PHOSPHORUS CYCLING IN FAST GROWING FOREST PLANTATIONS: AVAILABILITY, PLANT UPTAKE AND THE ROLE OF FOREST FLOOR

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en **Joan Romanyà i Socoró** per a optar
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A la Isabel, perquè
s'ho mereix.

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GENERAL INTRODUCTION

Fast growing forest plantations can arbitrarily be defined by those which mean annual increment exceeds 14 m^3 ha^{-1} yr^{-1}. Their area in 1981 totalled 6.8 million ha (2.4 of which was in radiata pine; *Pinus radiata* D.Don) and was confined to the tropics, the warm temperate Southern Hemisphere regions, and to the eucalypt (mostly *Eucalyptus globulus* Labill.) and radiata pine plantations of Spain and Portugal (Sutton, 1984). Fast growing plantations have much higher productivity and sensitivity to soil fertility (Crane, 1984) than natural forests and consequently they have a higher demand of soil nutrient and water. To achieve these high standards of productivity, intensive management practices are required. These particular characteristics of fast growing species have provided a convenient frame to develop the knowledge about forest nutrition and management. On the other hand, considerable doubt is expressed by both foresters and general conservationist groups, about the ability of fast growing plantations to sustain productivity through a number of rotations (Gessel, 1984). In fact, Keewes (1966), Florence (1967) and Bendall (1968) reported serious losses of productivity in the second rotation of radiata pine plantations in Australia. Further research proved that the second rotation decline was caused by a reduction of soil nutrient availability and it could be readily overcome by site preparation techniques, such as weed control or fertilizer application (Woods 1981, Stone 1982). Since fast

growing plantations high productivity is often highly dependant on site preparation techniques, unapropiate management of these plantations may easily jeopardize site fertility and cause remarkable productivity declines.

Nitrogen (N) and Phosphorus (P) deficiencies have been characterized to produce large reductions on forest growth. P deficiency has been found to be the most common deficiency in forest soils, especially in fast growing plantations (Will, 1985, Turner & Lambert 1986, Romanyà, 1992). In the soil-plant system, commonly more than 90 % of the P is retained in the soil (Ozanne, 1980). However, because of its low solubility and mobility, most of this phosphorus is not readily available for plants. Phosphorus interacts with soil in an array of different ways. In acid soils, inorganic orthophosphate reactions with Fe and Al are generally thought to control P availability (Syers & Iskandar, 1981), whereas in basic soils P mostly reacts with Ca. On the other hand, phosphorus is specifically sorbed at the surface of Fe- and Al-hydroxides and at the broken edges of phyllosilicates (Sposito, 1989). Phosphorus also reacts with Al complexed with humic and fulvic acids in soils (Arp & Meyer, 1985). Especially in forest soils, a large proportion of the total P often exists in organic form (Anderson, 1980). The cycling of organic P has a large impact on P availability and long term ecosystem productivity (Halstead & McKercher, 1975). Organic P interacts with the soil solid phase in a similar way than inorganic (orthophosphate) P. It is generally accepted that before becoming plant available organic P must be mineralized. Most of this

mineralization occurs as a result of the action of extracellular phosphatase that can be produced by roots and soil microorganisms.

Organic N pools contain most of the N in the soil. Unlike P, there are clear indications in the literature suggesting that some simple organic N compounds can be taken up by mycorrhizal fungi (Bowen & Smith, 1981), and subsequently transferred to plants. In spite of that, N mineralization still plays the most important role in supplying this nutrient to plants. Some of the N organic pools are fairly labile and mineralize readily while others are very recalcitrant due to complex chemical structures and physical occlusion in soil microagregates (Sollins et al., 1984). The major sources of inorganic N for plants are Nitrate (NO_3-N) and ammonium (NH_4-N). NH_4-N is retained in the soil cation-exchange sites while NO_3-N is a highly mobile compound in the soil.

To take up low mobility nutrients, plants have developed several mechanisms. Because of the extremely low mobility and solubility of P, most of these mechanisms seem to be addressed to obtain phosphorus from the soil. Romer et al. (1988) showed that root length strongly relates to plant phosphate absorption and, Mclauglin & James (1991) showed significant relationships between root length and P availability (McLauglin & James, 1991). Plant roots are able to exudate phosphatase enzims to mineralize P and organic anions, such as citrate or oxalate, which owing to their chelating abilities with Fe, Al and Ca can

increase P solubility (Rovira & Davey, 1971). Competition between these anions and phosphate esters has also been mentioned as a mechanism for greater P solubility (Earl et al. 1979). On the other hand, plant roots can induce a pH decrease in the soil-root interface, mainly due to the higher net inflow of cations than anions (Hedley et al. 1982a). These root induced pH changes can affect soil organic and inorganic P depletion. Unlike P, N mineralization is mostly driven by the structure of the organic N-binding compounds and is not regulated by the N availability for plant roots (McGill & Cole, 1981). Therefore, plants have no way to deliberately improve soil N availability.

Mycorrhizal infection effects in increasing plant growth have been well documented by several authors (Tinker, 1978, Gianinazzi-Pearson et al. 1981). Although the beneficial effects of mycorrhiza on plant growth have been mostly attributed to an increase of low mobility nutrient availability, especially P, N availability can also be increased by mycorrhiza (Binkley & Vitousek, 1989). Barber (1984) and Tinker (1984) showed that mycorrhizal infection can increase N availability by allowing mycorrhizal roots to compete more efficiently for inorganic N with soil microorganisms. The mechanisms by which mycorrhiza enhance the ability of the root system to obtain P can be of physical or chemical nature (Bolan, 1991). The physical mechanisms consist of the large increase of root-mycorrhiza soil exploration zone and therefore, to reduce the distance for P diffusion and to increase the surface of uptake. The chemical mechanisms introduced by

mycorrhiza consist of releasing large quantities of organic chelating anions (Harley, 1975) or enhancing P mineralization by increasing phosphatase activity (Hetrick, 1989). Most of the research about mycorrhiza deals with the effects of mycorrhiza on P uptake (Allen, 1991), and their effect on the effect of other nutrients has received less attention.

Most temperate forest tree species are mycorrhizal (Kottke & Oberwinkler, 1986). Although only a 3 % of phanerogams have ectomycorrhizal associations, in some families like Pinaceae, Salicaceae, Betulaceae and Fagaceae, ectomycorrhizal associations are thought to be essential for tree survival under natural conditions (Meyer, 1973). However, most of the work carried out with mycorrhizal associations have been dealing with Vessiculo-Arbuscular (VA) mycorrhizae and under controlled conditions confined to a few fungi selected strains. A better understanding of the effects of native ectomycorrhizal associations in radiata pine forest soils nutrient cycling and plant growth response would help to the nutrition management of these plantations.

The evaluation of site fertility is a major need to optimize the management of fast growing plantations. Accurately evaluating site fertility requires an understanding of the factors affecting nutrient uptake. The use of mathematical models has provided to some extend the possibility to integrate these different factors by using theoretical considerations. Hence, mathematical nutrient uptake

simulations have been based on the mass flow diffusion theory for ion transport in soil to the root surface and on root growth and uptake kinetic characteristics (Baldwin et al. 1973; Claassen & Barber, 1976). Although mechanistic uptake models do not consider a lot of processes actually occurring in soils, such as those related to mycorrhiza, mineralization, root exudates and so on, they have been proved to be able to predict nutrient uptake in many different cases for either agronomic crops or forest species seedlings (Silberbush and Barber 1983, Schenk & Barber, 1980, Rengel & Robinson, 1990; Kelly et al. 1992). Nonetheless, most of these studies have been carried out either in low or high nutrient availability conditions and none of them has been applied simultaneously in contrasted fertility conditions. On the other hand, the effects of mycorrhiza on nutrient uptake have never been approached from a modelling point of view.

The most distinctive characteristics of forest soils is the large amount of organic matter they have in their surface horizons known as forest floor. This organic matter is structured in layers with different stages of decomposition. Forest floor horizons and their characteristic microflora and fauna are the most dynamic phase of the forest environment (Pritchett & Fisher, 1987). The activity of the forest floor is essential for the maintenance of nutrient cycles, particularly N and P. Baule & Fricker (1970) reported a large decrease on site productivity after raking the forest floor. Because of its high dynamism and handiness, the forest floor is one of the elements in the forest which alteration has mostly been used to manage soil

fertility. Although there is information in the literature about the effects of forest and slash management techniques on soil fertility (Flinn et al. 1980; Attiwill et al. 1985; Smethurst & Nambiar, 1990), little information is available about the amounts and quality of nutrients annually leached from the forest floor.

Prescribed fires or slash burning techniques are among the site preparation activities commonly used that alter the forest floor. Despite a large proportion of nutrients contained in the forest floor are lost during fires (Raison et al. 1985), following fires soil nutrient availability is normally high (Ellis and Graley, 1983; Wilbur and Christensen, 1985; Simms, 1987). While short term effects of fire on N cycling are reasonably well understood (Walker et al. 1986), the effects of fire on P cycling have received less attention.

The research reported here originated from the PhD dissertation presented by Cortina (1992). He studied soil fertility transformations and nutrient allocation by first rotation radiata pine plantations, growing in Typic and Dystric Xerocrept soils (US soil taxonomy), developed from granitic and granodioritic parent materials respectively in Catalonia. Although granodioritic soils appeared to be quite fertile, they had very low organic matter content (carbon content always <1.5 %) and similar to granitic soils were shallow (often less than 50 cm). Fructifications from one of the most beneficial ectomycorrhizal fungi for radiata pine growth (*Rhizopogon* sp.) (Chu-Chou & Grace, 1988) were found in some of these sites,

and field observations revealed that most of the roots were infected by ectomycorrhizae. These facts suggested that naturally ocurring mycorrhizal associations may play an important role in supplying nutrients to trees.

Unlike plantations growing in granodiorite soils, those growing in granite soils were P deficient. In these plantations the amount of P in the mineral soil was low. Cortina (1992) showed that a significant proportion of soil nutrient pools was retained in the forest floor, and that organic matter decomposition was high. Under these conditions, it is likely that nutrient dynamics in the forest floor play an important part in the cycling of nutrients.

Therefore, the main two objectives in the following research were: (i) To study the role of naturally occurring mycorrhizae in the supply and uptake of nutrients in radiata pine plantations growing in soils derived from granodiorite. And (ii), evaluate the role of the forest floor in the cycling of nutrients in a mature radiata pine plantation, and assess the effect of altering the forest floor by burning, on soil P fertility, in a P deficient mixed *Eucalyptus* sp. forest.

To address the above mentioned objectives the following research was divided into three different parts dealing with different aspects of soil P fertility and plant uptake in fast growing species forest soils.

The first part which address the first main objective, is a study of native mycorrhiza effects on soil fertility and on seedlings growth morphology and nutrition in natural low P conditions, and after fertilizing with superphosphate. This part is divided into three chapters. The first one centrates in the effects of mycorrhiza and superphosphate addition on soil fertility parameters, whereas the second one describes different growth morphological and nutritional responses of *Pinus radiata* seedlings infected and uninfected with native mycorrhiza under contrasted P fertility conditions. Chapter three incorporates the results of the previous two chapters through Barber & Cushman (1981) nutrient uptake model. In this chapter the ability of this model to predict seedlings uptake under contrasted soil P availability conditions, as well as the suitability of the model to skip the effect of mycorrhiza on P and Mg uptake are tested.

The second part address the second main objective. This part centrates in field measurements and examines the relative importance of forest floor leachates on forest N and P nutrition in a mature *Pinus radiata* plantation. Chapter four studies the characteristics of litter leachates, paying special attention to organic matter exported in solution from the forest floor. And chapter five, qualitatively compares the different signature of P forms in litter leachates and mineral soil surface solution by using a proposed new chromatographic technique to fractionate P forms using anion exchange resins.

While *Pinus radiata* has been used to carry out the research in the first two parts, the research in this third part (Chapter 6) has been centrated in a different type of fast growing forest (Mixed *Eucalyptus* sp. forest from Orbost, SE Australia). This part also adress the second main obective. After the quantification of the role of forest floor on forest nutrient cycles carried out in the second part, this last chapter deals with the effects of altering the forest floor on surface soil P availability and sorption and desorption. The alteration of the forest floor consisted on clearfelling and burning the remaining slash.

Part I

CHAPTER 1

EFFECTS OF MYCORRHIZAL INFECTION AND SUPERPHOSPHATE FERTILIZATION ON NITROGEN AND PHOSPHORUS AVAILABILITY IN FOREST SOILS.

INTRODUCTION

Substantial advances in the understanding of mycorrhiza physiology and host-fungi relationships have been made but the role of mycorrhiza in the functioning of forest ecosystem is still not well understood. Because of the complexity of the mycorrhizal associations, most of the research has been carried out under controlled and simplified experimental conditions, and further research is needed to understand the importance of mycorrhiza to an ecosystem scale (Vogt et al., 1991).

It has been demonstrated that mycorrhizal fungi can transfer nitrogen (N) and phosphorus (P) from the soil to the host plant (Skinner and Bowen, 1974, Finlay and Read, 1986, Finlay et al., 1988, Plassard et al., 1991, Bolan, 1991). Moreover, they may produce phosphatases and phytases (Dighton, 1983), which may decompose organic matter (Giltrap 1982, Trojanowski et al., 1984, Dighton, 1991), therefore releasing P. Very few studies have attempted to explain the role of mycorrhizal associations on soil nutrient availability under natural conditions, probably due to the intrinsic experimental complexity. Entry et al. (1991) examined needle decomposition and nutrient fluxes in areas dominated by hyphal mats in Douglas-fir forests. They found that the rates of N and P released from needles were higher in the samples incubated in mat layers, but their transfer from the forest floor to the mineral soil did not differ between mat and non-mat soils. A

possible explanation for these results could be the rapid nutrient uptake by the mycorrhizal fungi. On the same site, Griffiths et al. (1989) found increased mineralizable nitrogen in mat soils. However, from those experiments it is not possible to differentiate between the effects of mycorrhizal activity itself and the spatial heterogeneity that their development promoted.

Mycorrhizal fungi have been found to produce phosphatase and phytase type enzymes (Theodorou, 1971; Kroehler et al., 1988) to increase phosphorus availability through organic P mineralization. Increased orthophosphate levels can result in lower phosphatase production (Dighton 1983), decreased mycorrhiza infection (Arias et al. 1991) and changes in the type of mycorrhiza (Newton and Pigott, 1991; Arias et al., 1991). Although these effects are far from being general (Dighton, 1983; Arias, et al. 1991; Danielson, 1991).

