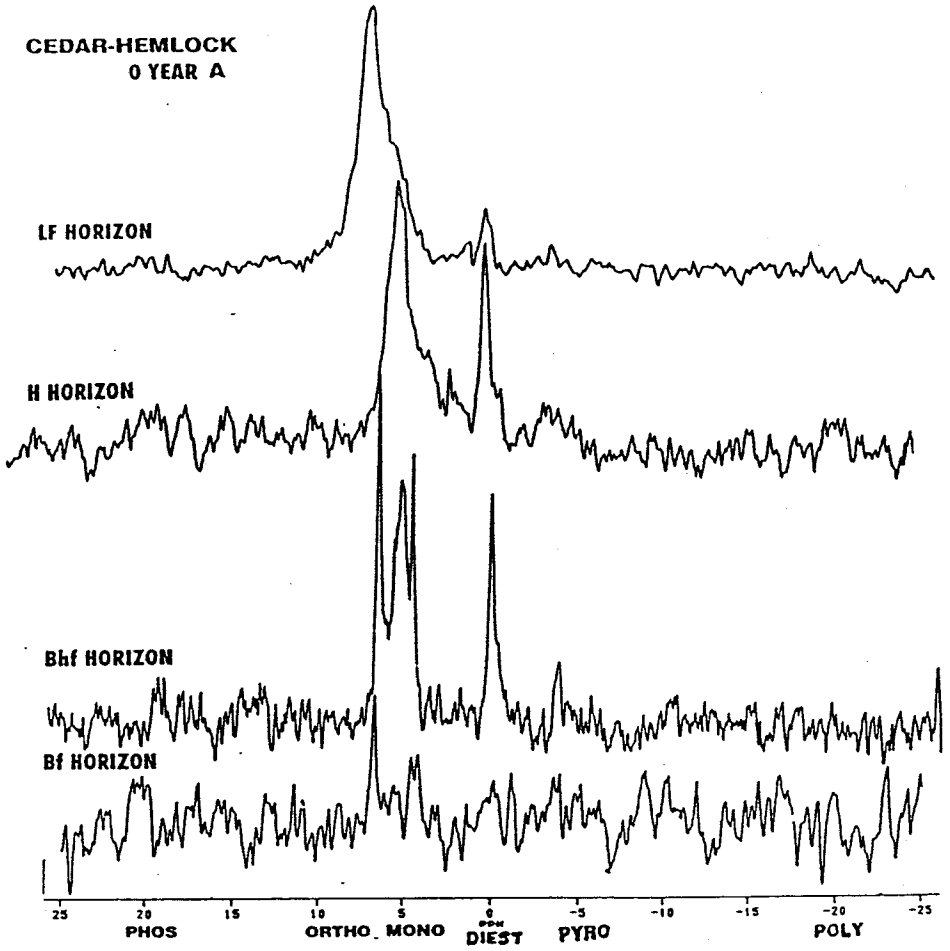
percentages. Diester and pyrophosphate peaks also occurred, but there were no phosphonate or polyphosphate peaks in any horizon.

On the recently burned sites (Fig. 7-3 A and B; Table 7-13), there were differences in the spectra for the two profiles. The orthophosphate and monoester peaks were distinctly separated in the

Bhf and Bf horizons of profile A, but not in the LF and H horizons, and they were not clearly separated in any horizon of profile B. Orthophosphate was the predominant P form in the LF of profile A. Monoesters were also present, as well as traces of diester P and pyrophosphate. In the H horizon, the percentages of monoester, diester and pyrophosphate P increased, while orthophosphate decreased. In the Bhf horizon, orthophosphate increased, while diester and pyrophosphate were similar to the H horizon. In the Bf, orthophosphate was the predominant P form, with a small amount of monoester. The LF and H horizons of profile B had orthophosphate and diester at equal levels, and a higher percentage of monoester. In the Bhf, phosphonate and pyrophosphate were present, in addition to orthophosphate, monoester and diester P. The P of the Bf horizon occurred mainly as monoesters, with some orthophosphate and diester P.

Five years after burning (Fig. 7-4 A and B; Table 7-14), monoester phosphate was the predominant P form in all horizons of profile A. The LF and H also contained diester, orthophosphate and polyphosphate peaks. There was a phosphonate peak in the H horizon, and pyrophosphate peaks in the LF and Bhf. In the Bf, the main P form was monoester, with some orthophosphate and diester P. In profile B, peaks were seen only for orthophosphate, monoester and diester P. Monoesters were the dominant P form in all but the Bf, where diester P was highest.

A)



B)

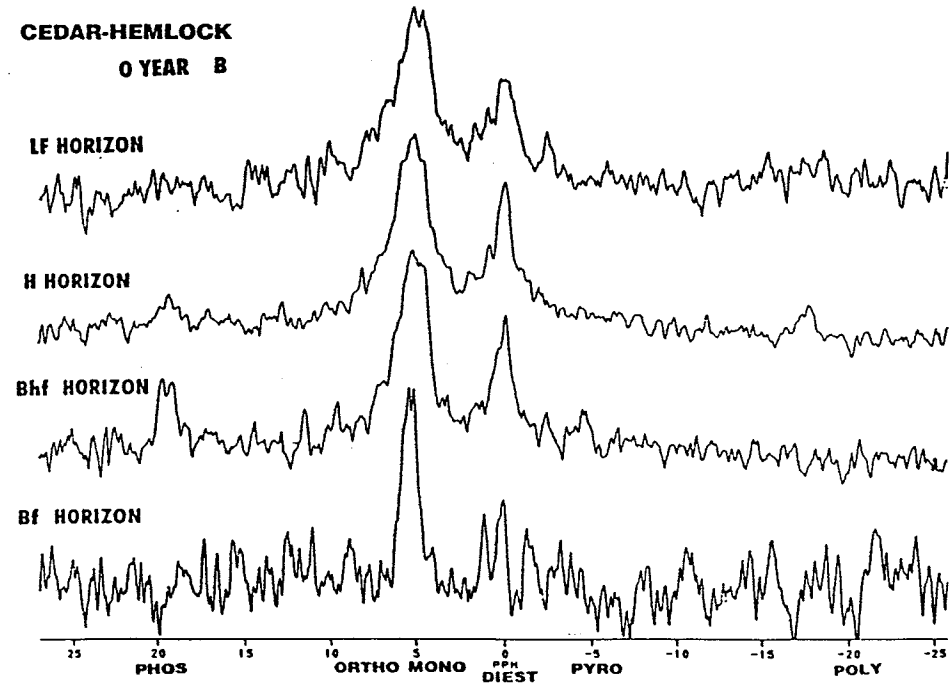
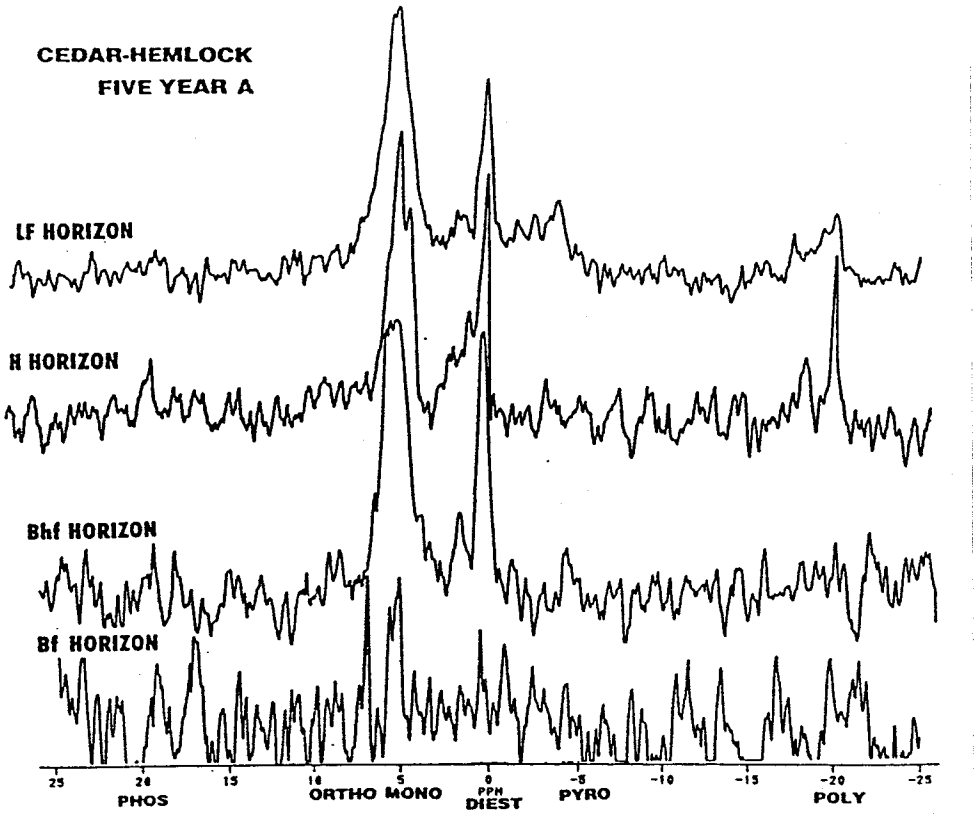
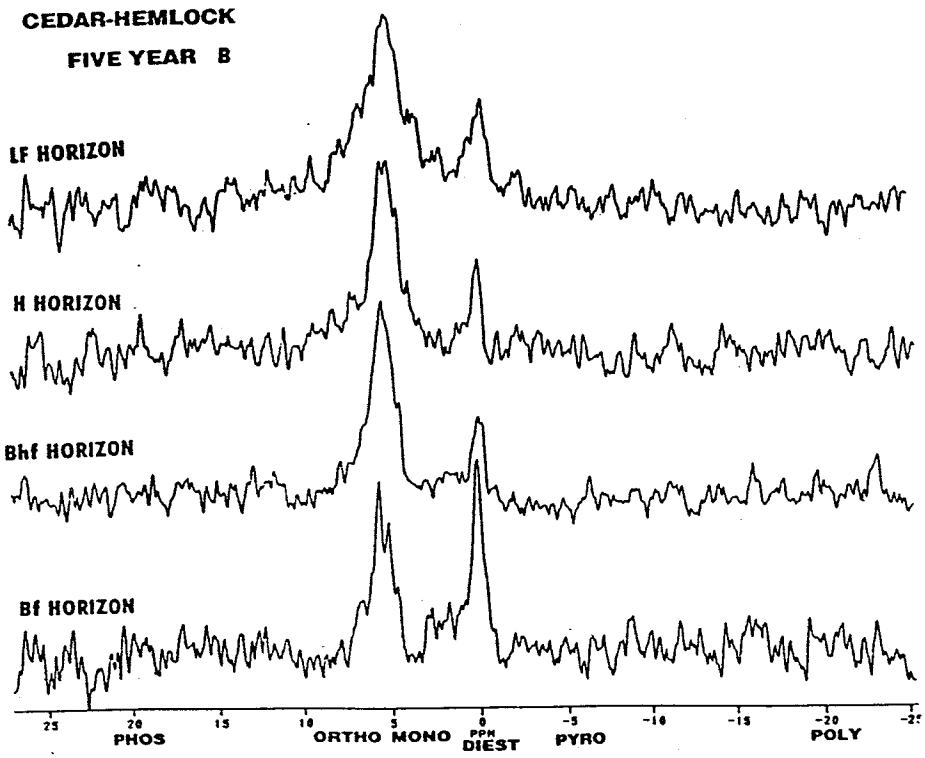


Figure 7-3: ^{31}P NMR spectra for two soil profiles from recently burned CH sites, extracted with NaOH-EDTA.