Pinus radiata in Catalonia (NE Spain) is largely planted in shallow soils with very low content of organic matter (Cortina 1992). Field observations in these plantations indicated that pine roots were largely infected with a great variety of ectomycorrhizal fungi. Under these conditions we designed a pot experiment, in an attempt to examine changes in soil phosphorus and nitrogen availability after suppression of native mycorrhizae, under two different phosphorus levels. To maintain the experimental conditions as close to the field situation as possible, we used the soil (including the forest floor) collected from a radiata pine (*Pinus radiata*

D.Don) plantation. Radiata pine seedlings were planted in this soil that was reinoculated with the saprophytic microflora after autoclaving.

MATERIAL AND METHODS

Description of the site where the soil was collected

The soil was collected from a 23 year old first rotation radiata pine plantation located 50 Km N from Barcelona (Close to Vallgorguina). The bedrock is granodiorite that developed a sandy loam Typic Xerochrept soil (US Soil Taxonomy). Some properties of the soil are presented in Table 1.1. Prior to radiata pine the site was used as a vineyard. Climate is mediterranean, annual precipitation is about 740 mm, and mean annual temperature is 13.6°C.

Experimental design

The experiment was carried out in sixteen 20 cm deep 21 cm diameter pots containing soil collected from the above mentioned radiata pine plantation. The

experiment consisted of four replicates of a 2X2 of superphosphate fertilization, and mycorrhiza suppression arranged in a completely randomized design.

Soil and seedling preparation

Mineral soil and forest floor (Oi, Oe-Oa layers) were collected from the radiata pine plantation previously described. The mineral soil was sieved (1 cm) and thoroughly mixed. Next, half of the mineral soil and whole of the forest floor were autoclaved for 90 min at 120°C. After 24 h. a second autoclaving was applied to the samples to obtain a completely sterilized soil. Following, the 16 pots were filled up with homogenized soil and organic horizons were reconstituted on each pot surface.

Seeds were sterilized by immersing them in H_2O_2 30 % for 30 min, stratified for one month and germinated in artificial substrate (peat-perlite-vermiculite). Two weeks after germinating, 20 seedlings were planted in each plot. Following, to recolonize the sterilized soil with field existing saprophytic microflora, non-mycorrhizal treatment pots (M-) were watered with 100 ml of solution that was prepared by shaking for 30 minutes the fresh field collected samples from Oe-Oa horizon with deionized water (1/20 weight:volume), and filtered with Whatman No 3 filter paper (0.6 µm pore size) to eliminate any propagules from symbiotic fungi

(Danell & Willford, 1974). One week after, one half of the pots received 5.54 g of phosphorus as superphosphate (80.5 g of superphosphate per pot that accounted for 100 Kg P/ha). During the dry season pots were watered occasionally using regular water. The total amount of water added to the pots represented about 300 mm of rainfall, which is the rainfall difference between Barcelona (where the experiment was carried out) and the site where the soil was collected. After 11 months what remained from the forest floor horizons was collected and discarded. Following, the mineral soil was sampled at four depths (0-1 cm, 1-3 cm, 3-5 cm and 5-20 cm). 0-1 cm layer contained large amounts of autoclaved organic matter from Oe-Oa horizon which mixed with the surface mineral soil.

	Bulk density ($g\ cm^{-3}$)	pH (H_2O)	pH (KCl)	Total C (%)	Total N $mg\ Kg^{-1}$	Total P $mg\ Kg^{-1}$
0-15 cm	1.49	5.61	2.95	0.63	401.00	606.03
15-30cm	1.53	6.10	3.25	0.37	325.00	563.71

	Exchangeable cations cmol (+) Kg^{-1}				
	Ca	Mg	K	Na	Al
0-15 cm	4.95	1.79	0.13	0.85	1.34
15-30cm	5.66	1.79	0.09	1.11	0.50

Table 1.1. Some characteristics of the soil studied.

Laboratory analysis

Soil was stored in a cold room (4°C) and analyzed within 1 week of collection from pots. Total carbon was determined from air dried grounded soils with a CHNS elemental analyzer by combustion (Carlo Erba 1500). Fresh samples were used for the following analysis: Mineral nitrogen (NH_4^+-N and NO_3^--N) was extracted using 0.5 M K_2SO_4 (1:10, weight:volume) and the extracts determined by flow injection analysis (Tecator FIASTAR). Autoclave nitrogen was determined after autoclaving the samples at 121°C for 16 h. using the method suggested by Keeney (1990). Soil biomass flush of carbon was estimated by a modified fumigation extraction method (Vance et al., 1987), but Hexanol was used as a fumigant in place of Chloroform. Preceding the determination of dissolved organic carbon (D.O.C.), hexanol was removed from the extracts by evaporating it. Blanks were used to check for complete removal of hexanol. Carbon in the extracts was determined by using the $K_2Cr_2O_7$ and $Fe(SO_4)(NH_4)_2$ procedure (Nelson & Sommers 1982). Biomass was calculated from the difference in the carbon content of the fumigated K_2SO_4 extracts minus the unfumigated. No factor was applied to correct for any incomplete recovery. Soluble Ca, Mg and K were extracted with deionized water 1:10 weight : volume and determined with atomic absorption spectrometry.

Water soluble and bicarbonate soluble P were extracted with deionized

water (Humphreys and Pritchett, 1972) and $NaHCO_3$ (pH=8.5) (Olsen et al, 1954) respectively. Ratios between soil weight : volume of extract and the time of extraction were 1/10 for 16 h. for the water soluble P and 1/20 for 1/2 h for the bicarbonate soluble P. Reactive labile P in the extracts was determined by ascorbic acid blue method (Murphy and Riley 1962). Total P in the extracts was also determined by the method of Murphy and Riley (1962) following digestion with H_2SO_4 and H_2O_2. Non reactive P was estimated as the difference between total and reactive labile P. As Murphy and Riley (1962) P determination requires to be done at low pH conditions, reactive labile P fraction, often operationally defined as inorganic P, may include some organic phosphates subjected to hydrolysis. On the other hand, non reactive P (NRP) is also often operationally defined as organic P.

Phosphatase activity was assayed by using the p-nitrophenyl phosphate method (Tabatabai & Bremner, 1978) buffered with maleate at pH=6.5.

Statistical analysis

Variables were analyzed by using a two factors weighted ANOVA (SAS institute, 1982). LSD test was used to decide significant differences at a prob. < 0.05. Differences between treatments in the relationships between autoclave

nitrogen vs. total C concentrations were tested with ANOVA using C concentration as a covariate.

RESULTS AND DISCUSSION

Because autoclaving introduces some changes to the soil (Powlson & Jenkinson, 1976), we could not be certain whether the differences in soil N and P labile forms, observed between M+ and M- treatments, were caused by the mycorrhiza or by the effects of autoclaving. However, the most important changes autoclaving introduces to the soil are related to soil microbial biomass, and consist of increases in labile N and P forms (Powlson & Jenkinson, 1976). In our experiment most changes observed consisted of decreases in N labile forms. Moreover, Wolters & Joergensen (1991) presented evidences supporting that soil microbial biomass turnover was very short (few hours). Thus, the effects of autoclaving in our soil after one year were probably very small.

Ammonium concentration in soils supporting mycorrhizal and non-mycorrhizal seedlings were not significantly different except for a reduction in the 1-3 cm horizon (Fig. 1.1). Superphosphate fertilization, soil depth and the

interaction between both, had significant effects on ammonium concentration. P fertilization resulted in a decrease in ammonium concentration in the surface 0-1 cm soil. NH_4-N was considerably higher than NO_3-N mainly in the surface mineral soil.

NO_3-N concentration was low in P-fertilized soils in all but the deepest horizon (Fig. 1.1). Interestingly, mycorrhiza suppression resulted in an increase in NO_3-N concentration. Two mechanisms can be envisaged to explain this fact: 1) Inhibition of nitrification by mycorrhiza, and ii) high NO_3 uptake by mycorrhizal roots. Nitrification is frequently inhibited in forest ecosystems with well developed ectomycorrhizae (Alexander, 1983). Norton and Firestone (1991) in a field experiment observed an increase in gross nitrification rates when roots of ponderosa pine were excluded. They also found that plants were more effective than heterotrophic microbes when competing for NO_3-N. Plassard et al. (1989) observed higher uptake rates of NH_4-N than NO_3-N by two ectomycorrhizal fungi in *Pinus pinaster* (Soland in Ait), but suggested that ectomycorrhizal fungi may improve NO_3-N uptake rates by pine. Higher NO_3-N uptake relative to NH_4-N associates with higher uptake of cations. In our experiment the N uptake to cations uptake (sum of K, Ca, Mg and Al) ratio was not affected by mycorrhiza (data not shown, see next chapter) suggesting that low NO_3-N pools in M+ treatments resulted from the inhibition of nitrification caused by ectomycorrhiza instead of high NO_3-N uptake rates by mycorrhizal seedlings.

Our values of C in microbial biomass were similar to other biomass determinations in forest soils (Vance et al. 1987), and as Jenkinson and Ladd (1987) stated, represented between 1 and 3% of soil total organic C. C in microbial biomass in 0-1 cm horizon was increased by both mycorrhiza suppression and superphosphate addition whereas in 1-3 cm horizon, it was diminished by both factors. Microbial biomass 5-20 cm horizon behaved similarly to the above horizon except for the biomass increase in M-P+ treatment. In M+ treatments, C in microbial biomass in horizons with low organic matter content (from 1 to 20 cm depth; C=0.5 %, C/N = 15) correlated positively with autoclave nitrogen ($R = 0.52$, $p<0.0005$ for M+P- and $R = 0.54$, $p<0.0003$ for M+P+; $n=12$), whereas M- treatments did not show significant relationships. Thus, the high microbial biomass observed in pots with mycorrhizae may control N mineralization to a higher degree than biomass in non mycorrhizal pots. On the other hand, unlike in the deep horizons, autoclave nitrogen in high organic matter horizons (0-1 cm) (C= 3.5 %, C/N = 23.5) correlated negatively with microbial biomass ($R = - 0.2708$, $p<0.0388$) and positively with total C concentration (Fig. 1.3), indicating that autoclave nitrogen in these conditions was not released from soil microbial biomass but from soil bulk organic matter. Furthermore, organic matter in M+ treatments had higher amounts of autoclave nitrogen than in M- treatments (Fig. 1.3). It has been demonstrated that mycorrhizal fungi can participate in the decomposition of soil organic matter (Giltrap 1982, Trojanowski et al. 1984), and accelerate it (Dighton et al. 1987). On the other hand, organic matter decomposition may also be

retarded in the presence of active mycorrhizae (Gadgil and Gadgil 1975), or in combination of mycorrhizal roots and saprophytic fungi (Dighton et al. 1987). In our experiment, because the source of autoclave nitrogen in 0-1 cm horizon appeared to be soil bulk organic matter, the high amounts of autoclave nitrogen per g of C observed in M+ pots suggested that N contained in the organic matter was less depleted under mycorrhizal conditions. The increase of soil microbial biomass and autoclave nitrogen observed in M+ low organic matter horizons (1-3 cm & 5-20 cm) (Fig. 1.2) could be attributed to the contribution of mycorrhizal fungi to soil microbial biomass.

Reactive and non-reactive water soluble and bicarbonate soluble P forms increased after fertilization at all depths (Figs. 1.4 & 1.5). This large increase of non-reactive water or bicarbonate soluble P in fertilized treatments, is in agreement with Nommik (1978) and with Batten et al. (1979), who observed an increase in total soil organic P after fertilizing with P, and showed that labile organic P pools were also increased by inorganic P addition. However, bicarbonate extracted non-reactive P showed a much larger increase than water soluble non-reactive P.

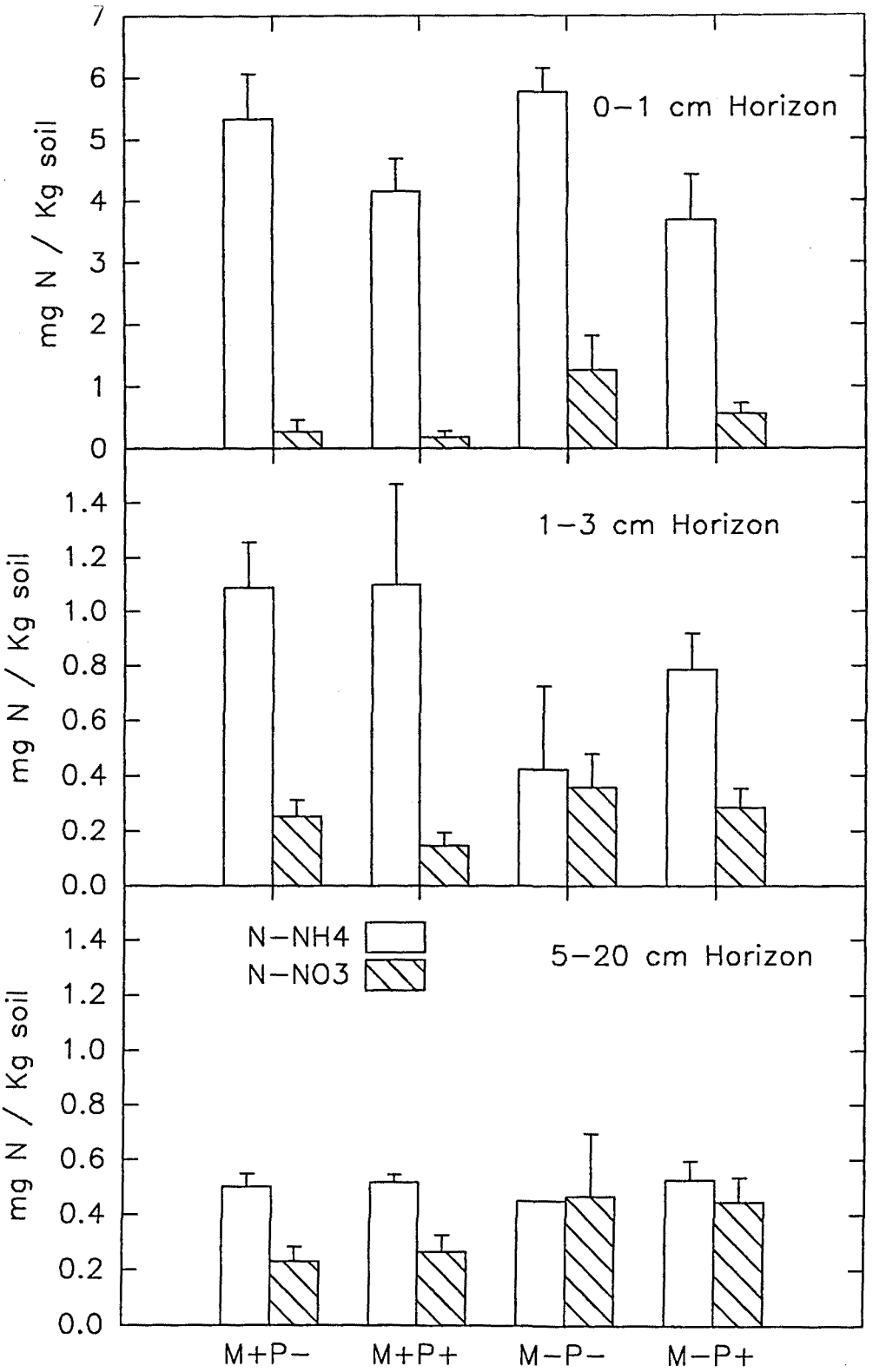


Fig. 1.1. Concentrations of Nitrate and Ammonia extracted with K_2SO_4 in soils across depths and treatments. Treatments: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in superphosphate fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. Bars represent the SE of the mean values.

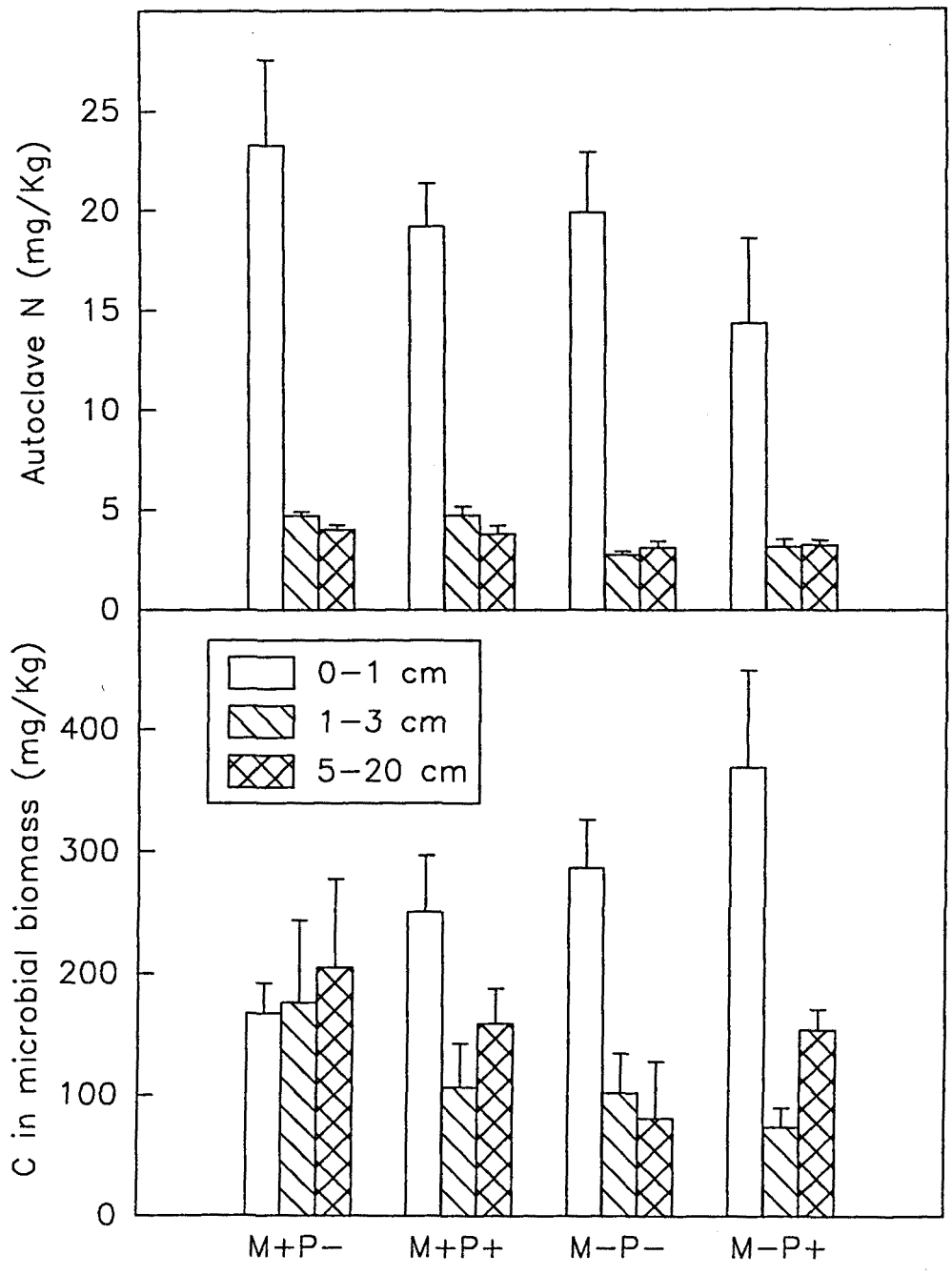


Fig. 1.2. C in microbial biomass and autoclave nitrogen. Treatments: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in superphosphate fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. Bars represent the SE of the mean values.

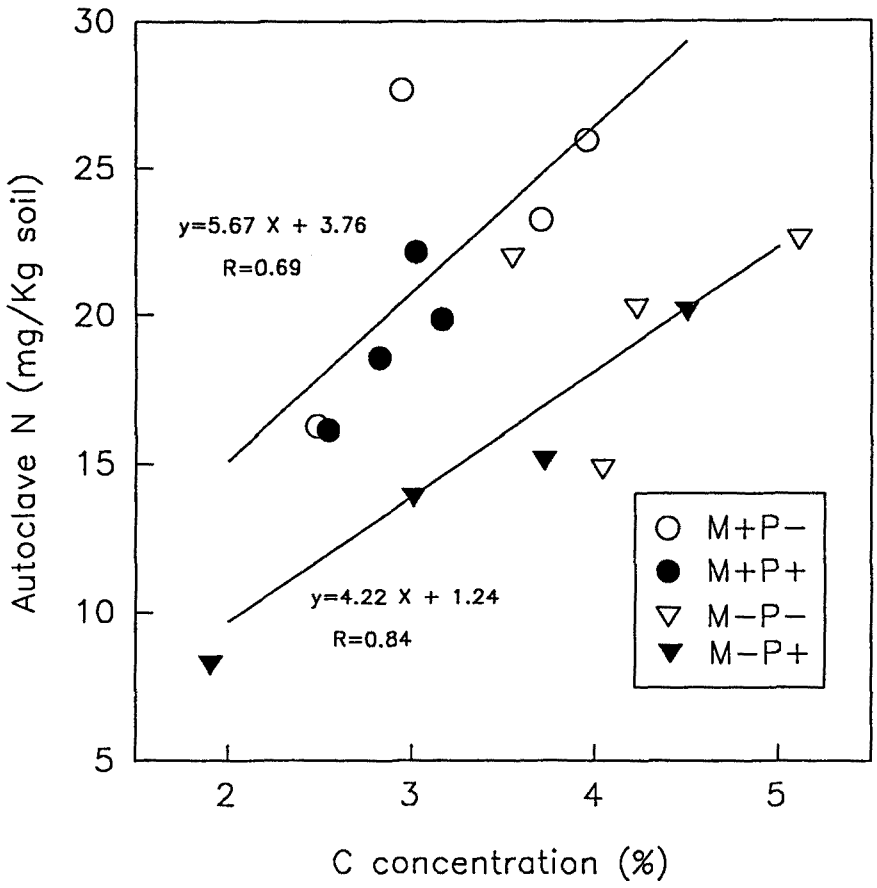


Fig. 1.3. Autoclave nitrogen vs. total C concentration in the pots at 0-1 cm soil horizon. Treatments: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in superphosphate fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. Linear regressions refer to M+ and M- pots respectively.

Acid phosphatase activity, was not affected by phosphorus addition (Fig. 1.5). Several authors have shown positive correlations between acid phosphatase activity and various forms of phosphorus under field conditions (Dalal, 1982; Baligar et al, 1988; Tarafdar et al., 1989). On the other hand, phosphatases are adaptative enzymes (Kroehler et al. 1988, Haussling and Marschner, 1989), and their activity has been related to the depletion of organic phosphorus (Tarafdar and Jungk 1987). Dighton (1983) found a negative relationship between phosphatase production and ortophosphate concentrations for *Betula pubescens* Ehrh. but not for *Pinus contorta* Dougl. seedlings. In our experiment, adequate phosphorus nutrition in mycorrhizal seedlings was supported by the lack of growth response after superphosphate addition, and by nutrient concentration in leaves (See chapter 2). Possibly because P was not limiting for mycorrhizal seedlings, phosphatase activity was not affected by phosphorus fertilization in these treatments. Moreover, bicarbonate extractable forms of organic P have been found to relate positively to phosphatase activity (Harrison, 1983). So, the expected inhibition caused by inorganic P addition, might have been counteracted by labile organic P increase. Our results showed a large decrease in soil phosphatase activity after mycorrhiza suppression (Fig. 1.6). As soil microorganisms are responsible for producing a significant amount of soil phosphatase enzymes (Dighton, 1983), decreases in soil microbial biomass after mycorrhiza suppression (Fig. 1.2) could explain the decrease in phosphatase activity in M- treatments. Doumas et al., (1968) (in Haussling and Marschner, 1987) reported a 40 %

decrease in phosphatase activity of non-mycorrhizal *Pinus halepensis* roots compared to mycorrhizal roots.

Correlation coefficient n=48	Water NRP	C in microbial biomass	Autoclave Nitrogen	NH_4^+-N
Water NRP	1 (0.0001)	0.357 (0.0139)	0.4013 (0.0046)	0.318 (0.0275)
Bicarbonate NRP	0.752 (0.0001)	0.532 (0.0001)	0.482 (0.0005)	0.4437 (0.0016)

Table 1.2. Correlations between Non reactive labile P (NRP) forms as extracted with water or bicarbonate ($NaHCO_3$). Figures in brackets indicate significance levels.

Mycorrhiza suppression did not affect reactive bicarbonate soluble and water soluble P forms at any depth. Correlation between the two methods was very high, especially for reactive soluble forms ($R = 0.908$ $p<0.0001$). Non-reactive P forms were less related (Table 1.2), but they showed significant correlations with soil biological parameters; microbial biomass, mineralizable nitrogen and ammonium. Non-reactive water soluble forms in the 0-1 cm horizon were slightly decreased by mycorrhiza suppression.

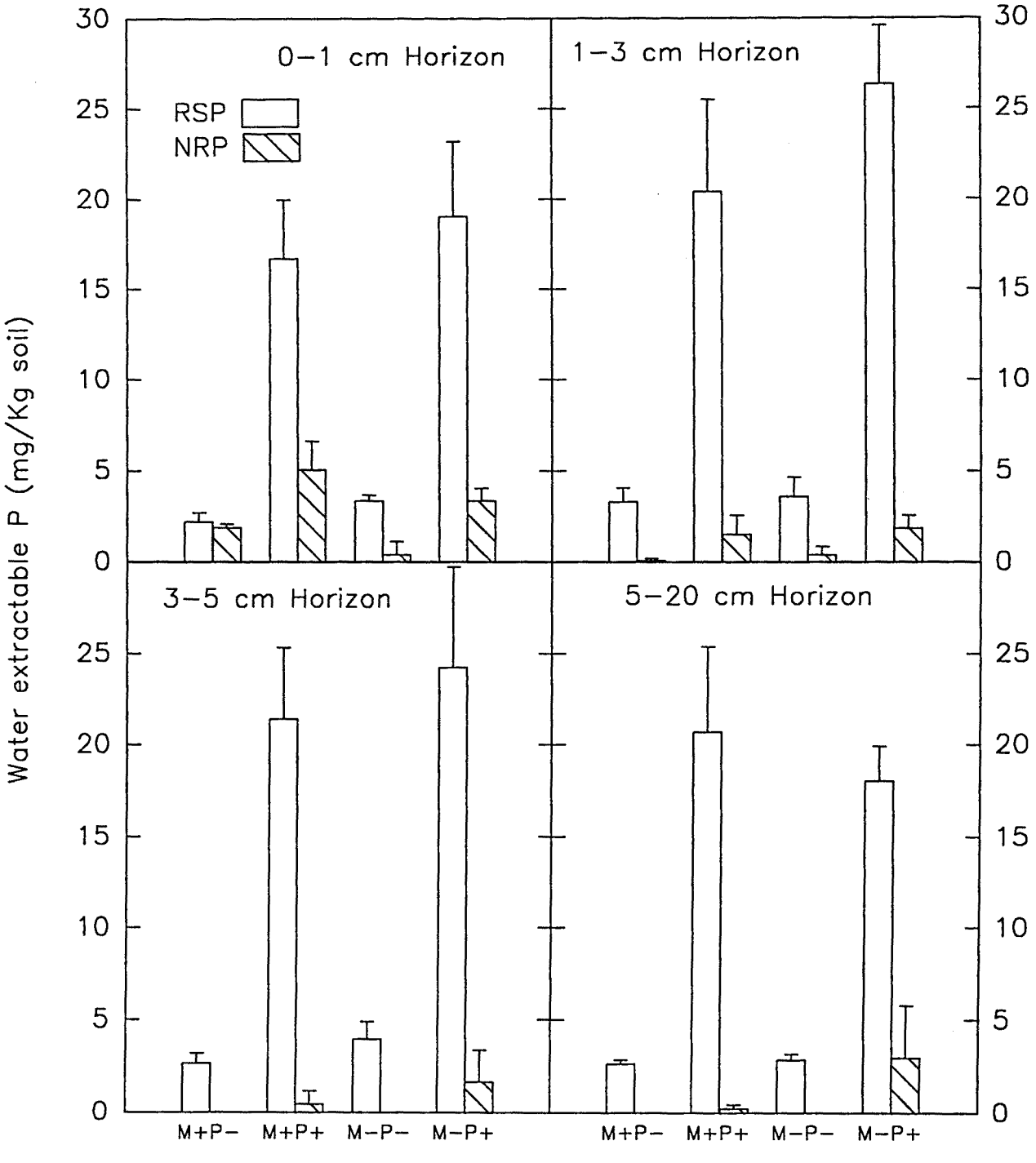


Fig. 1.4. Water extractable P. RSP; Reactive soluble P, NRP; non reactive soluble. Treatments: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in superphosphate fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. Bars represent the SE of the mean values.