A)



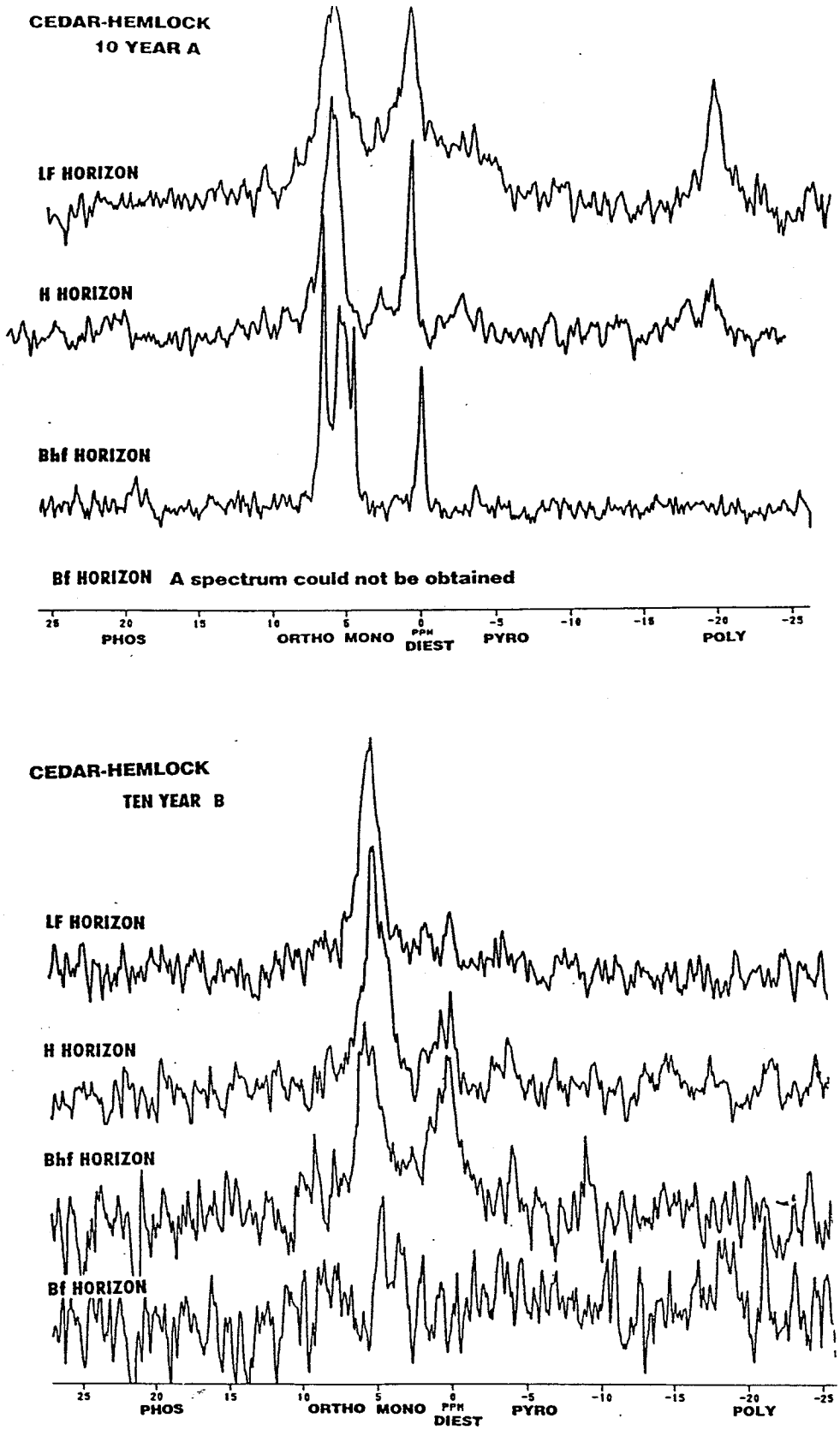
B)

Figure 7-4: ^{31}P NMR spectra for two soil profiles from CH sites 5 years after burning, extracted with NaOH-EDTA.

Figure 7-14: The proportion of P found within each P form, as calculated from the ^{31}P-NMR spectra by integration, for the 5-year and 10-year postburn profiles. Phos is phosphonate, orth is orthophosphate, mono is monoester P, diest is diester P, pyro is pyrophosphate and poly is polyphosphate.

AGE	HOR	Phos %	Orth %	Mono %	Dies %	Pyro %	Poly %
5-YR A	LF	0	23	40	18	7	12
	H	4	15	36	25	0	20
	Bhf	0	12	55	29	4	0
	Bf	0	30	60	10	0	0
5-YR B	LF	0	33	44	23	0	0
	H	0	23	59	18	0	0
	Bhf	0	23	51	26	0	0
	Bf	0	18	37	45	0	0
10-YR A	LF	0	17	27	32	10	14
	H	4	14	39	24	6	13
	Bhf	0	31	51	14	4	0
10-YR B	LF	0	36	53	11	0	0
	H	0	8	69	18	5	0
	Bhf	0	12	36	42	10	0
	Bf	0	65	25	0	0	10

The spectra for the two profiles from the 10-year sites were very different (Fig. 7-5 A and B; Table 7-14). In profile A, the orthophosphate and monoester peaks overlapped in the LF and H horizons, but were separate in the Bhf. A spectrum could not be obtained for the Bf horizon, due to the low P concentration in this soil, and the high concentration of interfering paramagnetic ions



A)

B)

Figure 7-5: ^{31}P NMR spectra for two soil profiles from CH sites 10 years after burning, extracted with NaOH-EDTA.

such as Mn. There was a range of P forms in the LF horizon of profile A. Diester P was highest, followed by monoester P. Orthophosphate and pyrophosphate were also present, as well as a sharp polyphosphate peak. The spectrum for the H horizon was similar to that of the LF, but with a smaller polyphosphate peak. A small phosphonate peak was also present in the H horizon. In the Bhf, most of the P occurred as monoesters. The percentages of orthophosphate were greater than in the LF and H, while diester P was less. There was also a small amount of pyrophosphate.

In profile B, the orthophosphate and monoester peaks were separated only in the Bf horizon. In the LF, the P was predominantly found as monoester phosphate, with smaller quantities of diester and monoester P. In the H horizon, the proportion of orthophosphate dropped, while the monoester and diester levels increased. Pyrophosphate was also present. The levels of monoester and diester P were almost equal in the Bhf, with lower levels of orthophosphate and pyrophosphate. The Bf horizon contained mainly orthophosphate, with low levels of monoester P and polyphosphate. The signal-to-noise ratio was low for this horizon, and it was difficult to distinguish peaks from the background noise. This was due to the low P concentration in this sample, and the high concentration of interfering paramagnetic ions.

Discussion

Prior to discussing the results of this research, it should be noted that a true chronosequence was not used. Although only CH forest types were sampled, differences between locations may have developed after burning which were independent of the burning effects. In addition, the 10-year sites were burned in the fall, while the 0- and 5-year sites were burned in the spring. The slash and litter were drier in the fall, resulting in a more intense burn. This was reflected in the depth to the Bf horizon, which was less on most of the 10-year sites than it was on the old growth, 0- or 5-year sites (personal observation). Fire intensity and duration, reflected in the quantity of slash and forest floor material consumed by the fire, are believed to determine the absolute amounts of nutrients lost via burning, through increased volatilization and convection losses (Boyle 1973; Brockley *et al.* 1992).

The choice of 10-year sites available for sampling was very limited, as most 10-year postburn sites had received N and P fertilizers to overcome the growth check problem. The locations used in this study were considered to be the "better" sites, with fewer growth problems, and thus had not been fertilized (P. Bavis, per. commun.). As well, the 10-year sites were replanted with different species from the 5-year sites. Sitka spruce (*Picea sitchensis* (Bong) Carr.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were found on the 10-year sites in addition to

western hemlock and western red cedar, which grew on the 5-year sites. There was a greater choice of 5-year sites than 10-year sites, because none of the 5-year sites had been fertilized. Site selection was based to some extent on ease of sampling: on many sites the salal was so dense that it made sample collection very difficult. The 5-year locations all had some salal, but the most densely covered locations were not used. Generally, there was less salal on the 10-year sites (personal observation). There was no choice of 0-year sites, as only three locations had been burned at the time of sample collection in the vicinity of the other postburn locations.

The availability of sampling locations largely determined the experimental design. A nested design was used, with locations nested within each age, which should take location effects into account. Despite many apparent trends in the data, some of the differences were not statistically significant. This may be due to insufficient sampling: the high variability of some of the elements investigated probably necessitates more than nine samples per treatment. This variability is due both to natural distribution patterns and to uneven heating and burning of slash and litter during each fire.

There was a significant pH increase in the surface horizon of the recently burned sites, as others have documented (Ahlgren and Ahlgren 1960; Kozlowski and Ahlgren 1974; Feller 1982; Ellis and Graley 1983; Macadam 1987; Tomkins *et al.* 1991; Brockley *et al.* 1992; Romanya *et al.* 1994). This is part of the so-called ashbed

effect of fire on soils (Humphreys and Lambert 1965; David 1987). The significant pH increase in the H horizon of the recent burn may reflect contamination of the samples with the ash layer during sampling. For the 5-year samples, the significant pH increase in the H horizon reflects movement of alkaline salts and ash down the horizon (Tomkins *et al.* 1991), which also accounts for the significantly elevated pH in the Bf of the 10-year postburn sites. Ellis and Graley (1983) and Tomkins *et al.* (1991) also reported pH increases at depth with time after burning.

There was a decrease in loss on ignition (LOI) in the LF horizon of the 0-year sites, as well as a lower C concentration, and a decrease in LOI in the H horizon 10 years after burning. As was discussed in Chapter 6, LOI is a measure of soil organic matter. Fire is expected to reduce organic matter content in surface horizons (Macadam 1987; Tomkins *et al.* 1991), especially in mor humus forms , where organic matter is not incorporated into soil, but accumulates on the mineral surface (Feller 1982). Litter inputs seem to bring the organic matter content of the LF horizons back to close to the old growth levels by 5 and 10 years after burning. However, LOI and C were lowest in the Bf horizon 10 years after burning, suggesting that burning the surface organic matter will affect the movement of organic matter through the profile. Macadam (1987) also reported a decrease in C concentration with time in mineral soil after fire.

The lowest total N concentrations in the LF were on the recent

burn; for the other horizons, total N was lowest on the 10-year sites. Those differences, however, were not significant. Reports in the literature on total N levels after fire are varied. Some researchers report decreases (Beaton 1957; Grier 1975; St. John and Rundel 1976; Ellis and Graley 1983; Khanna and Raison 1986; Macadam 1987; Brockley *et al.* 1991); others report increases (Vlamis *et al.* 1955; Mangas *et al.* 1992). Nitrogen is readily volatilized during fire (Mroz *et al.* 1980), and the amount of N lost is relative to the intensity of the burn. The correlation of total N to LOI and to other components of soil organic matter (such as organic P) suggests that the changes seen in this study reflect changes in organic matter, rather than volatilization losses of N. However, the lower N concentrations on the 10-year sites in the H, Bhf and Bf horizons may indicate some volatilization losses with the higher intensity burns used on these locations.

The results for Ca were highly variable, with no significant differences and no obvious trends, except in the LF horizon, where the Ca concentrations were slightly higher on the 0- and 5-year sites. Other researchers have reported significant increases in Ca concentration after fire (Vlamis *et al.* 1955; Beaton 1957; Boyle 1973; Grier 1975; St. John and Rundel 1976; Ellis and Graley 1983; Feller *et al.* 1983; Khanna and Raison 1986; Macadam 1987; Tomkins *et al.* 1991; Brockley *et al.* 1992; Rice 1993), with Ca increases proportional to fire intensity (Grier 1975; Brockley *et al.* 1992; Rice 1993).

Magnesium has also been shown to increase after burning (Grier 1975; Ellis and Graley 1983; Feller *et al.* 1983; Khanna and Raison 1986; Macadam 1987; Tomkins *et al.* 1991; Brockley *et al.* 1992; Mangas *et al.*1992; Rice 1993). In this study, Mg concentrations were significantly increased in the LF and H horizons of all of the postburn sites relative to the old growth. Although statistically there was a location effect, it occurred in the old growth data, as one location was higher in Mg in the LF and H horizons that the other two location used for this age. Becauses the Mg concentrations of the postburn sites were higher than even the highest old growth location, this apparent effect of fire on Mg is probably valid. The positive correlation of Mg to LOI, total N and organic P suggests that this increase was due to the release of Mg when organic matter was destroyed. The high Mg levels in the surface horizons 5 and 10 years after burning, with no increases at depth, indicate that it is not very mobile in these soils.

Significant differences in pyrophosphate-extractable Al and Fe were seen only in the mineral soil. Pyrophosphate-Fe was significantly lower in the 10-year sites in the Bhf and Bf horizons, while pyrophosphate-Al was significantly lower in the 10-year Bf. There were no significant differences in citrate bicarbonate dithionite (CBD)-extracted Fe and Al, nor in acid ammonium oxalate (AAO)-extracted Fe and Al. As discussed in Chapter 6, pyrophosphate extracts the organic-associated Fe and Al. In natural, unmodified podzols, Fe and Al in the Bhf and Bf

horizons are predominantly in organic form, reflecting the characteristic illuviation of organic matter and organo-metallic complexes in podzolic soils (Oades 1989). The presence of crystalline Fe but not Al is typical of podzols of Vancouver Island; the amorphous Fe and Al in the Bf horizons reflects the volcanic parent material of this region (Lewis 1976; Sanborn 1987). In the Bhf horizon, the 10-year sites had less organic Fe than the other ages. In the Bf, the 10-year locations had less organic Fe and more amorphous Fe, and significantly more amorphous Al. Amorphous Al and Fe concentrations were determined by subtracting the pyrophosphate extraction results from the AAO extraction values. The significantly lower pyrophosphate results may falsely elevate the amorphous Al and Fe concentrations. In light of the lack of differences in AAO-extracted Fe and Al, the apparent increase in amorphous Fe and Al is probably not a true effect of burning, unlike the change in organic Fe and Al. The intense burning of the 10-year sites appears to have altered the illuviation of organic matter and organo-metallic complexes. The same changes do not appear to have occurred on the 5-year sites, either because there has been insufficient time since burning for changes to manifest, or because the less intense burn on the 5-year sites destroyed less organic matter, leaving the illuviation processes unaffected. These changes in the Bf are probably only temporary, although they may persist for several years. The regrowth of vegetation on these sites will increase litter inputs to the surface, which should increase the cycling of organic matter

throughout the soil profile. There were no reports in the literature of changes in pyrophosphate-extracted Fe and Al. However, Humphreys and Lambert (1965) observed a decrease in oxalate-soluble Al in mineral soils nine years after burning, while Kwari and Batey (1991) demonstrated that heating soils in laboratory ovens could increase CBD-extracted Al and Fe, which they considered to be a measure of free Fe and Al oxides.

There was a significant increase in pyrophosphate-extracted Mn in the LF immediately after burning, as Mn was released from organic matter. This appears to have been a short-term effect, however, as the Mn concentration of the 5-year sites was only slightly increased, and there were no significant changes in any other horizon.

A significant increase in available P (P_A) after burning has been reported by other researchers, and is one of the most consistent effects of fire on soils (Ahlgren and Ahlgren 1960; Humphreys and Lambert 1965; Kozlowski and Ahlgren 1974; Feller 1982; Feller *et al.* 1983; Macadam 1987; DeBano and Klopatek 1988; Tomkins *et al.* 1991; Brockley *et al.* 1992; Mangas *et al.* 1992; Saa *et al.* 1993; Romanya *et al.* 1994). This increase in P_A is relative to the severity of the burn, and is of short duration, decreasing with time (Macadam 1987; DeBano and Klopatek 1988; Tomkins *et al.* 1991; Romanya *et al.* 1994). On these sites, P_A in all horizons had returned to old growth levels in the 10-year postburn sites. As was observed with soil pH, significant increases in P_A in the H

horizon of the recent burn probably reflect contamination with ash during sampling.

There were no significant differences among the ages for total P (P_T), and the variability was high. Generally, P_T was highest on the recently burned sites in the LF horizon. In the H horizon, all of the burned sites had elevated P_T levels relative to the old growth. In the Bf, P_T was lowest 10 years postburn. Beaton (1957), Ellis and Graley (1983), Tomkins *et al.* (1991) and Romanya *et al.* (1994) also report increased P_T in surface horizons after burning, while the results of Saa *et al.* (1993) were variable, showing no clear pattern.

Organic P was elevated relative to the old growth on the 0- and 5-year sites in the LF and H horizons. Previous studies report decreased P_O after fire (Kwari and Batey 1991; Saa *et al.* 1993; Romanya *et al.* 1994), which is believed to be due to the combustion of organic matter, and to the solubilization and transport of P_O down the profile, caused by the pH increase in the upper horizons (Romanya *et al.* 1994). The apparent increase in P_O found in this study is probably an artifact of the method used to determine P_O. In this procedure, organic matter is oxidized to P_I, and a subsequent colorimetric test measures the P_I in solution. This is then related back to the P_O content, without accounting for the P_I which may already be present. In the recently burned samples, where fire has oxidized the organic matter, releasing P_I, this would cause an overestimation of P_O. When the Saunders and

Williams (1955) ignition method which measured both P_o and P_I was used, it showed a significant increase in P_I and a significant decrease in P_o in the LF horizon of the 0-year sites relative to the other ages. This is supported by the NMR results, which showed mainly inorganic orthophosphate in the LF of recently burned soils. The methods used in this study are discussed in more detail in Chapter 4. The significant decrease in P_o in the Bf horizon of the 10-year sites reflects the altered illuviation patterns in these stands.

The ^{31}P NMR spectra show changes in P forms after fire. As was noted in Chapter 6, the NMR spectra are specific to each profile examined, making generalizations difficult. Better results might have been obtained by using composite samples. In the old growth samples, the LF and H horizons show spectra typical of wet areas with slow decomposition: the diversity of P forms is high, and relatively labile compounds such as diesters have persisted (Tate and Newman 1982; Condron *et al.* 1990a; Gil-Sotres *et al.* 1990). In the Bhf and Bf horizons, the diversity of P forms is reduced, and orthophosphate predominates, although organic P forms are found, even in the Bf. Immediately after burning, most of the P in the surface horizons was converted to orthophosphate, although some organic forms are still present. The lower horizons appear to be unchanged from those of the old growth, with the same general trends. Five years after burning, orthophosphate is the predominant P form in the LF and H horizons. Polyphosphate peaks are also present for one profile. Khanna and Raison (1986) suggest that the

P which is mineralized by soil heating may be deposited in ash in slowly soluble forms such as polyphosphates. Polyphosphates are easily formed in the laboratory by heating orthophosphate (Kulaev 1979), and their presence in these horizons may be an effect of burning. However, as discussed in Chapter 6, they are also storage products of ectomycorrhizae and soil microbes, and thus their origin may be biological. The spectra for the Bhf and Bf horizons are not unlike those of the old growth and recent burn profiles, except for the Bf of the 5-year A, which produced a poor quality spectrum due to its low P concentration. Litter deposits from reestablished vegetation resulted in spectra for the LF and H horizons of 10-year profile A which were comparable to those for the old growth. In profile B, though, monoester phosphate was the predominant P form in all but the Bf horizon. The most noticable difference of the 10-year sites, relative to the other ages, was the poor quality of the spectra obtained for the Bf horizon. In fact, a spectrum could not be obtained for the Bf of profile A. This was due to low P concentrations in these horizons, reflecting lower organic P levels. Romanya *et al.*(1994) have suggested that the increased pH of the surface horizons after fire may cause the solubilization and transport of P_o compounds down the soil profile. These NMR results negate that, however, as fewer P_o forms are found at depth with time after fire.