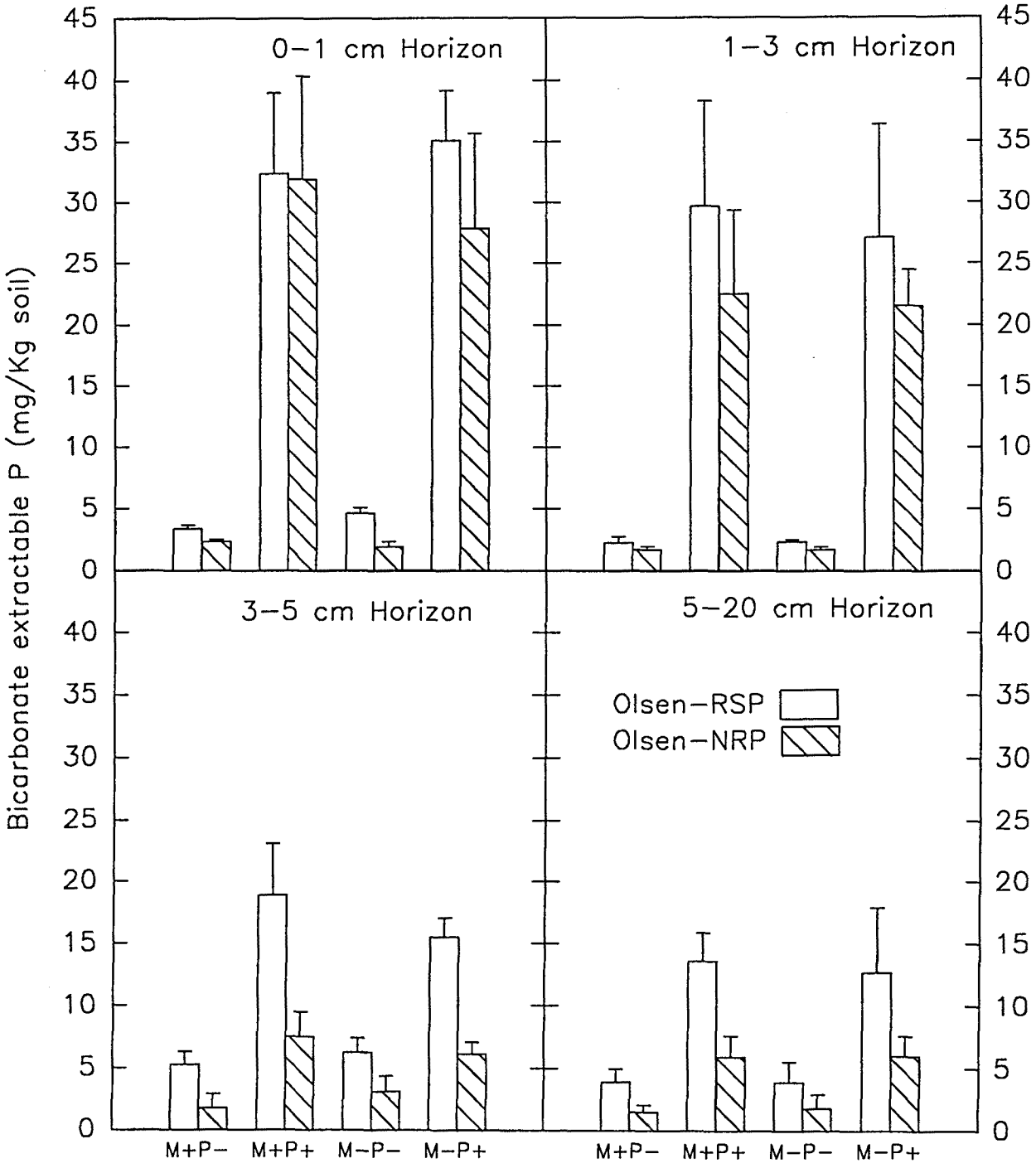


Fig. 1.5. Bicarbonate extractable P (Olsen et al 1954). RSP; Reactive soluble P, NRP; non reactive soluble. Treatments: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in superphosphate fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. Bars represent the SE of the mean values.

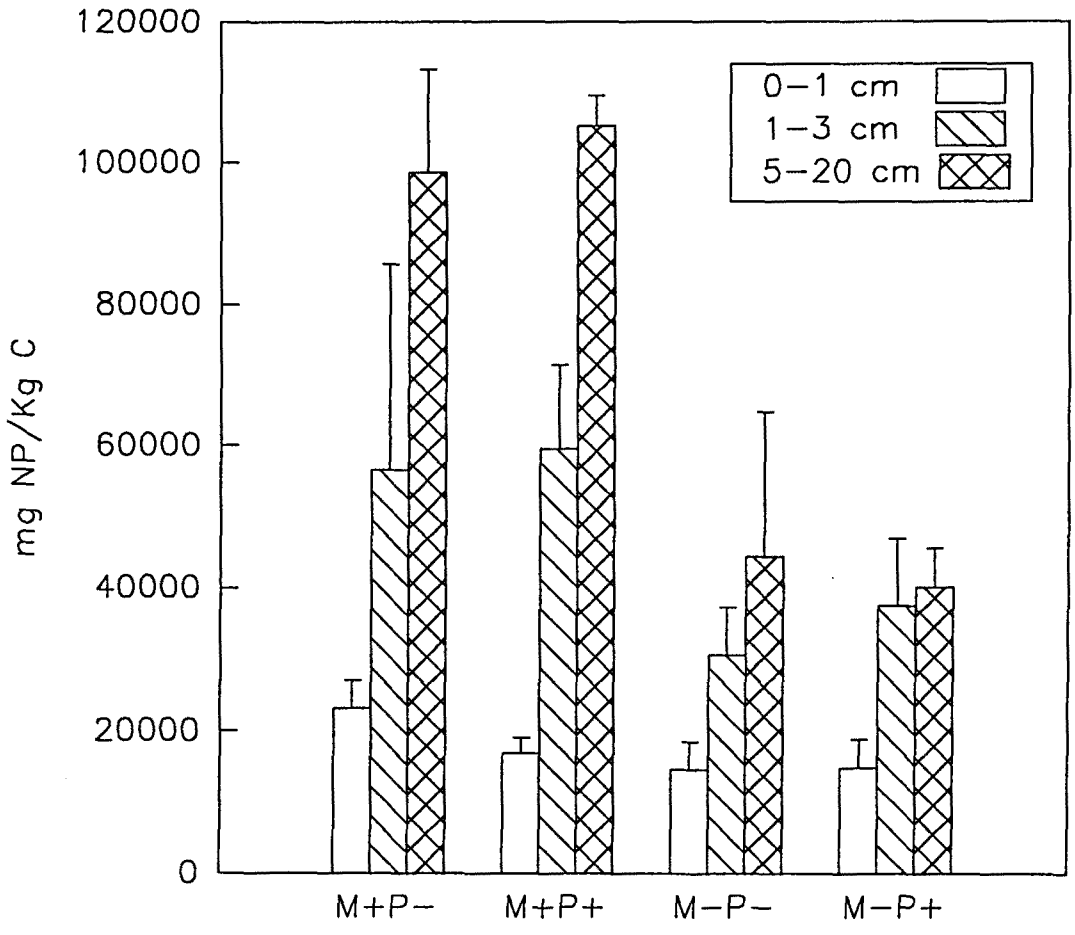


Fig. 1.6. Soil Phosphatase activity. Treatments: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in superphosphate fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. Bars represent the SE of the mean values. NP refers to p-nitrophenyl phosphate mineralized by phosphatase enzyms.

CONCLUSIONS

Mycorrhiza suppression did not affect soil pools of NH_4-N, but the inhibition of nitrification caused by ectomycorrhiza decreased soil NO_3-N. Increases in autoclave nitrogen and microbial biomass observed in low organic matter horizons in M+ treatments were possibly originated by mycorrhizal hyphae. In contrast, in high organic matter horizons, the lack of increase in autoclave nitrogen and microbial biomass probably resulted from the interactions between mycorrhizae and soil organic nitrogen. Consequently, soil surface organic matter (Oe-Oa horizon) showed high mineralizable N in M+ treatments.

Mycorrhiza had very little effect on labile P forms. Water test, possibly because of its mildness, appeared to be slightly more sensitive to the effects of mycorrhiza than bicarbonate test, as small differences in labile non-reactive P pools could be detected in the surface horizon. These P pools increased after inorganic P addition, and related to soil microbial biomass and nitrogen forms. On the other hand, sodium bicarbonate was able to extract much larger amounts of the increased non-reactive P pools resulted from P fertilization than water. Acid phosphatase activity was reduced by 69 % in M- soils compared to M+ soils. No effects of P addition on phosphatase activity were observed, probably indicating sufficient P supply for plant and microbes.

CHAPTER 2

EFFECT OF MYCORRHIZAL INFECTION AND SUPERPHOSPHATE FERTILIZATION ON *Pinus radiata* D. Don SEEDLINGS GROWTH, MORPHOLOGY AND NUTRIENT UPTAKE.

INTRODUCTION

It is well known that mycorrhizal infection can increase plant growth (Gianinazzi-Pearson et al., 1981; Abbott & Robson, 1984; Chakravarty & Chatarpaul, 1989), and facilitate nutrient uptake, particularly that of low mobility nutrients such as phosphorus (Heinrich et al. 1988; Rouseau & Reid, 1989; Koide, 1991). High availabilities of soil N and P have often been recognized to reduce mycorrhizal infection (Björkman, 1942; Dumbroff, 1968, Boxman & Roelofs, 1987; Newton & Pigott, 1991). Some of the mechanisms by which mycorrhiza increase nutrient uptake are related to the increase of the soil physical exploration provided by mycorrhizal hyphae, which causes a reduction of diffusion distances and largely increases the absorption surface (Bolan, 1991). Depending on the intrinsic characteristics of each nutrient (mobility, concentration, diffusibility) its uptake will be enhanced by mycorrhiza to a varying degree. Pritchett (1972) found that the uptake of P in pine seedlings planted in pots was more increased by mycorrhiza presence than the uptake of nitrogen. It has been often discussed whether seedlings response to mycorrhizal infection was caused by mycorrhiza itself or due to enhanced P nutrition (Rousseau & Reid, 1989; Kothari et al. 1990a). Most of the research about mycorrhiza deals with the effects of mycorrhiza on P uptake processes (Allen 1991), and their effect on the uptake of other nutrients has received less attention.

Both mycorrhizal infection and P availability have been shown to alter root morphology. While mycorrhizal infection can largely reduce total root length (Dosskey et al. 1992), high P supply has been reported to enhance root growth (McLaughlin & James, 1991), the frequency of root initiation and the extension rate of lateral roots (Bowen et al. 1974). Because mycorrhizal infection and P supply appear to have opposite effects in root growth, to study the interaction between both will help in the understanding of the significance of root elongation strategies.

The complex and difficult methods involved in studying mycorrhizae in relation to plant nutrition have caused that most of the research in this field has been carried out under controlled conditions and with VA mycorrhizal plants. Many times, solution culture experiments confined to a few different selected fungi strains have been the base of the experiments to study mycorrhiza (Sanders & Tinker, 1973; Jongbloed et al., 1992). Consequently, the purpose of the experiment reported here was to study the effect of field native mycorrhiza on the nutrition, growth and root morphology of *Pinus radiata* D.Don seedlings growing under natural (low P availability) and fertilized conditions (high P availability). To do this, *Pinus radiata* seedlings were grown in pots with soil collected from a mature radiata pine plantation.

MATERIAL AND METHODS

This experiment was carried out in pots using the experimental design described in chapter 1, which consisted of four treatments (with & without mycorrhiza and low & high P availability) arranged in a completely randomized experimental design with four replicates.

Seedling preparation and harvest

In July 1989, 320 recently germinated *Pinus radiata* seedlings (3 weeks old) were planted in the 16 pots (20 seedlings per pot), prepared by the method given in the previous chapter. The aboveground parts of 10 seedlings per pot, were harvested 7 months after planting (January 1990) and roots were left in the soil. Five month later, in May, the remaining 10 seedlings were harvested with roots. Roots were separated from soil by washing carefully with water. One root system per pot was kept in formalin (a mixture of acetone, ethanol and acetic acid 80:15:20) to preserve from decaying and to maintain the original shape and mycorrhizal condition of the roots. The remainder of the roots and shoots were oven dried at 60°C. After stripping needles from stems, roots, needles and stems were weighed separately. Next, each sample was ground for chemical analysis. Weight per needle was estimated by weighing 6 replicates of 15 needles per pot.

Laboratory analysis

Plant samples were wet digested with a mixture of concentrated HNO_3 and $HClO_4$ (3:1) by using a Tecator digester. Ca, Mg, Al, P, S and K were determined with ICP spectrometry. The C and N determinations in the undigested samples were carried out with a Carlo Erba 1500 CHNS elemental analyzer.

Root measurements

Percentage of mycorrhizal infection was determined by counting mycorrhizal and non mycorrhizal root tips in two plants per treatment. Weight:length relationships were worked out by measuring root length of fresh roots using a grid contact system (Tennant, 1975). Total root length was calculated from the length:weight ratio and the individual root weight per treatment. Root diameter was measured using a stereomicroscope (10X4) with a graduated ocular of 0.01 mm precision. For each treatment, 300 randomly selected root sections were measured. Root surface was calculated from the individual root length times the diameter mean for each treatment.