There are no published studies showing ^{31}P NMR spectra of forest soils following burning. Zech *et al.* (1987) state that cutting and burning of *Pinus mugo* and establishment of pasture did

not significantly influence the patterns of ^{31}P NMR spectra of the surface horizons, but they give no information on the length of time since burning, or the intensity of the fire, and they only looked at one sample.

There were no significant differences among the sites for P_{HCl}, which measured calcium phosphates, or P_{CBD}, which measured P occluded within Fe and Al oxides and hydroxides. In the Bhf, P_{NaOH} (non-occluded) was significantly higher in the samples from the recent burn than from the old growth or 10-year sites. There was also a significant location effect, as one of the recent burn locations had very high concentrations of P_{NaOH}, while one of the old growth locations had very low concentrations, and the variability was high. It is unlikely that this increased P_{NaOH} is an effect of fire, because only the surface layers were burned, and there had not been sufficient time or rainfall for burning effects to be experienced in the lower horizons. In the Bf, the 10-year postburn samples appeared to have increased levels of P_{HCl}, and reduced P_{NaOH} and P_{CBD} relative to the other ages. The formation of calcium phosphates in surface soils after fire has been reported elsewhere (Ellis and Graley 1983; Kwari and Batey 1991; Saa *et al.* 1993). It is possible that the higher intensity fires on the 10-year sites, together with the elevated pH, caused the Ca and P released from organic matter by burning to recombine into calcium phosphates. These subsequently moved down the soil profile to the Bf horizon, where they have persisted because the pH is still significantly elevated over old growth levels. However, the positive correlation

of P_{HCl} to Al extracted by acid ammonium oxalate and citrate bicarbonate dithionite suggests that the extraction procedure for P_{HCl} may be measuring P associated with Al rather than Ca. Increased aluminium phosphate concentrations have been reported after fire (Humphreys and Lambert 1965; Khanna and Raison 1986; Kwari and Batey 1991), as well as increased P sorption capacity, which is thought to be due to Al released from organic matter after burning (St. John and Rundel 1976; Kwari and Batey 1991; Romanya *et al*. 1994). Although phosphate sorption capacity was not measured in this study, Yuan and Lavkulich (1994) have demonstrated that it can be predicted from the concentrations of acid ammonium oxalate-extracted Fe and Al. Because these were not significantly different among the ages, it is unlikely that fire has significantly altered the P sorption capacity of these soils.

One major drawback to this research is that the effects of harvesting and burning could not be separated, because unburned, harvested sites for each age were not available. However, for the 10-year sites, one location was scarified and the other two locations were not. This did not show up as a location effect during statistical analysis, and the standard deviations of the data on the 10-year sites were low. The observed changes were also consistent with burning effects reported by other researchers. This suggests that the burning effects had more impact on the measured data than did harvesting. However, the distinction between harvesting and burning effects were not addressed by the scope of this research, and warrants further research.

Conclusions

After clear-cutting and burning, the soils of the CH forest types experience an ashbed effect, with increased pH and higher concentrations of available P, Ca, Mg and Mn in the surface horizons. These increases are only temporary, and most of these factors return to preburn levels within ten years. The combustion of organic matter is responsible for much of the ashbed effect. Destruction of organic matter in the more intense fall burnings appears to disrupt illuviation processes throughout the soil profile, and may produce longterm changes in lower mineral horizons. Although total P levels were not changed, there was a shift from organic P forms to inorganic P forms, and changes in P forms with time at depth in the profile. These changes in the P cycle may contribute to the growth check observed on these sites.

CHAPTER EIGHT

THE USE OF ORGANIC PHOSPHORUS BY WESTERN RED CEDAR

Introduction

As discussed in previous chapters, a growth check which may be overcome with N and P fertilization occurs in Sitka spruce and western hemlock trees replanted onto clearcut and slash-burned cedar-hemlock (CH) sites. Studies by Weetman *et al.* (1989a, b) suggested that western red cedar were performing better than Sitka spruce and western hemlock when replanted on the CH sites, and showed less response to P fertilization than the other species. Pot seedling bioassays confirmed that cedar was relatively insensitive to nutrient availability (Messier 1993); apparently, cedar was able to access nutrients of low availability in CH soils. Western red cedar is unusual in that it forms vesicular-arbuscular (VA) mycorrhizae rather than ectomycorrhizae (Curran and Dunsworth 1988). This symbiotic association may give this species some advantage on CH sites.

Previous research (Chapters 6 and 7) showed that much of the P in the soils of the CH sites is in organic form. Organic P (P_o) must be hydrolysed to orthophosphate before it can be used by plants (Tate 1984). This mineralization is catalysed by phosphatases, which are grouped into phosphoric monoester hydrolases (EC 3.1.3), phosphoric diester hydrolases (EC 3.1.4) or acid anhydride hydrolases (EC 3.6.1) (Speir and Ross 1978; Tabatabai 1982). The monoester hydrolases include: acid and

alkaline phosphatases, characterized by the pH at which they are most active and acting on compounds such as inositol phosphates, sugar phosphates and glycerophosphate; and enzymes such as phytase, which catalyses the removal of phosphate from inositol hexaphosphate (phytic acid) (Speir and Ross 1978; Tabatabai 1982). The diester hydrolases include enzymes which act on nucleotides and phospholipids. The acid anhydride hydrolases act on phosphoryl-containing anhydrides such as ATP and pyrophosphate (Speir and Ross 1978; Tabatabai 1982). Enzymes which can hydrolyse soil P_o compounds have been demonstrated in soil microorganisms, including fungi and bacteria (Szember 1962; Ko and Hora 1970; Greenwood and Lewis 1977; Dick and Tabatabai 1978, 1984; Browman and Tabatabai 1978; Beever and Burns 1980; Helal and Dressler 1989; Fox and Comerford 1992); in higher plants (Saxena 1964; Hasegowa *et al.* 1976; McLachlan 1980a, b; Basha 1984; Tarafdar and Claasen 1988; Tarafdar and Jungk 1987; Helal 1980; Garci and Ascencio 1992; Adams and Pate 1992; Dinkelaker and Marschner 1992; Barrett-Lennard *et al.* 1993; Tadano *et al.* 1993; Fernandez and Ascencio 1994; Pant *et al.* 1994); in ectomycorrhizae (Bartlett and Lewis 1973; Ho and Zak 1979; Alexander and Hardy 1981; Dighton 1983; Antibus et al. 1986; 1992; Kroehler *et al.* 1988; Ho 1989; Bae and Barton 1989; Haussling and Marschner 1989; Kropp 1990; Meysselle *et al.* 1991; Pasqualini *et al.* 1992; Tam and Griffiths 1993; McElhinney and Mitchell 1993); in ericoid mycorrhizae (Straker and Mitchell 1986; Shaw and Read 1989; Read 1991; Dighton and Coleman 1992) and in orchid

mycorrhizae (Antibus and Lesica 1990). Although phosphatase production has been demonstrated in VA mycorrhizae (Gianinazzi-Pearson and Gianinazzi 1976; MacDonald and Lewis 1978; Gianinazzi *et al.* 1979;Kapoor *et al.* 1988; Dodd *et al.* 1987; Jayachandran *et al.* 1992; Thiagarajan and Ahmad 1994; Khalil *et al.* 1994; Tarafdar and Marschner 1994), it is believed that VA mycorrhizal and non-mycorrhizal roots obtain P from the same soil P pools (Bolan 1991; Jennings 1995).

The objective of this research was to investigate the ability of mycorrhizal and non-mycorrhizal western red cedar seedlings to grow when supplied with organic P compounds such as phytic acid, glycerophosphate and ATP, and with inorganic pyrophosphate and KH_2PO_4. In addition to measuring changes in growth parameters and foliar nutrient concentrations, the activities of several phosphatase enzymes were also determined.

Materials and Methods

Plants

Two-year-old western red cedar seedlings, in styroblock containers, were purchased from Koksilah Nursery, Duncan BC, in March 1992. In June, 1992, the seedlings were transplanted into a sand-peat mixture, with four seedings in each 5 L pot. The seedlings were grown in a greenhouse with supplemental fluorescent and incandescent lighting, at 15-25°C. The seedlings were watered with tap water, and were fed weekly with modified Long Ashton

solution (Hewitt 1966), with P at 1/4 strength. In Feb. 1993, the seedlings were transplanted into open pots containing a 3:1 mixture of medium sand and Turface, a calcined montmorillonite clay (Applied Industrial Materials Corporation, Deerfield, IL), with one tree per 5 L pot. They were irrigated with tap water, and were fed weekly with modified Long Ashton nutrient solution.

Feeding Experiment

As all of the seedlings were colonized by VA mycorrhizal fungi (species unknown), a systemic fungicide was applied to half of the pots to suppress the mycorrhizal fungi, to produce a 'non-mycorrhizal' treatment. Benomyl [(1-butyl-carbamoyl)-2-(benzimidazole) carbamic acid, methyl ester; ($C_{14}H_{18}N_4O_3$); sold as 'Benlate' (50 WP) by Later's Chemicals Ltd., Richmond, BC] was applied at a rate of 30 L/pot of a 5 g/L solution, every 9 days beginning April 20, 1993.

The trees were given 300 mL of tap water every third day, plus a light overhead misting to increase the humidity. Beginning May 14, 1993, the watering every ninth day was replaced with 200 ml of modified Long Ashton solution, containing KNO_3 (400 mg/L), K_2SO_4 (350 mg/L), $Ca(NO_3)_2.5H_2O$ (900 mg/L), $MgSO_4.7H_2O$ (500 mg/L), $MnSO_4.4H_2O$ (2.25 mg/L), $CuSO_4.5H_2O$ (0.25 mg/L), $ZnSO_4.7H_2O$ (0.30 mg/L), H_3BO_3 (3.0 mg/L), NaCl (5.0 mg/L) and one of eleven P treatments. These treatments were:

1. No P
2. phytic acid high (inositol hexaphosphoric acid, dodecasodium salt; $C_6H_6O_{24}P_6Na_{12}$; Sigma P-3168), 50 mg P/L

3. phytic acid low, 10 mg P/L

4. ATP high (Adenosine 5'-Triphosphate, disodium salt, Grade II; $C_{10}H_{14}N_5O_{13}P_3Na_2$; Sigma A-3377), 50 mg P/L

5. ATP low, 10 mg P/L

6. glycerophosphate high (disodium salt; $C_3H_7O_6PNa.5H_2O$; Sigma G-6501), 50 mg P/L

7. glycerophosphate low, 10 mg P/L

8. pyrophosphate high ($Na_4P_2O_7.10H_2O$; ACS Fisher S390) 50 mg P/L

9. pyrophosphate low, 10 mg P/L

10. orthophosphate high (KH_2PO_4, BDH ACS 657) 50 mg P/L

11. orthophosphate low, 10 mg P/L

At the time of feeding, the benomyl-treated plants were given 30 mL of a 5 g/L solution of benomyl and 70 mL of tap waster, while the mycorrhizal plants were given 100 mL of tap water. In the winter months, the seedlings were watered with only 200 mL of tap water, and the extra water was eliminated from the benomyl-treated plants at feeding time, The mycorrhizal plants were given only 30 mL of extra tap water, to match the liquid from the fungicide which the benomyl-treated plants received.

There were 5 mycorrhizal and 5 benomyl-treated seedlings for each P treatment; for a total of 110 seedlings. The experiment was arranged in the greenhouse in a completely randomized design, and the pots were rearranged periodically to maintain randomness of greenhouse effects.

Laboratory Analyses

The trees were harvested May 9-20, 1994, after 52-53 weeks of growth. Initially, 2 trees from each treatment were harvested. Immediately after harvest, approximately half of the roots and 50 g soil were assayed for acid and alkaline phosphomonoesterase, phosphodiesterase and pyrophosphatase activity, as described in Tabatabai (1982). Three replicates and one control sample were used per assay and the results for each plant and soil were averaged. For all plants in the experiment, a portion of randomly sampled roots was frozen for later analysis and the soil was air-dried. The above-ground biomass was oven-dried at 60°C for 48 hours, after which the dry weight was measured. The foliage was removed from the branches, ground with a stainless steel coffee grinder, and digested in glass tubes using the Parkinson and Allen (1975) method. The N in the digests was determined colorimetrically using a LaChat Flow Injection Analyzer, while P, Ca, Mg, Cu, Fe, Al and Zn were read using an Inductively Coupled Argon Plasma (ICAP) spectrophotometer (Fisher Scientific Co.). The soils were analyzed for available P using the Bray P1 method (Olsen and Sommers 1982), and for pH in water (1:1, w/v) on one sample per treatment (McLean 1982). Thawed roots were cleared and stained in trypan blue with a modified version of the Phillips and Hayman (1970) method. Roots were cut into 1-2 cm pieces, spread evenly in destaining solution over the bottom of a Petri plate, and counted as per the gridline-intersect method of Giovanetti and Mosse (1980). The tree heights from the root plug, and diameters at

25 cm above the root plug, were measured at the start and end of the experiment.

Statistical Analysis

Statistical analyses were done using the Systat program (Wilkinson 1990) to perform analysis of variance and Tukey's tests. Homogeneity of variance was tested by plotting residuals against estimates. Where necessary, log and log (n+1) transformations were performed.

Results

The diameter changes and mean dry weights at the end of the feeding experiment are shown in Table 8-1, and the results from the analysis of variance can be found in Table 8-2. In Table 8-1 and in subsequent tables where the TREATMENT*MYCORRHIZAL interaction was not significant, but the treatment differences were significant, both the mycorrhizal and benomyl-treated plants were used for each treatment to calculate the means. There were no significant differences among the treatments in tree height or diameter at the start of the feeding experiment (Table 8-2). One year later, the trees receiving the high rate of ATP had the greatest diameter increase while those receiving no P increased the least. The only other treatment in which the diameter increase was significantly different from the no P control was the high orthophosphate treatment. The highest dry weights were found in the treatments receiving high levels of ATP and orthophosphate, while the lowest dry weights were measured in the treatments fed no

Table 8-1: The means and (standard deviations) for diameter change and dry weight of above ground material after the feeding experiment. Ortho is orthophosphate, phyt is phytic acid, glyc is glycerophosphate and pyro is pyrophosphate. L is 10 mg P/*l*, H is 50 mg P/*l*. Different letters in a column indicate statistically significant treatment effects at $p<0.05$. (n=10)

Treatment	Diameter Change (mm)	Dry Weight (g)
No P	3.0 (1.05) c	59.5 (18.6) c
Ortho L	4.6 (1.52) abc	68.6 (10.0) bc
Ortho H	5.4 (1.26) ab	98.3 (14.1) a
ATP L	4.4 (1.26) abc	65.6 (18.0) bc
ATP H	5.6 (1.51) a	91.5 (7.88) a
Phyt L	3.2 (0.79) bc	50.2 (10.3) c
Phyt H	3.4 (1.71) bc	56.7 (15.8) c
Glyc L	3.6 (1.78) bc	60.7 (21.0) c
Glyc H	4.9 (1.29) abc	86.6 (15.0) ab
Pyro L	3.6 (0.97) abc	65.1 (6.41) bc
Pyro H	4.2 (1.48) abc	81.9 (20.0) ab

P, low glycerophosphate, and both rates of phytic acid.

Significant mycorrhizal effects were observed for height change, dry weight, and diameter change (Tables 8-2, 8-3). In all cases, the measurements for the benomyl-treated plants were greater than those for the mycorrhizal plants.

There were no significant differences in concentrations of foliar Al, Mg or Fe at the end of the feeding experiment (Table 8-2). The mean foliar Al concentration was 94.4 ug/g, the mean foliar Mg concentration was 2430.2 ug/g, and the mean foliar Fe

Table 8-2: Analysis of variance results for height and diameter at the start of the feeding study, height and diameter change, dry weight at harvest, and foliar P, Ca, Mg, Cu, Fe, Al, Zn and N. A * indicates significance at $p<0.05$.

	Treat	Myc	Treat*Myc	Mult R_2	N
Ht Start	0.062	0.065	0.749	0.246	110
Ht Change	0.503	0.031*	0.176	0.245	110
Diam Start	0.086	0.287	0.618	0.232	110
Diam Change	0.000*	0.027*	0.201	0.377	110
Dry Weight	0.000*	0.010*	0.315	0.608	110
Foliar P	0.000	0.000	0.046*	0.807	110
Foliar Ca	0.000*	0.227	0.372	0.415	110
Foliar Mg	0.132	0.340	0.759	0.208	110
Foliar Cu	0.002*	0.473	0.103	0.352	110
Foliar Fe	0.280	0.120	0.466	0.219	110
Foliar Al	0.725	0.228	0.448	0.173	110
Foliar Zn	0.000*	0.049*	0.718	0.357	110
Foliar N	0.000	0.000	0.007*	0.625	110

Table 8-3: The means and (standard deviations) for mycorrhizal and benomyl-treated effects on height change, dry weight, diameter change and foliar Zn after the feeding experiment. Different letters in a column indicate statistically significant differences at $p<0.05$. (n=55)

Mycorrhizal Treatment	Height Change (cm)	Dry Weight (g)	Diameter Change (mm)	Foliar Zn (ug/g)
Mycorrhizal	29.7 (12.10) b	67.7 (22.03) b	3.93 (1.78) b	19.31 (6.56) b
Benomyl-Treated	34.6 (11.67) a	75.0 (19.01) a	4.42 (1.24) a	21.39 (5.49) a

Table 8-4: The means and (standard deviations) for concentrations of Ca, Cu and Zn in foliage after the feeding experiment. Ortho is orthophosphate, phyt is phytic acid, glyc is glycerophosphate and pyro is pyrophosphate. L is 10 mg P/*l*, H is 50 mg P/*l*. Different letters in a column indicate statistically significant treatment effects at $p<0.05$. (n=10)

Treatment	**Foliar Ca** (ug/g)	**Foliar Cu** (ug/g)	**Foliar Zn** (ug/g)
No P	9774.8 (871.9) b	6.7 (1.3) bc	17.2 (3.3) b
Ortho L	10950.4 (5650.3) b	8.2 (4.4) abc	18.5 (6.7) ab
Ortho H	10768.8 (1898.4) b	8.9 (2.7) abc	18.2 (4.3) ab
ATP L	9533.9 (1625.4) b	6.7 (1.4) bc	17.6 (2.8) b
ATP H	9207.0 (1502.9) b	8.1 (1.1) abc	21.5 (5.3) ab
Phyt L	8988.6 (1348.9) c	6.5 (1.2) c	17.6 (2.1) b
Phyt H	9323.6 (1665.8) b	7.7 (1.6) abc	17.9 (4.9) b
Glyc L	14585.3 (4484.8) a	9.8 (3.3) a	26.2 (10.3) a
Glyc H	11778.5 (1678.7) ab	9.3 (2.1) ab	20.5 (5.9) ab
Pyro L	11694.9 (2214.8) ab	8.1 (1.3) abc	22.6 (2.8) ab
Pyro H	12413.5 (3438.3) ab	9.1 (3.1) ab	26.1 (6.5) a

concentration was 118.0 ug/g. Significant treatment effects occurred for foliar Ca, Cu and Zn (Tables 8-2, 8-4). The highest foliar Ca concentrations were seen with both levels of

glycerophosphate and pyrophosphate. The plants fed the low level of phytic acid had the lowest foliar Ca concentrations. Foliar Cu was highest when glycerophosphate, at either level, was the P source, and lowest when the trees received the low rates of phytic acid and ATP, or no P. Foliar Zn was highest with the low glycerophosphate and high pyrophosphate treatments, and lowest when the plants were fed no P, low ATP and both levels of phytic acid. There was also a significant mycorrhizal effect for foliar Zn (Tables 8-2, 8-3). Higher concentrations of Zn were found in the foliage of benomyl-treated plants than mycorrhizal plants.

There were significant TREATMENT*MYCORRHIZA interactions for foliar N and foliar P (Tables 8-2, 8-5). The benomyl-treated plants had higher foliar N concentrations for every treatment except the high rate of ATP. The benomyl-treated plants receiving the low rate of phytic acid had the highest N concentrations; the mycorrhizal trees fed high levels of orthophosphate had the lowest foliar N concentrations. Generally, there were no significant differences in foliar P between mycorrhizal and benomyl-treated plants for each P treatment. The exception was the low rate of orthophosphate: the mycorrhizal trees had higher P concentrations than the benomyl-treated seedlings. Foliar P concentrations were higher when the trees were given the higher rate of the P source for all but phytic acid. The low rates of ATP, phytic acid and pyrophosphate resulted in foliar P concentrations which were not significantly different from the controls receiving no P.