Calculation of DRIS index, nutrient content and uptake

The Diagnostic and Recomendation Integrated System (DRIS index) is a method of diagnosing nutrient element deficiencies and imbalances from the mineral composition of plant tissue. This method is based on ratios of nutrient element concentrations in plant tissues (Jones, 1981) and often produces more acurate diagnoses than conventional systems of plant analysis based on critical concentrations. We calculated DRIS index for N, P and S using the norms given for *Pinus radiata* needles by Truman & Lambert (1980) for Sunny Corner forests NSW (Australia). Nutrient uptake was calculated by the addition of each fraction times its nutrient concentration. To calculate nutrient uptake between January and May, nutrient concentration in the roots in January was assumed to be the same as in May. Root biomass in January was calculated by multiplying the single treatment root:shoot ratio worked out at the end of the experiment (May 1990), times the aboveground biomass measured in January 1990. Needle weight was used to calculate nutrient content in needles, and nutrient uptake by needles between January and May.

Statistical analysis

The results were analyzed by a two factor ANOVA (SAS Institute Inc. 1985). Significant differences were also tested by using Student's T-Test. In order to normalize proportions of mycorrhizal tips, they were transformed to angles (arcsin square root), whereas root diameter was transformed to (arcsin square root (root diameter))$^{-1}$. Kolmogorov-Smirnov test was used to test for normality. Linear regressions in fertilized and unfertilized treatments were compared by analysis of covariance.

RESULTS AND DISCUSSION

Seedling growth

The percentage of mycorrhizal tips, 11 months after planting, was drastically reduced in autoclaved treatments for any depth but autoclaving did not entirely avoid mycorrhizal infection (Fig. 2.1). As expected, the application of superphosphate reduced the ectomycorrhizal infection (Dumbroff, 1968, Thomson et al., 1986). The highest mycorrhiza concentration was always in roots growing in 0-5 cm soil.

Mycorrhiza suppression decreased the biomass production at both harvests (January and May), whereas superphosphate application did not show any significant effect on seedling growth at 0.05 level (Fig 2.2). At the second harvest, the effects of superphosphate application were significant at 0.1 level ($p<0.059$) and T-test revealed that under M- conditions, superphosphate addition significantly (at 0.05 level) increased seedling growth. Nevertheless, the decrease in growth caused by mycorrhiza suppression was observed at any time in both, low and high P availability conditions. This result contrasted with Rouseau & Reid (1989) study in *Pinus taeda* L. seedlings, which showed that the addition of P had similar effect on seedling growth than mycorrhizal infection.

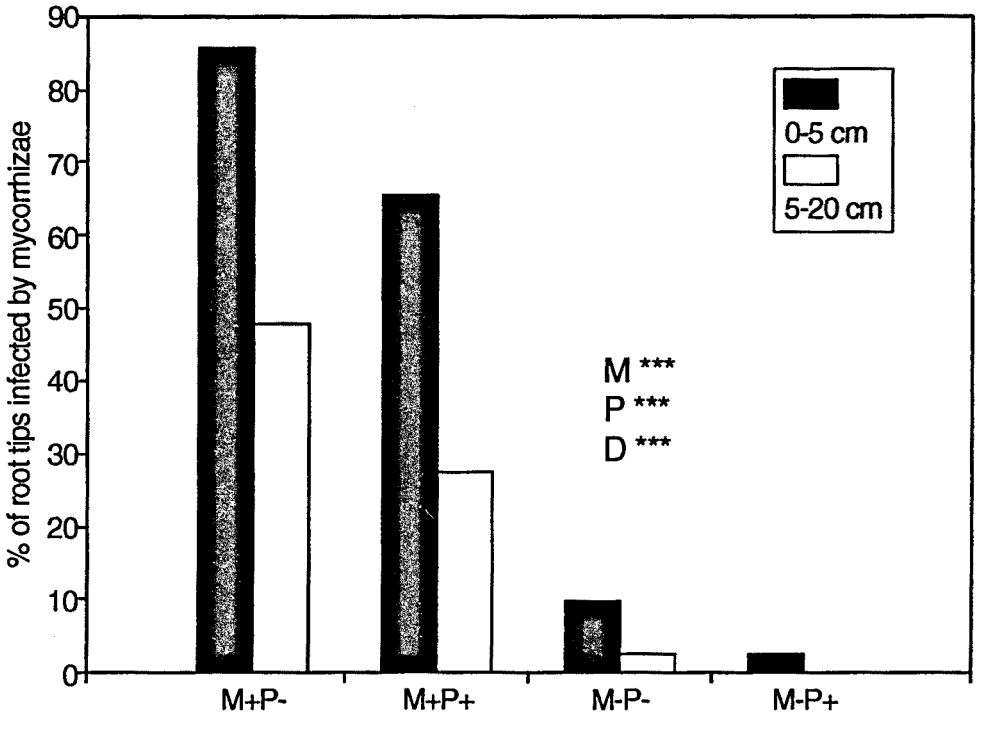


Fig. 2.1. Percentage of root tips infected by mycorrhizae at two soil depths. Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. D refer to depth. *,, *** refer to significant main effects and interactions significant at $p<0.05$, 0.01 and 0.001 respectively.**

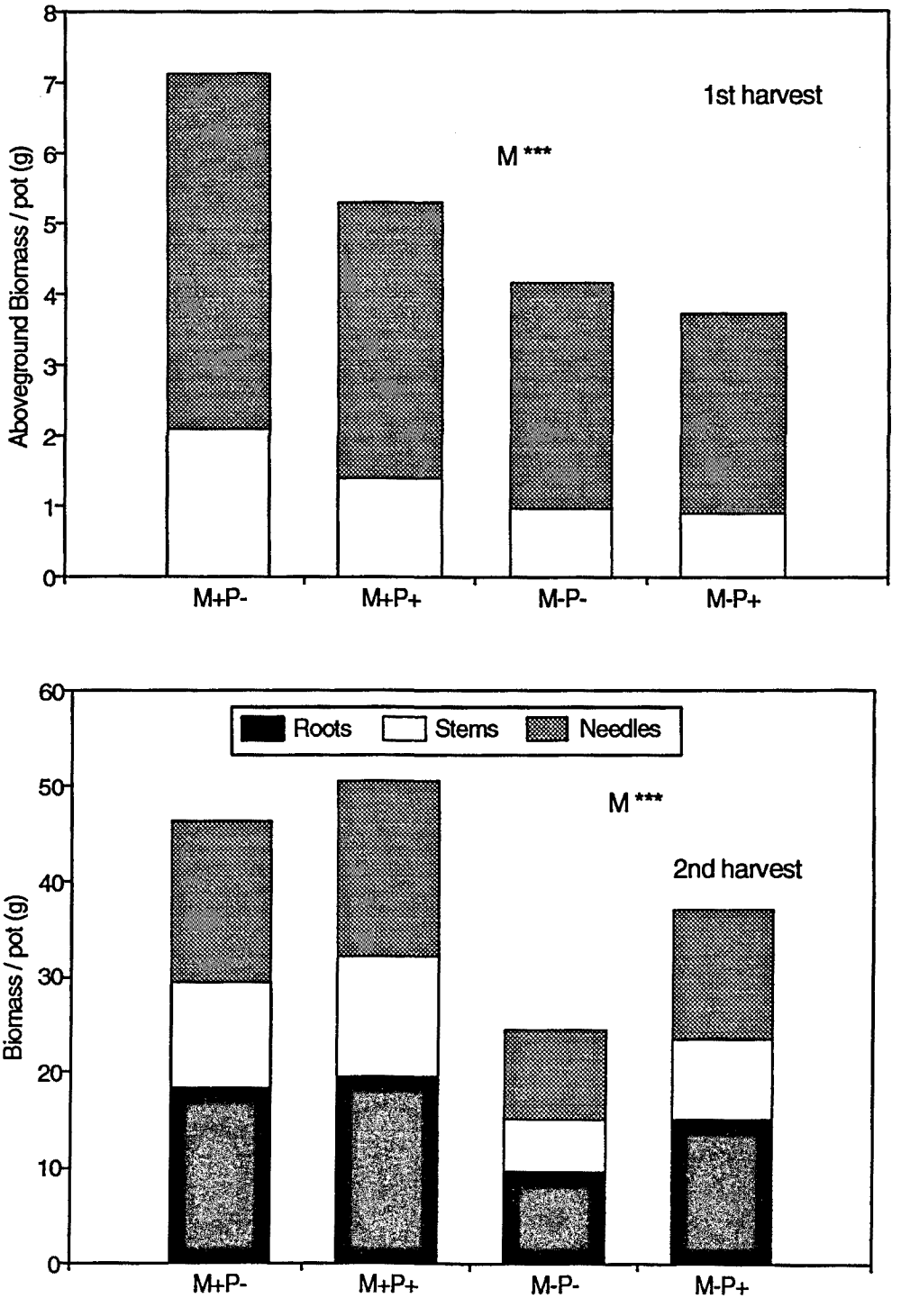


Fig. 2.2. Biomass production in the 1st harvest (January) and in the 2nd (May). Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. *,, *** refer to significant main effects and interactions significant at $p<0.05$, 0.01 and 0.001 respectively.**

In January all pots had the same number of needles per pot, but the average needle weight was less by 33.84 % in the M- treatments compared to M+ treatments (Table 2.1). In contrast, the average needle weight in May was not affected by any treatment and the number of needles per pot in M-P- treatment was largely decreased (42.76 % in respect to the other treatments). The decrease in needle biomass after mycorrhiza suppression observed in seedlings from the first harvest, corresponded to a decrease in average needle weight, while in the second harvest it was caused by a decrease in the number of needles. This fact indicated that the number of needles in early stages of seedlings growth was not affected by the mycorrhizal condition nor by superphosphate addition. Differences in average needle weight observed in the first harvest could be attributed to a delay on needle growth in M- conditions. Because fertilizer addition did not affect average needle weight, it is suggested that the delay on needle growth was not caused by the lack of P. Althought several authors have interpreted differences in leaf growth as caused by mycorrhiza mediated hormonal influences (Allen et al., 1980; Baas & Kupier, 1989), large variations in needle nutrient concentrations (Table 2.3) suggested that this delay in needle growth was caused by variations on nutrient availability. Unlike juvenile needles, the average needle weight of mature needles (2nd harvest) was not dependant on the mycorrhizal condition nor on P availability. Fife & Nambiar (1987), also with radiata pine needles, found the size of mature needles (g/needle) to be independent of its nutrient content. During the period between January and May all treatments except M-P-, produced new

needles resulting in 29.84 % increase in M+P-, 50.3 % in M+P+ and 51.95 % in M-P+. On the contrary, M-P- treatment showed a 30% loss of needles although average needle weight per pot was increased to the level of other treatments. Hence, this loss in needle number represented a Leaf Area Index (LAI) decrease for this treatment and possibly originated the large decrease in seedling biomass observed in M-P- pots. Unlike total seedling biomass, the considerable loss of needles observed in M-P- treatment was completely overcome after superphosphate addition. This fact suggested that, although under M-P- conditions, P was limiting for both, mature needle production and seedling growth, P appeared to play a more notable role in controlling the former than the latter. Contrasting with the first harvest, at this stage of growth (11 month after seeding), seedlings controlled needle biomass by adjusting the number of needles to the existing conditions of fertility.

	Treatments				ANOVA		
	M+P-	M+P+	M-P-	M-P+	M prob.<	P prob.<	MxP prob.<
	First harvest						
Needle biomass (g pot^{-1})	5.02 a	3.92 a	3.19 b	2.83 b	0.0010	ns	ns
No needles pot^{-1}	2389 a	2278 a	2570 a	2102 a	ns	ns	ns
Average needle weight (mg needl.$^{-1}$)	2.09 a	1.81 ab	1.24 b	1.35 b	0.0029	ns	ns
	Second harvest						
Needle biomass (g pot^{-1})	17.01 a	18.53 a	9.34 b	13.01 ab	0.0032	ns	ns
No needles pot^{-1}	3103 a	3424 a	1855 b	3195 a	ns	0.0500	ns
Average needle weight (mg needl.$^{-1}$)	5.48 a	5.41 a	5.04 a	4.23 a	ns	ns	ns

Table 2.1. Needle biomass, Number of needles per pot and average needle weight in 7 (first harvest) and 11 (second harvest) months old radiata pine seedlings. Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. ns refers to non significant differences at 0.05 level. Letters underneath figures refer to Student T-test comparisons.

Similar to other fractions of plant biomass, root biomass showed the same pattern of variation between treatments than total biomass. Interestingly, it behaved differently than root length and root surface. Mycorrhiza suppression had a highly significant effect in reducing root biomass (42.9% in P- treatment and 25.45% in P+), but it did not affect root length and root surface (Table 2.2). On the other hand, the effect of fertilizer application on root biomass was not significant ($p<0.0555$), but it increased root length and surface by 26.49% in M- and 27.43% in M+ treatments for root length, and 35.86% in M+ and 52.92% in M- for root surface when compared to the unfertilized treatments. Specific root length (cm g^{-1}) increased to about two fold after mycorrhiza suppression. Fertilizer application increased the specific root length in M+, while decreased it in M- treatments, because the reduction of biomass was greater than the increase in length. Although root:shoot ratio was not affected by mycorrhiza suppression both, root length:shoot weight and root surface:shoot weight ratios were greatly increased in M- treatments (Table 2.2). Because mycorrhizal hyphae can largely increase the physical exploration of the soil (Bolan, 1991), and absorb nutrients as far as 12 cm from roots (Skinner & Bowen, 1974, Li et al., 1991), they could possibly account for the decrease on root length (and surface):shoot ratio observed in M+ treatments. Root length is known to be negatively correlated to plant P concentration (Foehse & Junk, 1983). However, in our experiment, the high plant P concentration following superphosphate addition (Table 2.3) resulted in absolute root length and surface increases (Table 2.2). McLaughlin & James (1991) reported increases in root length in wheat caused by an increase of P supply.

	Treatments				ANOVA		
	M+P-	M+P+	M-P-	M-P+	M prob.<	P prob.<	MxP prob.<
Root biomass ($g\ pot^{-1}$)	18.19 a	19.54 a	10.38 b	14.57 b	0.0013	ns	ns
Root length ($m\ pot^{-1}$)	185.18 a	234.24 ab	221.12 ab	281.80 b	ns	0.0452	ns
Root surface ($cm^2\ pot^{-1}$)	3141.57 a	4268.21 b	2778.72 a	4249.37 b	ns	0.0032	ns
Specific root length ($cm\ g^{-1}$)	1018.10 a	1199.12 b	2187.81 c	1936.68 d	0.0001	ns	0.0003
Root: shoot ratio	0.66 a	0.68 a	0.74 a	0.74 a	ns	ns	ns
Root length: shoot ratio ($m\ g^{-1}$)	6.73 a	7.62 a	14.47 b	13.71 b	0.0001	ns	ns
Root surface: shoot ratio ($cm^2\ g^{-1}$)	114.19 a	138.76 a	181.79 b	206.73 b	0.0027	ns	ns
Root diameter (mm)	0.54 a	0.58 a	0.40 b	0.48 c	0.0001	0.0001	ns

Table 2.2. Root measurements from 11 months old radiata pine seedlings grown in pots as affected by treatments. Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. ns refers to non significant differences at 0.05 level. Letters underneath figures refer to Student T-test comparisons.