Significant TREATMENT*MYCORRHIZA interactions were also found

for mycorrhizal colonization, soil P and root acid phosphatase activity (Tables 8-6, 8-7). Colonization was generally higher in the mycorrhizal plants than in the benomyl-treated plants for each treatment, although significant differences were seen only for the high rates of glycerophosphate and pyrophosphate. The highest level of colonization was in the mycorrhizal treatment without P; the lowest in the benomyl-treated trees fed high rates of orthophosphate, glycerophosphate and pyrophosphate. The concentration of available soil P at the end of the feeding experiment was highest when the plants were fed the high rate of glycerophosphate, pyrophosphate or orthophosphate. Available P was lowest in the soil receiving the low rate of phytic acid (mycorrhizal and benomyl-treated). The mycorrhizal low phytic acid treatment was significantly lower in available soil P than the no P mycorrhizal treatment. The mean soil pH in the pots at the end of the feeding experiment was 5.56.

Root acid phosphatase activity, in *ug p*-nitrophenol/g of roots/hour, was highest in the mycorrhizal plants receiving the low rate of orthophosphate and the high rate of ATP, and in the benomyl-treated seedlings fed the low rate of glycerophosphate and the high rate of orthophosphate (Tables 8-6, 8-7). The other treatments were not significantly different from one another. Soil acid phosphatase activity was significantly higher in the pots containing benomyl-treated plants than in the pots of mycorrhizal trees (Tables 8-7, 8-8). A significant treatment effect was also observed for root alkaline phosphatase activity (Tables 8-7, 8-9).

Table 8-5: The means and (standard deviations) for foliar N (%), and foliar P (ug/g) after the feeding experiment. Ortho is orthophosphate, phyt is phytic acid, glyc is glycerophosphate and pyro is pyrophosphate. L is 10 mg P/*l*, H is 50 mg P/*l*. M is mycorrhizal, NM is benomyl-treated. Different letters for a parameter (eg foliar N), including M and NM indicate statistically significant treatment effects at $p<0.05$. (n=5)

Treatment	Foliar N M	Foliar N NM	Foliar P M	Foliar P NM
No P	2.37 (0.37) bc	2.43 (0.33) b	581.8 (73.6) fg	534.6 (86.0) g
Orth L	1.72 (0.37) ij	2.59 (0.25) a	991.9 (385.8) cdef	556.1 (121.3) g
Orth H	1.67 (0.20) j	1.87 (0.29) hi	1554.5 (288.6) abc	1346.2 (264.1) abcd
ATP L	2.12 (0.14) def	2.61 (0.31) a	694.1 (132.4) efg	727.7 (109.7) efg
ATP H	2.11 (0.10) defg	2.21 (0.13) de	1401.0 (326.4) abcd	1139.4 (367.1) bcde
Phyt L	2.06 (0.19) fg	2.59 (0.15) a	581.5 (86.5) fg	606.9 (50.6) fg
Phyt H	2.06 (0.08) fg	2.40 (0.18) b	1138.4 (180.2) bcde	861.9 (158.1) defg
Glyc L	2.11 (0.14) defg	2.40 (0.19) b	1167.1 (410.7) bcde	743.7 (142.9) efg
Glyc H	1.90 (0.19) h	2.08 (0.21) efg	2034.1 (422.2) a	1555.7 (309.2) abc
Pyro L	2.01 (0.34) fg	2.67 (0.28) a	874.6 (90.0) defg	680.6 (166.1) efg
Pyro H	1.96 (0.16) gh	2.25 (0.37) cd	1615.1 (360.4) abc	1743.4 (497.7) ab

Table 8-6: Means and (std. deviations) for colonization (%), soil P (mg/g) and root acid phosphatase (Rt Ac Pase) activity after the feeding experiment. Orth=orthophosphate, phyt=phytic acid, glyc=glycerophosphate, pyro=pyrophosphate. L=10 mg P/*l*, H=50 mg P/*l*. M is mycorrhizal, NM is non-mycorrhizal. Different letters for a parameter, including M and NM indicate statistical significance at $p<0.05$. (n=5)

Treatment	Col M	Col NM	Soil P M	Soil P NM	Rt Ac Pase M	Rt Ac PaseNM
No P	38.6 (10.9) a	18.4 (7.5) abcde	16.2 (2.0) cd	13.3 (1.5) cde	538 (122) cde	520 (67) e
Orth L	24.8 (7.9) abcd	10.6 (4.7) defgh	18.6 (5.1) bc	11.6 (0.9) cde	1192 (160) a	380 (61) e
Orth H	17.0 (7.0) abcdef	6.8 (2.2) fgh	31.0 (5.5) a	30.1 (7.8) a	592 (116) cde	540 (156) e
ATP L	26.8 (10.2) abc	28.0 (7.1) abc	11.2 (1.8) de	12.0 (1.9) cde	586 (374) cde	628 (353) bcde
ATP H	8.4 (3.6) efgh	15.9 (6.1) bcdef	26.8 (6.3) ab	26.2 (4.7) ab	933 (35) abc	427 (239) e
Phyt L	15.4 (5.0) bcdef	17.0 (5.5) abcdef	9.7 (3.0) e	10.3 (0.8) de	492 (188) e	412 (26) e
Phyt H	28.2 (14.2) abc	14.2 (5.3) cdefg	14.2 (2.3) cde	10.1 (2.6) e	722 (349) bcde	701 (73) bcde
Glyc L	35.8 (13.1) ab	17.0 (3.9) abcdef	15.7 (2.1) cd	13.7 (2.2) cde	376 (17) e	954 (135) ab
Glyc H	20.0 (7.9) abcde	5.8 (2.2) gh	28.3 (3.5) ab	30.9 (8.8) a	538 (111) e	541 (110) e
Pyro L	27.2 (13.0) abc	14.6 (5.6) cde	13.7 (1.6) cde	12.1 (1.3) cde	535 (192) e	581 (173) cde
Pyro H	33.4 (13.4) abc	5.2 (1.3) h	29.2 (3.6) a	26.6 (2.8) ab	575 (416) de	906 (612) abcd

Table 8-7: Analysis of variance results for percent colonization, soil P at the end of the feeding trial, root and soil alkaline phosphatase (Pase), root and soil acid phosphatase, root and soil diesterase, and root and soil pyrophosphatase. A * indicates significance at $p<0.05$.

	Treat	Myc	Treat*Myc	Mult R^2	N
Colonization	0.000	0.000	0.000*	0.720	110
Soil P	0.000	0.005	0.036*	0.851	110
Root AlkPase	0.000*	0.803	0.425	0.735	44
Soil AlkPase	0.078	0.319	0.998	0.498	44
Root AcidPase	0.664	0.491	0.034*	0.589	44
Soil AcidPase	0.060	0.000*	0.301	0.801	44
Root DiesPase	0.659	0.662	0.548	0.424	44
Soil DiesPase	0.963	0.387	0.933	0.226	44
Root PyroPase	0.063	0.893	0.578	0.568	44
Soil PyroPase	0.602	0.477	0.268	0.491	44

Table 8-8: The means and (standard deviations) for mycorrhizal and benomyl-treated effects on soil acid phosphatase activity (*ug p*-nitrophenol/g/h) after the feeding experiment. Different letters in a column indicate statistically significant differences at $p<0.05$. (n=55)

Mycorrhizal Treatment	Soil Acid Phosphatase
Mycorrhizal	14.3 (9.3) b
Benomyl-Treated	106.0 (140.5) a

The highest root alkaline phosphatase activity was associated with plants fed the high rate of phytic acid, both rates of glycerophosphate, and the low rate of pyrophosphate. Root alkaline phosphatase activity was lowest in the no P, low orthophosphate and low ATP treatments. There were no significant differences among

the treatments in the activities of soil alkaline phosphatase, root and soil diesterase, and root and soil pyrophosphatase. The mean soil alkaline phosphatase activity was 20.1 *ug* *p*-nitrophenol/g/h. The mean soil diesterase activity was 7.9 *ug* *p*-nitrophenol/g/h, and that of roots was 303.0 *ug* *p*-nitrophenol/g/h. The mean soil pyrophosphatase activity was 3.7 *ug* P/g/h, and the mean root pyrophosphatase activity was 29.7 *ug* P/g/h. The variability was high within and among the P treatments for these enzyme assays. Table 8-10 shows the correlation matrix for the results from this greenhouse study. Root acid phosphatase activity was positively correlated with root diesterase activity and with diameter change. The activities of soil and root alkaline phosphatase were positively correlated, and there was a positive relationship between soil alkaline phosphatase and soil pyrophosphatase. The diameter and height at the start of the feeding experiment were positively correlated, as were the changes in diameter and height at the end. Dry weight correlated positively with diameter change, foliar P and soil P. Foliar Ca, Cu, Mg and Zn were positively correlated with one another, and foliar P was positively correlated with soil P and with foliar Ca, Cu and Zn. There was a high positive correlation of foliar Al to foliar Fe. Foliar N was negatively correlated with soil P and foliar P.

Table 8-9: The means and (standard deviations) for root alkaline phosphatase activity (*ug p*-nitrophenol/g/h) after the feeding experiment. Ortho is orthophosphate, phyt is phytic acid, glyc is glycerophosphate and pyro is pyrophosphate. L is 10 mg P/*l*, H is 50 mg P/*l*. Different letters in a column indicate statistically significant treatment effects at $p<0.05$. (n=10)

Treatment	Root Alkaline Phosphatase	
No P	82.2 (39.3)	b
Ortho L	97.7 (2.6)	b
Ortho H	141.8 (21.2)	ab
ATP L	120.6 (17.0)	b
ATP H	197.1 (69.7)	ab
Phyt L	159.1 (64.6)	ab
Phyt H	217.6 (45.1)	a
Glyc L	273.8 (57.8)	a
Glyc H	257.5 (122.9)	a
Pyro L	259.5 (20.5)	a
Pyro H	155.1 (79.3)	ab

Table 8-10: Correlation matrix. Acidrt and acidsl are root and soil acid phosphatase activity; alkrt and alksl are root and soil alkaline phosphatase activity; col is colonization; diamch is change in diameter; diamst is diameter at start; diesrt and diessl are root and soil diesterase activity; drywt is dry weight; folal, folca, folcu, folfe, folmg, foln, folp and folzn are foliar Al, Ca, Cu, Fe, Mg, N, P and Zn; htchg is height change; htst is height at start; pyrort and pyrosl are root and soil pyrophosphatase activity; and soilP is the soil P content.

	acidrt	acidsl	alkrt	alksl	col	diamch	diamst	diesrt	diessl	drywt	folal	folca
acidrt	1.000											
acidsl	-0.180	1.000										
alkrt	0.027	-0.186	1.000									
alksl	-0.234	-0.118	0.448	1.000								
col	0.069	-0.264	-0.211	-0.053	1.000							
diamch	0.485	-0.087	0.073	-0.265	-0.365	1.000						
diamst	-0.401	0.211	-0.037	0.052	0.132	-0.166	1.000					
diesrt	0.454	0.017	0.227	0.100	0.168	0.268	-0.077	1.000				
diessl	0.108	0.087	0.019	-0.100	-0.126	0.081	0.156	0.149	1.000			
drywt	0.272	0.037	0.125	-0.189	-0.326	0.757	0.213	0.214	0.068	1.000		
folal	-0.295	-0.102	-0.132	-0.037	-0.073	-0.011	-0.109	-0.302	-0.142	-0.011	1.000	
folca	-0.020	-0.268	0.334	0.078	0.096	0.011	-0.036	0.025	-0.043	0.059	0.011	1.000
folcu	-0.085	-0.073	0.379	0.331	0.014	0.105	-0.057	0.101	-0.131	0.182	-0.128	0.692
folfe	-0.271	-0.180	-0.057	0.023	-0.048	0.005	-0.083	-0.288	-0.094	0.033	0.922	0.180
folmg	-0.183	-0.026	-0.083	0.168	0.049	0.040	-0.090	-0.190	0.070	0.003	0.068	0.739
foln	-0.107	0.179	0.033	0.082	-0.062	-0.158	0.076	-0.086	0.088	-0.289	-0.089	-0.185
folp	0.027	-0.193	0.218	0.161	-0.166	0.316	0.013	-0.015	0.072	0.560	0.045	0.403
folzn	0.178	-0.176	0.255	0.019	-0.051	0.120	0.033	-0.116	-0.052	0.252	0.100	0.615
htchg	0.091	-0.123	0.089	-0.054	-0.251	0.438	0.039	-0.047	-0.051	0.335	-0.125	-0.062
htst	-0.212	0.148	-0.048	-0.028	-0.092	0.000	0.514	-0.158	0.001	0.225	-0.047	-0.082
pyrort	-0.001	-0.047	0.297	-0.043	0.175	0.170	0.231	0.176	0.270	0.367	-0.079	-0.045
pyrosl	0.036	-0.007	0.232	0.420	0.223	0.051	0.060	0.218	0.156	-0.029	-0.067	-0.191
soilp	0.107	-0.015	-0.003	0.141	-0.226	0.379	0.084	-0.001	0.015	0.611	-0.032	0.087

	folcu	folfe	folmg	foln	folp	folzn	htchg	htst	pyrort	pyrosl	soilp
folcu	1.000										
folfe	-0.022	1.000									
folmg	0.679	0.160	1.000								
foln	-0.182	-0.107	-0.255	1.000							
folp	0.485	0.180	0.303	-0.472	1.000						
folzn	0.536	0.261	0.434	0.030	0.392	1.000					
htchg	-0.018	-0.109	-0.062	0.139	0.029	0.091	1.000				
htst	-0.071	-0.103	-0.086	0.138	-0.074	-0.011	-0.167	1.000			
pyrort	0.013	0.044	-0.114	-0.002	0.178	0.076	-0.012	0.036	1.000		
pyrosl	0.152	-0.031	0.268	-0.104	0.019	-0.098	-0.148	-0.276	0.317	1.000	
soilp	0.223	0.012	0.060	-0.465	0.687	0.105	0.124	0.024	0.084	-0.006	1.000

Discussion

Benomyl, a systemic fungicide, was used to produce a 'non-mycorrhizal' control, and colonization rates were generally higher in mycorrhizal plants than benomyl-treated ones. Colonization rates were also influenced by P source, with the highest rates of colonization in mycorrhizal plants not fed P, and the lowest levels in plants receiving high rates of orthophosphate, glycerophosphate and pyrophosphate.

Benomyl is known to decrease VA mycorrhizal colonization in many plant species (Bailey and Safir 1978; Rhodes and Larson 1981; Hale and Saunders 1982; Verkade and Hamilton 1983; Fitter 1986; Fitter and Nichols 1988; Sukarno *et al.* 1993), and is thought to be well-suited to generating non-mycorrhizal control plants (Fitter 1986; Sukarno *et al.* 1993). It reduces the number of living internal hyphae, arbuscules and living external hyphae, although the fungus is still able to colonize the root to some extent (Sukarno *et al.* 1993). This was seen in the benomyl-treated seedlings of this study, which still had low levels of mycorrhizal colonization. A side-effect of benomyl treatment, which in retrospect should have been anticipated, was the apparent N enrichment of non-mycorrhizal plants. Benomyl contains a benzimidazole group, and N comprised 19.3% of the benomyl molecule. This appears to be supplying the non-mycorrhizal plants with extra N, at a calculated rate of 0.029 g N/pot at each feeding. While there has been no overt discussion of the effects of benomyl on

plant nutrition in the VA mycorrhizal literature, Verkade and Hamilton (1983) and Fitter (1986) both report improved plant growth, with increased foliar N concentrations, in benomyl-treated plants. Because benomyl was applied to the seedlings in this study for more than one year, the effects of benomyl on plant N many be more pronounced than in an experiment of shorter duration. The negative correlation of foliar N to foliar P and soil P probably also reflects the N-enrichment of benomyl-treated plants.

The benomyl-treated plants were generally larger than the mycorrhizal trees, with greater increases in dry weight and diameter. This may be due to the N from benomyl, but may also reflect the cost to the plant for the benefits of mycorrhizal colonization: Marschner and Dell (1994) estimate that 10-20% of net photosynthates are required for formation, maintenance and function of mycorrhizal structures. Shoot and root dry weights are usually increased by inoculation with VA mycorrhizal fungi (Harley and Smith 1983; Tarafdar and Marschner 1994), but this is usually relative to completely non-mycorrhizal plants, which may be severely P deficient. Since the benomyl-treated trees were not completely uncolonized, P stress in these plants would be lessened relative to tree roots with no colonization. The increased growth of the benomyl-treated plants suggests that the cedar trees used in this experiment were N-limited.

The source of phosphorus had a major influence on plant growth and nutrition in this study, as did colonization, but to a lesser extent. The only significant difference between mycorrhizal and

benomyl-treated seedlings for foliar P occurred with the low orthophosphate treatment: the mycorrhizal plants had higher foliar P concentrations than benomyl-treated plants. Generally, foliar P was higher with higher rates of each P source, and the low rates of ATP, phytic acid and pyrophosphate resulted in foliar P concentrations which were not significantly different from the no P control. There appears to be some P available to No P plants as well, because plant height and dry weight increased with this treatment. Although available P was not measured in the growth medium prior to the start of the experiment, Jayachandran *et al.* (1992) indicate that the available P concentration of a similar growth medium was 14 mg/kg. This, combined with translocation, prevented these trees from showing severe P deficiency symptoms.

The poorest tree growth resulted from the phytic acid treatments, especially at the low application rate. These trees had the lowest foliar P concentrations, as well as low levels of foliar Ca, Zn and Cu. In animal nutrition, phytic acid is known to form complexes with essential metals such as Ca, Zn, Fe, Mg, Mn and Cu, causing deficiencies (Cosgrove 1980; O'Neill *et al.* 1980). This also appears to be occuring with the cedar in this experiment. As a calcium accumulator (Weetman *et al.* 1988), cedar is especially sensitive to Ca availability, and the symptoms which appeared in the trees receiving the low phytic acid treatment were consistent with Ca deficiency (Weetman *et al.* 1988). In the literature, some researchers report that plants and mycorrhizae can use phytic acid

as a P source (Saxena 1964; Helal 1990; Mitchell and Read 1981; Adams and Pate 1992; Antibus *et al.* 1992; Pasqualini *et al.* 1992; Jayachandran *et al.* 1992; Tam and Griffiths 1993; Tarafdar and Marschner 1994), but others report that the P of phytic acid is not available to plants and mycorrhizae (Thomas *et al.* 1982; Adams and Pate 1992; Barrett-Lennard *et al.* 1993). There were no reports of other nutrient deficiencies with phytic acid as a P source, possibly because of the increased sensitivity of cedar to Ca deficiencies, or the shorter duration of other experiments. Interestingly, cedar grew better with the high rate of phytic acid than the low rate, which is the reverse of what would be expected if growth problems were caused by the complexing of other nutrients. Findenegg and Nelemans (1993) also report better growth with high levels of phytic acid relative to low levels. This may be related to enzyme induction, which will be discussed in more detail below. Although phytic acid and other inositol hexaphosphates are relatively abundant in soils (Stevenson 1986), they are adsorbed to soil surface and are probably not readily available to plants. In this experiment, Na-phytate was used, which is soluble, while in soil the insoluble Fe- and Al-phytates are more common (Read 1991), making inferences about phytic acid as a P source in soil difficult from this study.

The greatest plant growth was achieved with the high rate of ATP. The plants in this treatment also contained foliar N concentrations than other treatments, suggesting that the improved

growth was due to N as much or more than P from the ATP. The trees were also able to use glycerophosphate and inorganic pyrophosphate, which are known to provide a P source to plants and mycorrhizae (Bartlett and Lewis 1973; McKercher and Tollefson 1978; Beever and Burns 1980; Thomas *et al.* 1982; Tarafdar and Classen 1988; Pasqualini *et al. 1992; Jayachandran* et al. 1992; Barrett-Lennard *et al.* 1993; Tam and Griffiths 1993; McElhinney and Mitchell 1993). Growth was improved with all P sources when they were supplied at the higher rate.