Root diameter was significantly decreased by mycorrhiza suppression, and increased by superphosphate addition (Table 2.2). After mycorrhiza suppression, the number of roots thinner than 0.5 mm of diameter increased, while the number of roots thicker than 0.5 mm decreased (Fig. 2.3). Superphosphate addition only affected the number of roots of intermediate diameter. It decreased the number of roots between 0.2 and 0.5 mm thick and increased the number of roots between 0.5 and 1mm of diameter.

The increase of root length per g of aboveground biomass was due to the elevate number of thin roots in non-mycorrhizal root systems. This increase was also observed in high P fertility conditions, and M+P+ treatment showed luxury levels of P in leaves (Table 2.3). Thus, the increase of root length per g of shoot under high P availability conditions was not explained by the plant necessity to obtain P from the soil. Despite changes in root morphology and structure introduced by ectomycorrhiza are much more obvious than those from VA mycorrhiza, the specific root length (cm root/g root) increment introduced by ectomycorrhiza suppression was within the same order of magnitude than the ones reported for VA mycorrhiza (115% for P- treatments and 60% for P+ treatments; 60 % for VA mycorrhiza, Kothari et al. 1990a), suggesting that the increase of the soil physical exploration provided by both types of mycorrhiza is of similar magnitude relative to g of infected root.

Fertilizer application increased root length and surface but it did not have any effect on these parameters when related to shoot biomass. Because the class of roots between 0.5 mm to 1 mm thick was the only fraction increased by superphosphate addition (Fig. 2.3), it is suggested that the increase in root length in P+ treatments was made up with roots of this size.

Aboveground biomass from seedlings harvested in January showed high coefficient of variation under mycorrhizal conditions (ANOVA, $p<0.0026$) (data not shown). In the 2nd harvest, only root surface showed this high coefficient of variation in the presence of mycorrhiza (ANOVA, $p<0.0133$). Burgess and Malajczuk (1989) found that mycorrhizal inoculation of *Eucalyptus globulus* largely reduced its growth variability. It can be suggested that in our experiment under M+ conditions, seedlings were not equally infected by soil naturally occurring mycorrhiza. This fact would lead to an increase of competition among trees and growth variability.

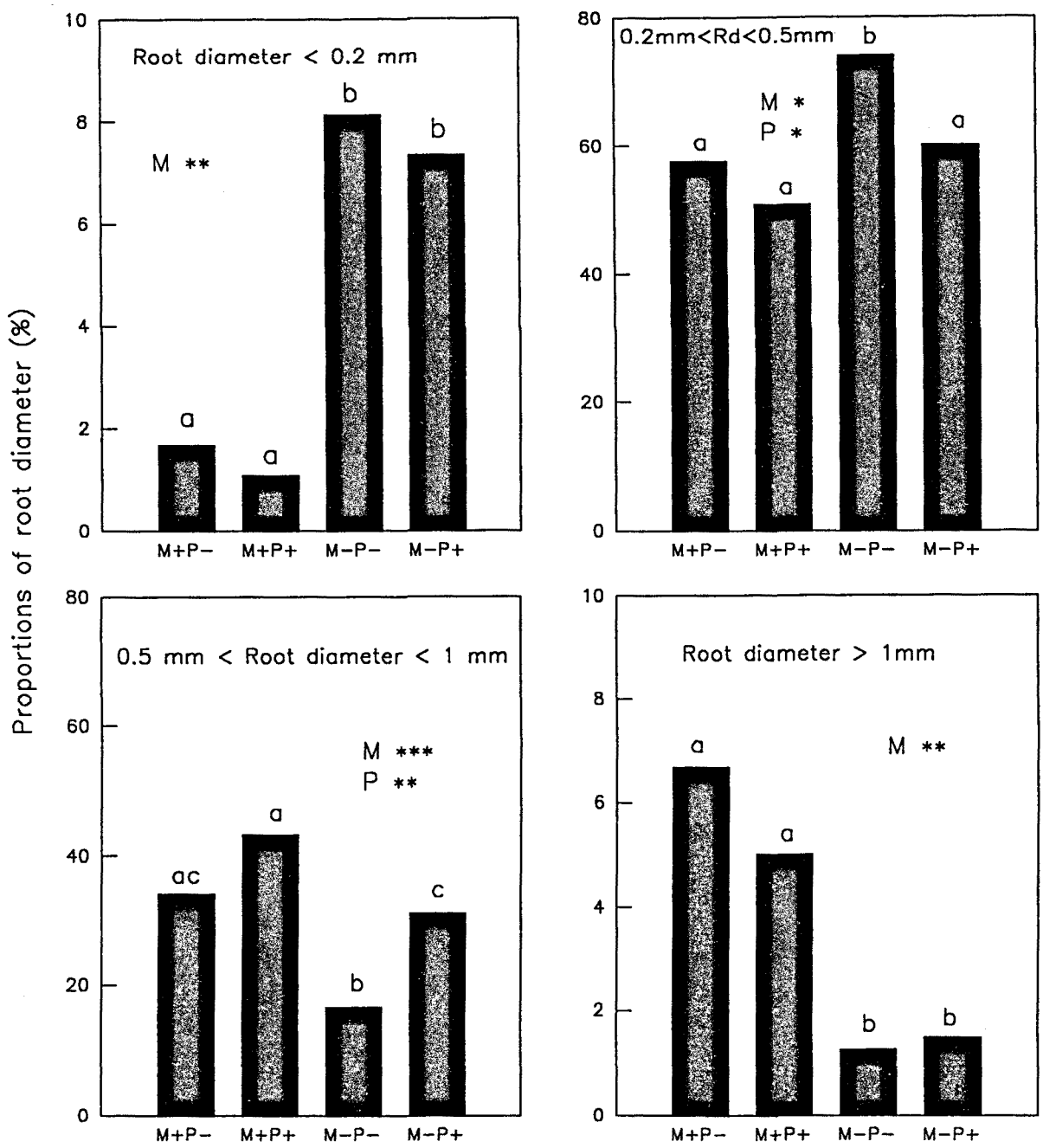


Fig. 2.3. Distribution of root diameter in percentage. Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. D refers to depth. *,, *** refer to significant main effects and interactions significant at p<0.05, 0.01 and 0.001 respectively. Letters refer to student T-test comparisons.**

Foliar nutrient concentration and content

Needles collected in the second harvest (May) showed concentrations of nutrients comparable to radiata pine mature needles (Will 1985). According to the critical levels of nutrient sufficiency in needles of radiata pine seedlings given by Hopmans & Flinn (1983), needles collected in May (mature needles) showed that our seedlings had sufficient levels for all nutrients except N and Mg in any treatment. Levels of P in the unfertilized and mycorrhiza suppressed treatment were also deficient. DRIS index based on concentrations of N, P and S in mature needles, indicated that N was the most limiting nutrient except in M-P- treatment in which P was slightly more limiting (Table 2.3 & 2.4). DRIS nutritional imbalance is the sum of the absolute value of the DRIS index for each element. In our experiment, in the needles collected in January (juvenile needles) the imbalance was greatly increased by both mycorrhiza suppression and superphosphate fertilization (Fig. 2.4). In M-P+ treatment, nutritional imbalance of juvenile needles was by far the greatest observed in any treatment. DRIS imbalance in mature needles was not affected by treatments.

Foliar nutrient concentration and content per needle contribute to interpreting plant nutritional status (Timmer & Stone, 1978). Both, needle concentration and nutrient content per needle in juvenile needles were more sensitive to treatments than in mature needles (Table 2.3 & 2.5). Nutrient content

per needle in juvenile needles was largely decreased by mycorrhiza suppression for any nutrient, whereas only N, P, S and K were decreased in mature needles. P, K and S concentrations were increased with fertilizer addition at both times, while Ca concentration only was increased in the juvenile needles. P concentration in unfertilized seedlings at both harvests, was decreased by mycorrhiza suppression whereas in fertilized seedlings it was increased. High concentrations of P in M+ and fertilized (P+) treatments indicated luxury consumption levels for P, especially in M-P+ treatment. Because the addition of superphosphate did not show any effect on needle biomass, the high concentration of P in the foliage of P+ treatments can be interpreted as an accumulation of P. The fact that mycorrhiza suppresion increased P concentration in P+ treatments could be atributed to the decrease in needle biomass in respect to M+ treatments after mycorrhiza suppresion.

While element concentration in needles was highly decreased between 6 months and 11 months old seedlings, except Al, all studied elements (Al included) showed an absolute increment per needle after this four month period (Table 2.3 & 2.5). This fact could be atributed to the dilution effect of needle growth (Timmer & Stone, 1978). Increase in Aluminum content per needle was much higher than of any other element, even increasing in concentration that is overcoming dilution by needle growth. P and Al needle uptake from January to May were not affected by any treatment, whereas needle uptake of N, Ca, Mg, S and K were especially

increased in M-P- treatment (Fig. 2.5). The lack of increase in P needle uptake in M-P- pots suggested again the lack of P for this treatment.

Similar to average needle weight (Table 2.1), needle nutrient concentration and imbalance (Fig. 2.4) in seedlings harvested in January were very sensitive to treatments, whereas in seedlings harvested in May, they were sizably controlled by the plant. In M- treatments, fertilizer application reduced N, Mg and Ca content in mature needles and largely increased the average needle weight. Because N and Mg in M-P- needles were already under critical levels, the low N and Mg content in M-P+ needles indicated that superphosphate addition diluted these nutrients in needles and possibly exacerbated N and Mg deficiencies. Because the addition of P in M- treatments was not suficient to achieve the growth of M+ pots (Fig. 2.2), and because the lack of Ca and Mg does not normally affect much seedling growth (Will, 1985), it is suggested that growth was mostly N limited.

	Treatments				ANOVA		
	M+P-	M+P+	M-P-	M-P+	M prob.<	P prob.<	MxP prob.<
	Needles (1st Harvest)						
%C	48.86	48.29	48.85	47.61	ns	0.0001	ns
%N	2.18	2.31	1.98	1.88	0.0334	ns	ns
P	3069.16	4636.01	1558.29	5191.51	0.0528	0.0001	0.0002
S	2617.03	3331.98	2413.01	3670.66	ns	0.0031	ns
K	18337.57	19496.61	15086.50	18533.02	0.0418	0.0484	ns
Ca	4874.34	6086.78	5698.57	6571.48	ns	0.0285	ns
Mg	1514.15	1574.35	1656.95	1799.41	0.0222	ns	ns
Al	261.42	251.35	312.67	282.88	0.0078	0.0094	ns
	Needles (2nd Harvest)						
%C	46.19	46.39	45.88	45.90	ns	ns	ns
%N	1.04	1.09	1.04	0.92	ns	ns	ns
P	1752.53	2192.92	819.11	2473.65	0.0043	0.0001	0.0001
S	1408.17	1632.56	1207.87	1595.66	ns	0.0080	ns
K	9919.14	10587.48	9511.89	11788.14	ns	0.0142	ns
Ca	3572.74	4478.49	4891.57	4283.88	ns	ns	ns
Mg	979.54	1076.12	1142.64	1050.06	ns	ns	ns
Al	846.95	668.52	969.44	907.77	ns	ns	ns
	Stems (1st harvest)						
%C	48.94	48.78	49.02	48.07	ns	ns	ns
%N	0.88	1.00	0.98	1.08	ns	ns	ns
P	3268.88	4477.93	1911.25	5022.91	ns	0.0001	0.0018
S	1047.81	1397.75	1229.01	2172.17	0.0080	0.0019	ns
K	11806.97	13858.00	12602.31	17952.68	0.0247	0.0034	ns
Ca	2191.71	2459.58	2643.96	3241.38	0.0004	0.0124	ns
Mg	962.14	1011.56	1185.86	1441.68	0.0001	ns	ns
Al	435.08	487.74	436.90	298.56	ns	ns	ns
	Stems (2nd harvest)						
%C	48.67	48.30	50.30	48.50	ns	ns	ns
%N	0.55	0.44	0.61	0.58	ns	ns	ns
P	1884.24	2697.54	667.73	3429.19	ns	0.0001	0.0033
S	838.57	1177.09	1004.83	1640.33	ns	0.0221	ns
K	7757.48	10274.43	8527.92	11796.98	ns	0.0422	ns
Ca	2562.75	3051.26	3038.19	3685.53	0.0208	0.0185	ns
Mg	830.68	1017.35	1195.40	1247.70	0.0383	ns	ns
Al	168.43	241.70	276.66	271.31	0.0330	ns	ns
	Roots (2nd harvest)						
%C	39.03	37.86	44.74	44.34	0.0030	ns	ns
%N	0.93	0.96	1.24	0.91	ns	ns	ns
P	1498.48	2393.62	820.83	1903.53	0.0004	0.0001	ns
S	1003.01	1088.81	963.38	1261.33	ns	0.0365	ns
K	7103.37	8252.23	5724.71	7119.74	0.0205	0.0193	ns
Ca	9066.79	10435.16	8280.08	7409.98	0.0008	ns	ns
Mg	3414.81	3628.93	2884.17	2339.03	0.0489	ns	ns
Al	10317.27	10621.90	8306.61	6963.21	0.0395	ns	ns

Table 2.3. Mean values of concentration of nutrient-element in needles, stems, and roots from seedlings harvested in January and May. C and N are presented in %. P, S, K, Ca, Mg and Al are presented in mg Kg^{-1}. Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. ns refers to non significant differences at 0.05 level.

	concentration in needles			DRIS Index			Nutrient imbalance	Order of deficiency
	%	mg/Kg						
	N	P	S	N	P	S		
M+P-	1.04	1752.53	1408.17	-92.05	30.67	61.38	184.10	N>P>S
M+P+	1.09	2192.92	1632.56	-122.51	47.48	75.03	245.02	N>P>S
M-P-	1.04	819.11	1207.87	-33.79	-52.94	86.72	202.90	P>N>S
M-P+	0.92	2473.65	1595.66	-153.94	92.24	61.70	334.56	N>S>P

Table 2.4. Mean concentrations and DRIS index for N,P and S from needles collected at the second harvest (May). Nutritional imbalance corresponds to the sum of absolute values of DRIS index. Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil.

	Treatments				ANOVA		
	M+P-	M+P+	M-P-	M-P+	M	P	MXP
ug/needle					prob.<	prob.<	prob.<
	First harvest						
C	1030.00	880.00	610.00	640.00	0.0001	0.0001	0.0001
N	44.89	41.98	24.63	25.39	0.0001	ns	ns
P	6.37	8.15	1.93	7.00	0.0001	0.0001	0.0015
S	5.42	5.33	3.00	4.95	0.0017	0.0209	0.0125
K	39.10	35.38	18.73	24.97	0.0001	ns	0.0259
Ca	9.80	9.08	7.08	8.86	0.0045	ns	0.0121
Mg	3.15	2.68	2.06	2.42	0.0001	ns	0.0005
Al	0.55	0.44	0.39	0.38	0.0001	0.0048	0.0104
	Second harvest						
C	2530.00	2510.00	2310.00	1940.00	0.0001	0.0001	0.0001
N	56.93	58.93	52.30	39.13	0.0002	0.0365	0.0077
P	9.61	11.86	4.12	10.47	0.0001	0.0001	0.0013
S	7.72	8.83	6.08	6.75	0.001	ns	ns
K	54.38	57.28	47.89	49.89	0.0161	ns	ns
Ca	19.59	24.23	24.63	18.13	ns	ns	0.0435
Mg	5.37	5.82	5.75	4.44	ns	ns	0.0422
Al	4.64	3.62	4.88	3.84	ns	ns	ns

Table 2.5. Nutrient-element content per needle. Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. ns refers to non significant differences at 0.05 level. Letters underneath figures refer to Student T-test comparisons.

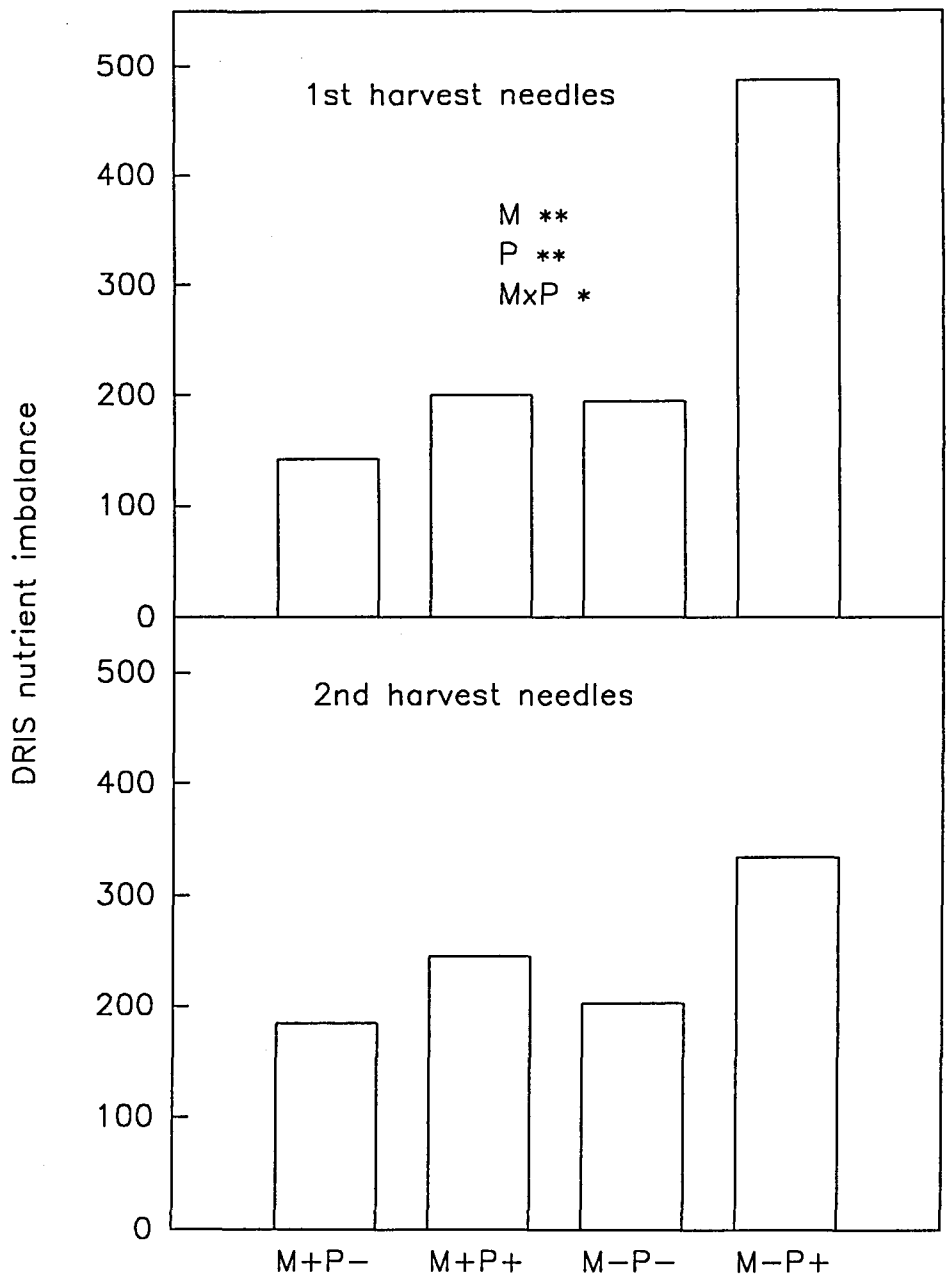


Fig. 2.4. DRIS nutritional imbalance in young needles (January) and mature needles (May). Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. D refers to depth. *,, *** refer to significant main effects and interactrions significant at p<0.05, 0.01 and 0.001 respectively.**

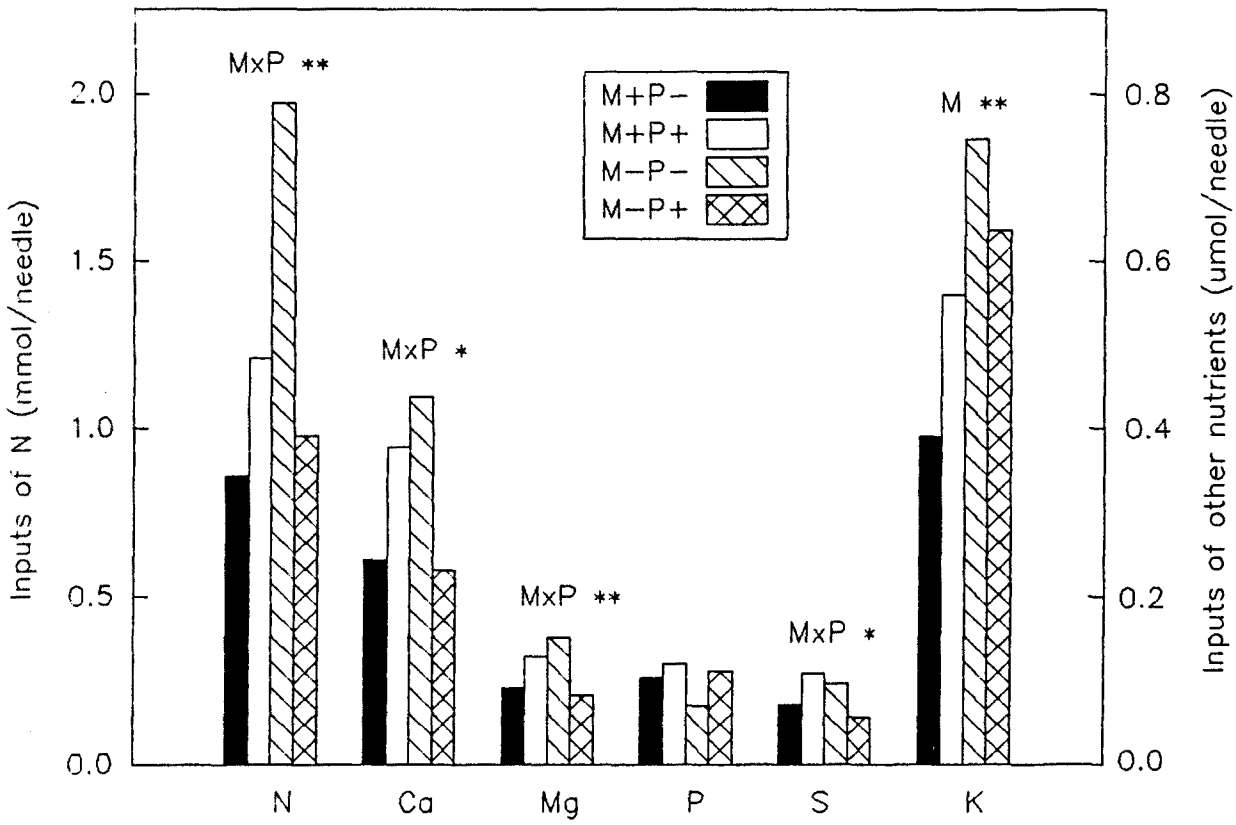


Fig. 2.5. Needle nutrient uptake from January to May. Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. D refer to depth. *,, *** refer to significant main effects and interaction significant at $p<0.05$, 0.01 and 0.001 respectively.**

Nutrient concentration in stems

P, K, S and Ca concentrations in stems were increased in both harvests after fertilizer application. Similar to needles, stem P concentration in P- treatments was diminished after mycorrhiza suppression but increased in P+ treatments. Because stem growth was affected by mycorrhiza suppresion and superphosphate addition similarly to needle biomass, the same discussion applies here. In the first harvest, mycorrhiza suppression increased S, K, Ca, and Mg concentrations, whereas in the second one Ca, Mg and Al concentrations were increased (Table 2.2). Ca, Mg and Al uptake is often by passive processes, suggesting that they were affected by the lack of dilution in M- treatments.

Like needles, stem nutrient concentration was decreased from 7 to 11 month old seedlings for most nutrients. Only Ca showed a significant increase (from 13 to 24 %), and Mg did not show any change, indicating that these nutrients accumulated in stems.

Nutrient concentration in roots

In roots of 11 months old seedlings, P, K, Ca, Mg and Al concentrations decreased due to mycorrhiza suppression, contrasting with stems where Ca Mg

and Al increased due to this factor. This decrease of mineral substances could explain the increase in C concentration observed in M- roots. Like needles and stems, P, K and S concentrations in roots were increased after superphosphate fertilization (Table 2.2).

Concentrations of most elemets (P, K, Ca, Mg and Al) in mycorrhizal roots were higher than in non mycorrhizal roots. This trend was not observed in mature needles nor in second harvest stems (Table 2.3). Interestingly, Ca, Mg and Al concentrations in mycorrhizal second harvest stems were low. These results agree with those from Kothari et al., (1990b) in maize VA mycorrhizae, and indicates that root-shoot transport in mycorrhizal and non-mycorrhizal seedlings was not paired. Kothari et al. (1990b) concluded that this unpaired transport was the result of the decrease in root length and lateral roots observed under mycorrhizal infection, which allowed Ca and Si to stay immobile in the apoplast. In our experiment, the increase on Ca, Mg and Al concentrations in M+ roots coincided with high root diameter and P concentration. Moreover, concentrations of Ca Mg and Al in roots were considerably higher than in shoots (Table 2.3). Therefore this unpaired increase on Ca, Mg and Al in M+ roots could be possibly caused by an increase on P-Ca, P-Mg and P-Al compounds being immobilized in root apoplast. Truman et al. (1986) with radiata pine seedlings showed an accumulation of P-Al compounds in root apoplast. These P-Al compounds also could accumulate in root cell walls (Jensen et al. 1989). The decrease in C concentration shown by M+

roots could be attributed to the high accumulation of inorganic compounds in root apoplast under M+ conditions.

Nutrient uptake

Because nutrient uptake general trends for the 11 months whole growth period were similar to those of the 4 months period between January and May, here are only reported the figures corresponding to this latter period.

Nutrient uptake was decreased by mycorrhiza suppression for all nutrients except N, which decrease was not statistically significant (Table 2.6). Superphosphate addition increased the uptake of any element except N and Al. Possibly because seedlings were harvested at early stages of growth, the amount of fertilizer taken up by plants was always very low (ranging from 0.79 % to 2.73 % of the total added). However, it was increased by mycorrhiza (data not shown).

	Treatments				ANOVA		
	M+P-	M+P+	M-P-	M-P+	M prob.<	P prob.<	MxP prob.<
N	25.00	27.98	15.56	19.98	ns	ns	ns
P	1593.35	2807.18	344.77	2098.73	0.0003	0.0001	ns
S	1000.50	1491.43	447.38	1158.72	0.0023	0.0002	ns
K	6033.75	9016.80	2922.23	6891.88	0.0017	0.0002	ns
Ca	4571.12	6588.80	2355.76	3968.78	0.0009	0.0067	ns
Mg	2548.31	3421.06	1237.23	1917.57	0.0009	0.0325	ns
Al	5605.27	6852.16	2439.21	3741.25	0.0070	ns	ns

Table 2.6. Total uptake. N is presented in mmol pot^{-1} whereas P, S, K, Ca, Mg and Al are presented in umol/pot. ns no significant at 5 %. Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. ns refers to non significant differences at 0.05 level.

To better understand the relative contribution of the treatments to nutrient acquisition, nutrient uptake was related to root surface area (specific uptake rate: umol uptake cm^{-2} root). The specific uptake rate was decreased by mycorrhiza suppression for all nutrients (Fig. 2.6). This decrease was larger than the absolute uptake decrease and was different for each nutrient. Fertilizer application increased P, S, K and Ca specific uptake rate. P, S and Ca were added to the soil in the fertilizer (superphosphate). The increases of soil soluble P (see chapter 1) and of exchangeable Ca and K observed after fertilizing (Fig. 2.7), could explain this increase in uptake. The relative effect of mycorrhiza suppression in element uptake in high and low P fertility conditions is illustrated in Fig. 2.8. In P- treatments, P was the element that uptake was most increased by mycorrhizal infection, followed by Al, S, Mg, K, Ca and N, whereas in P+ Al uptake was the one most increased by mycorrhiza, followed by Mg, Ca, N, P, K and S. The high specific uptake rate observed in M+ treatments relative to M- treatments, decreased when soil nutrient availability was high (Fig. 2.8). This decrease could be partly explained by the parallel decrease on the proportion of mycorrhiza infected root tips observed after adding fertilizer. It is generally believed that P is the nutrient which uptake is most largely enforced by mycorrhiza (Koide, 1991; Bolan, 1991). In our experiment mycorrhizae largely enforced P uptake only in low P availability conditions (Fig. 2.8).