The use of organic P compounds by western red cedar appears to be facilitated by phosphatase enzymes, as shown by the correlation of root acid phosphatase to the change in plant height and diameter. Enzyme activities were higher on roots than in bulk soil, suggesting that these enzymes are produced by roots, mycorrhizae or rhizosphere microorganisms. It should be noted that the "root" phosphatases discussed here are more accurately "rhizosphere" phosphatases, as the roots were not washed prior to enzyme assay. Because this experiment was not conducted under sterile conditions, these root phosphatase assays reflect enzyme activities of plant roots, mycorrhizae and rhizosphere microorganisms. In an attempt to separate rhizosphere and root phosphatase activity, a duplicate portion of the roots was shaken in an antibiotic solution prior to the enzyme assays. Unfortunately, this produced results which were highly variable, with no obvious patterns, and so these data were not included in

this report. "Soil" phosphatase activities were measured in the bulk soil, and were all very low. Rhizosphere phosphatase activities tend to be higher than activities remote from plant roots, due to greater microbial numbers in the rhizosphere and possibly because of higher phosphatase activities of rhizosphere organisms and the excretion of plant root enzymes (Spier and Ross 1978).

Phosphatases are inducible by phosphate depletion (Goldstein 1992). They are high in soils with high organic C and organic P contents (Appiah and Thomas 1982; Rojo *et al.* 1990; Baligar *et al.* 1991), are sensitive to soil moisture levels (Spier and Cowling 1991; Baligar *et al.* 1991), and are strongly inhibited by inorganic phosphate (Spier and Ross 1978). Higher plants do not produce alkaline phosphatases (Speir and Ross 1978; Tabatabai 1982). In this study, enzyme differences between mycorrhizal and benomyl-treated seedlings were found only for root acid phosphatases, and these were influenced by P source. The P source also affected the activities of root alkaline phosphatase, while root diesterases and pyrophosphatases were present but were not significantly influenced by P treatment. There are varying reports in the literature as to the production of alkaline phosphatases by mycorrhizae. All types of mycorrhizae have been shown to produce acid phosphatase (eg Read 1991; Dodd *et al.* 1987), but alkaline phosphatase activity has been reported by only a few researchers (Gianinazzi-Pearson and Gianinazzi 1976, 1978; Ho and Zak 1979; Krishna *et al.* 1983;

Kapoor *et al.* 1988; Tarafdar and Classen 1988; Bae and Barton 1989; Read 1991; Pasqualini *et al.* 1992; McElhinney and Mitchell 1993; Thiagarajan and Ahmed 1994; Tarafdar and Marschner 1994). In VA mycorrhizae, alkaline phosphatases specific to the VA mycorrhizae and of suspected fungal origin have been reported (Gianinazzi-Pearson and Gianinazzi 1978). Thiagarajan and Ahmed (1994) found extra peaks for mycorrhizal but not non-mycorrhizal alkaline and acid phosphatases by column chromatography. There are also reports of ATPases in plants and mycorrhizae (Tikhaya *et al.* 1990; McArthur and Knowles 1993), which may be responsible for the hydrolysis of ATP in the present study.

Both P source and rate of application influence phosphatase activity in this study. Acid phosphatase is higher in mycorrhizal plants, and in mycorrhizal plants is stimulated by low orthophosphate and high ATP, while in benomyl-treated seedlings it is stimulated by low glycerophosphate and high pyrophosphate. Alkaline phosphatase activity was increased by glycerophosphate, low levels of pyrophosphate and high levels of phytic acid. The hydrolysis of phytic acid by alkaline phosphatases at high levels may account for increased growth despite nutrient deficiencies, as previously discussed. The enzyme may be inducible only when there is sufficient substrate present, and hydrolysis may release some of the Ca, Zn and Cu, in addition to P. The variability in enzyme activity with the different levels of P substrates suggests that a number of enzymes may be operating as acid and alkaline

phosphatases, each induced with different rates of substrate.

In a study of phosphatase activities in soils of Douglas-fir and hemlock stands of southern Vancouver Island, Pang and Kolenko (1986) reported that phosphomonoesterase activity was highest in the forest floor, and decreased with depth and with fertilization. Neutral phosphatases were present on the sites, as well as alkaline phosphatase activity under Douglas-fir. In the current study, phosphatase activity was not measured in the soils of the research sites on northern Vancouver Island.

It is apparent from these results that cedar can access P_o compounds, with or without mycorrhizae. Plants grown under sterile conditions, without mycorrhizae,can use organic P forms such as phytic acid if the P form is supplied at a sufficiently high rate (Findenegg and Nelemans 1993). Lupins (*Lupinus* spp.), which never form mycorrhizae, can also obtain P from organic P sources (Adams and Pate 1992). As noted by Jayachandran *et al.* (1992), the P sources which reduce colonization of mycorrhizal plants tend to stimulate growth and P uptake in non-mycorrhizal plants. Apparently, mycorrhizal plants benefit from access to P_o, but a P_o source which can be used directly by the plant may reduce reliance on the symbiosis. As cedars in forests are always colonized by VA mycorrhizae (Curran and Dunsworth 1988), it may be that soil levels of nutrients, both inorganic and organic, are too low for the plant to access directly. One drawback to the current study is that it is impossible to separate the effects of rhizosphere microorganisms from those of plants or mycorrhizae. However, in the forest, cedar

would also have associated rhizosphere organisms, so the results from this study may better reflect field conditions than a study conducted under aseptic conditions. In forests, synergistic interactions between bacteria and VA mycorrhizae may be important in facilitating P mobilization (Read 1991).

Some of the apparent differences in this study between mycorrhizal and benomyl-treated plants, such as elevated Zn concentrations in benomyl-treated trees, may be due to the improved N nutrition of the benomyl-treated trees from the benomyl treatments. Unfortunately, this may have also affected some differences in plant P. Future studies using benomyl to produce non-mycorrhizal controls would be well-advised to compensate for the increased N from benomyl by supplementing the mycorrhizal plants.

Conclusions

Western red cedar mediated the hydrolysis of the organic P compounds glycerophosphate and ATP, and inorganic pyrophosphate, particularly when these compounds were supplied at high rates. The trees grew very poorly with phytic acid, which may have complexed Ca, Zn and Cu from the nutrient solutions, inducing Ca deficiencies. Although phophatases were produced, it was impossible to distinguish those of the plant and mycorrhizae from the enzymes of rhizosphere microorganisms. Mycorrhizal colonization improved foliar P content, but other mycorrhizal effects may have been masked by N enrichment from benomyl, the fungicide used to produce non-mycorrhizal control trees.

CHAPTER NINE

GENERAL CONCLUSIONS

This study was initiated to investigate the role of phosphorus in the growth check problem of trees replanted onto cedar-hemlock (CH) stands after logging and slash-burning, and to investigate the use of organic P forms by western red cedar, which did not experience a growth check on these siges to the same degree as other species such as western hemlock and Sitka spruce.

Prior to the onset of this thesis research, it was hypothesized that some procedures to determine organic, total and available P might be better suited to the Orthic Ferro-Humic Podzols of the CH and HA forests of northern Vancouver Island than would other procedures. The results of this study indicate that the Parkinson and Allen digestion procedure removes more total P from soil samples than the Saunders and Williams ignition and extraction method. Both the Saunders and Williams method and the Bowman and Moir extraction procedure appeared to overestimate organic P in these soils. This was established by a comparison of the P-extraction techniques to the P forms revealed by ^{31}P NMR spectroscopy. Either of the Bray P1 or Mehlich 3 procedures were suitable to determine available P.

A second hypothesis was that some extraction procedures for ^{31}P NMR spectroscopy might be better suited to these soils than other extraction procedures. It would appear from this reasearch than an ideal soil extractant for ^{31}P NMR does not yet

exist. The new extraction procedure for organic P developed by Bowman and Moir, which had not been used as an extractant for ^{31}P NMR spectroscopy prior to this study, shows great promise because it extracts more of the total organic P than do the convential extractants NaOH and Chelex. However, this extractant also maintains paramagnetic ions such as Mn in solution, causing line broadening and reducing the quality of the spectra. Thus a compromise must be made to obtain reasonably interpretable spectra and representativeness of the organic phosphorus forms.

A third hypothesis of this thesis was that the soils of the mature CH forests contained different P forms from the mature HA stands, as well as differences in other aspects of the soil chemistry. This study revealed that, although significant differences exist between the two forest types for pH, C concentration, loss on ignition, C/N and C/P ratios and extractable LCa concentration, significant differences in P concentration and forms did not exist.

The fourth hypothesis of this research was that the soils of the CH stands 10 years, 5 years and immediately after burning contained different P forms and concentrations from one another and from the mature stands, as well as differences in other aspects of the soil chemistry. Immediately after clear-cutting and burning, the soils of the CH forest types experienced an ashbed effect, which temporarily increased the pH and the concentrations of available P, Ca, Mg, and Mn in the surface

horizons. By 10 years after cutting and burning, the concentrations of these nutrients had returned to preburn levels. However, significant decreases in organically-bound Fe and Al, and organic P, were observed in mineral horizons 10 years postburn. These decreases were attributed to altered illuviation patterns in these podzolic soils from the removal of organic matter by burning. Although total P concentrations were unchanged, there was a shift from organic P forms to inorganic P forms, and changes in P forms with time at depth in the profile. This may have a significance in altering the movement of sesquioxides through the soil profile and the relationship of organic P to inorganic P at lower depths in the soil. This would indicate that more orthophosphate may accumulate in B horizons after burning. Over time, however, the effect of the burn becomes less pronounced.

The final hypothesis of this research was that western red cedar were able to use organic P forms in addition to, or instead of, inorganic forms, and that mycorrhizal trees used different P forms from non-mycorrhizal trees. Although N enrichment of the 'non-mycorrhizal' seedlings from benomyl interfered wtih the results of this study, the trees were able to grow when supplied with the organic compounds glycerophosphate and ATP, and the inorganic compounds KH_2PO_4 and pyrophosphate, especially when these compounds were supplied at high rates. Phosphatases were produced, but it was impossible to distinguish those of the plant and mycorrhizae from the

enzymes of rhizosphere microorganisms.

This study suggests a number of research directions. Phosphorus-31 NMR spectroscopy should be tied into conventional fractionation and partitioning techniques, to further our understanding of soil organic matter. To further develop extraction techniques for ^{31}P-NMR, other combinations of extractants and resins could be tried, in an attempt to achieve the high percentage of total P extracted by the NaOH-EDTA procedure, but without the concommitant extraction of paramagnetic ions. It would be very interesting to resample the 5-year and 10-year postburn sites used in this study in 5 or 10 years, to determine if the 5-year sites would eventually resemble the 10-year sites in soil chemistry, and to determine how long-term the effects of burning are. The results of the greenhouse study also warrant further investigation, in a simpler study with better controls on rhizosphere organisms, and perhaps with a different fungicide to control mycorrhizal colonization.

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