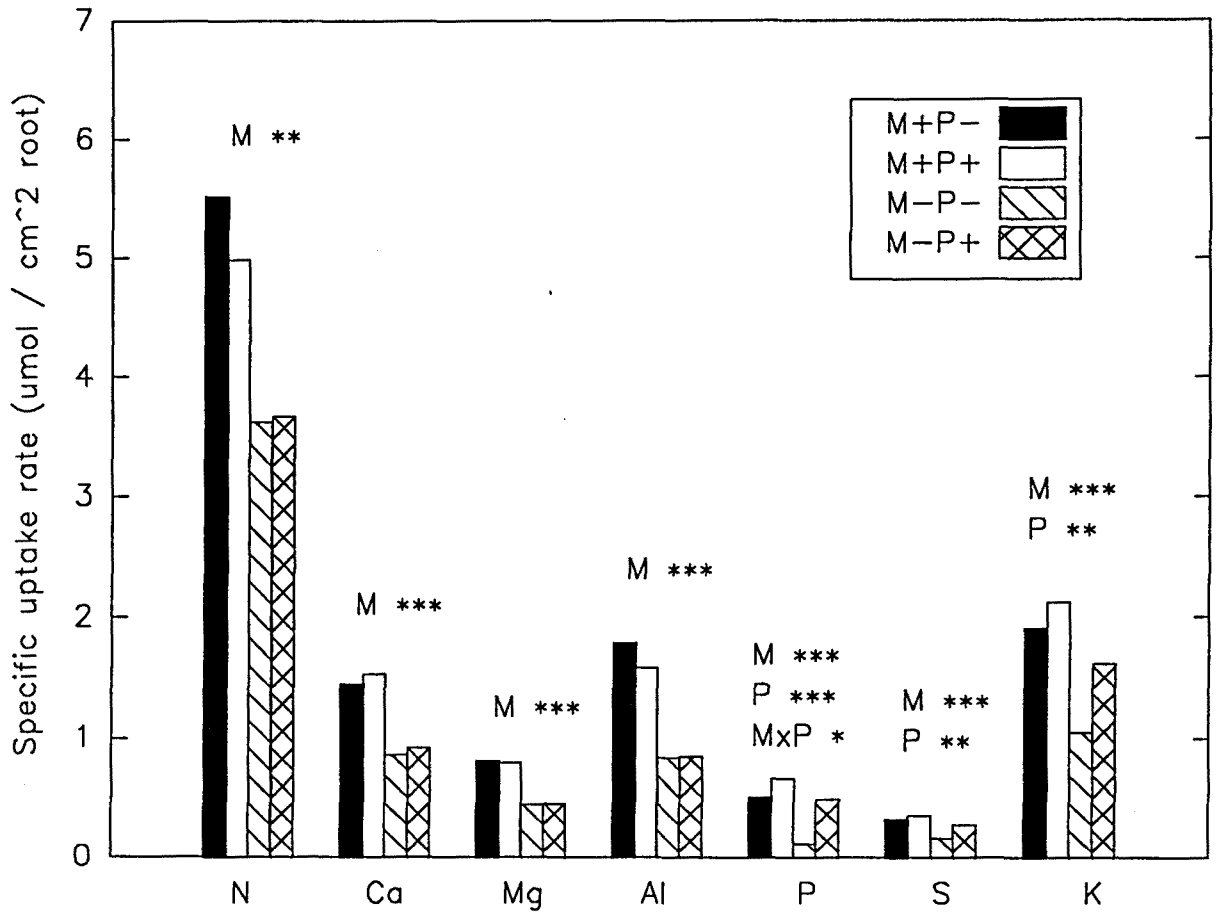


Fig. 2.6. Specific uptake rate (umol uptake cm^{-2} root). Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. *,, *** refer to significant main effects and interactions significant at $p<0.05$, 0.01 and 0.001 respectively.**

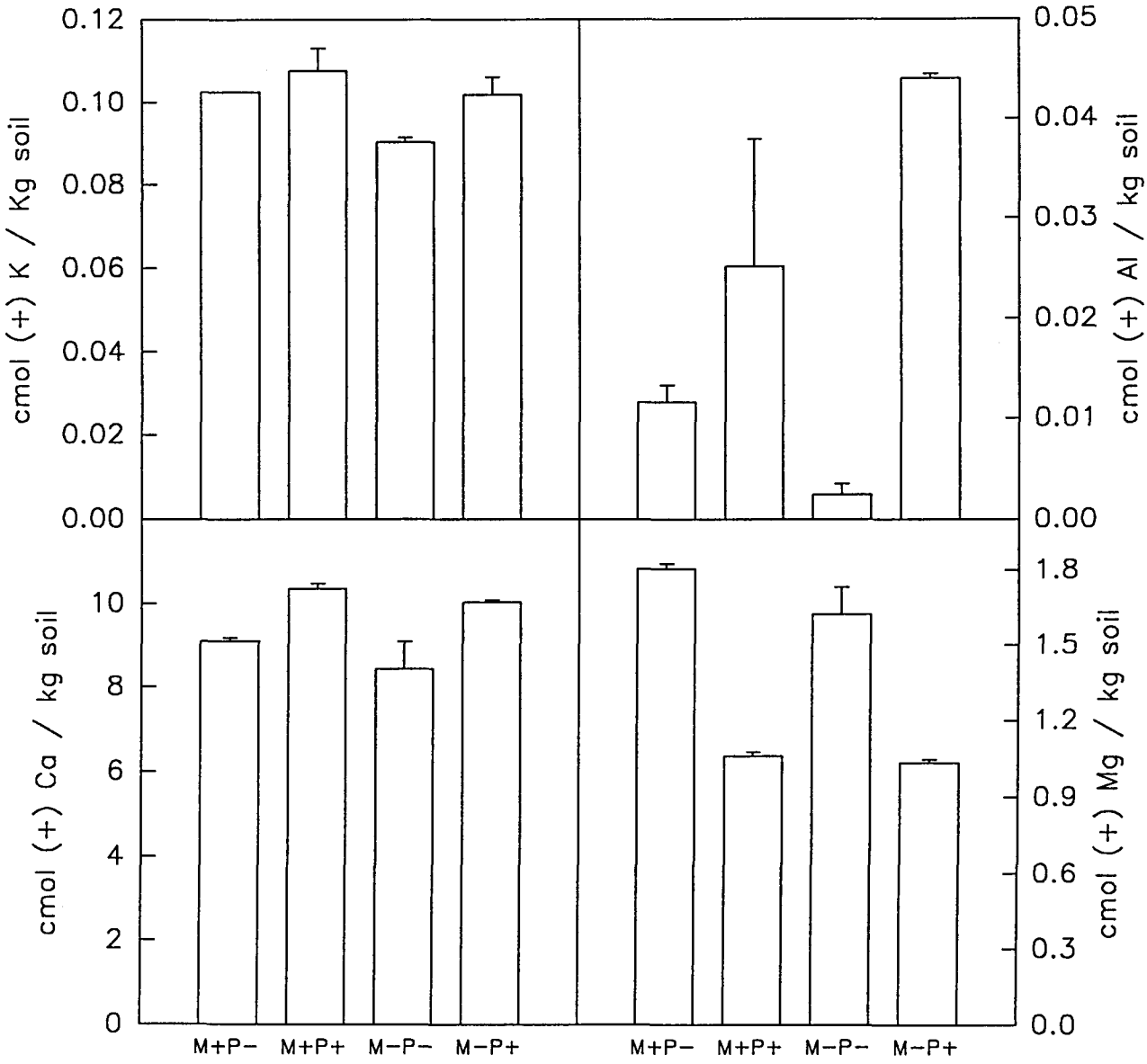


Fig. 2.7. Soil exchangeable cations as affected by treatments. Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. Bars refer to standard deviation.

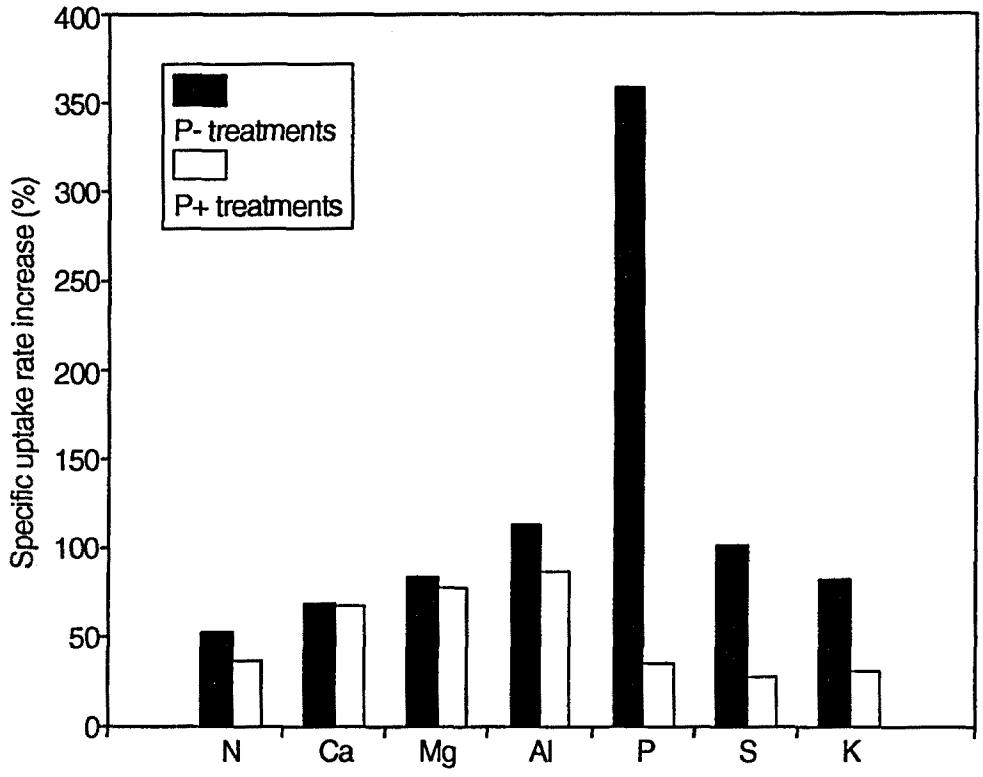


Fig. 2.8. Relative effect of mycorrhiza suppression, expressed in percentage, on nutrient specific uptake rate (umol uptake cm^{-2} root) in fertilized (P+) and unfertilized treatments (P-).

Ratios comparing the uptake of P to the other elements were only affected by mycorrhiza suppression in P- treatments (Table 2.7). This fact indicated that under low P availability conditions, the suppression of mycorrhiza had an important effect in decreasing the supply of P to the plant in relation to all other nutrients.

Nutrient uptake correlated well with root surface especially in M- treatments (Table 2.8). For most nutrients, the curves were statistically parallel, but their intercept diminished after mycorrhizae suppression, indicating that the high efficiency in taking up nutrients, in M+ treatments, was independent from root surface. Ca was the only nutrient that showed a slope decrease by a factor of 2 (Fig. 2.9).

In M+ treatments the slope of the relationship between Ca uptake and root surface two folded the same relationship in M- treatments (Fig. 2.9). Khotari et al. (1990a) reported a twofold water uptake increase caused by VA mycorrhizal infection in maize. Because Ca is mostly taken up by mass flow processes (Barber, 1984), it is reasonable to think that the increase in Ca uptake would result of the increase in water uptake in mycorrhizal plants compared to non-mycorrhizal. This fact also would explain why the uptake of Ca in M+ treatments increased with root surface at a rate twice higher than in M- treatments.

Nutrient uptake ratios	M+P-	M+P+	M-P-	M-P+	M prob.<	P prob.<	MxP prob.<
N/P	10.86 a	7.52 b	33.33 c	7.35 b	0.0017	0.0001	0.0007
S/P	0.62 a	0.53 a	1.65 b	0.56 a	0.0027	0.0011	0.0038
K/P	3.78 a	3.22 a	11.24 b	3.32 a	0.0039	0.0017	0.0046
Ca/P	2.92 a	2.35 a	9.41 b	1.87 a	0.0147	0.0023	0.0063
Mg/P	1.69 a	1.23 a	4.87 b	0.92 a	0.033	0.0031	0.0129
Al/P	3.76 a	2.46 a	9.23 b	1.75 a	ns	0.0073	0.0428

Table 2.7. Ratios of nutrient-element uptake. Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil. ns refers to non significant differences at 0.05 level. Letters underneath figures refer to Student T-test comparisons.

Nutrient	Treatment	slope	intercept	r^2	prob.<
N	M+	3.77 a	5.37 a	0.420	ns
	M-	5.79 a	-6.79 b	0.857	0.0010
P	M+	0.91 a	-1175.39 a	0.756	0.0050
	M-	0.78 a	-1527.53 b	0.790	0.0031
S	M+	0.40 a	-244.49 a	0.765	0.0045
	M-	0.35 a	-418.04 b	0.800	0.0027
K	M+	2.49 a	-1684.85 a	0.758	0.0049
	M-	1.98 a	-2032.27 b	0.860	0.0009
Ca	M+	2.08 a	-2128.93 a	0.883	0.0005
	M-	1.06 b	-563.19 b	0.855	0.0010
Mg	M+	0.88 a	-228.51 a	0.485	ns
	M-	0.51 a	-201.64 a	0.973	0.0001
Al	M+	1.64 a	194.74 a	0.274	ns
	M-	1.25 a	-1330.51 b	0.743	0.0059

Table 2.8. Relationships between nutrient-element uptake and root surface in treatments fertilized with superphosphate (M+) and in unfertilized treatments (M-) (n=8); ns refers to the non significant regressions at 0.05 level. Regressions were compared by using analysis of covariance. Letters next to figures compare the parameters of the regression for each nutrient at 0.05 level.

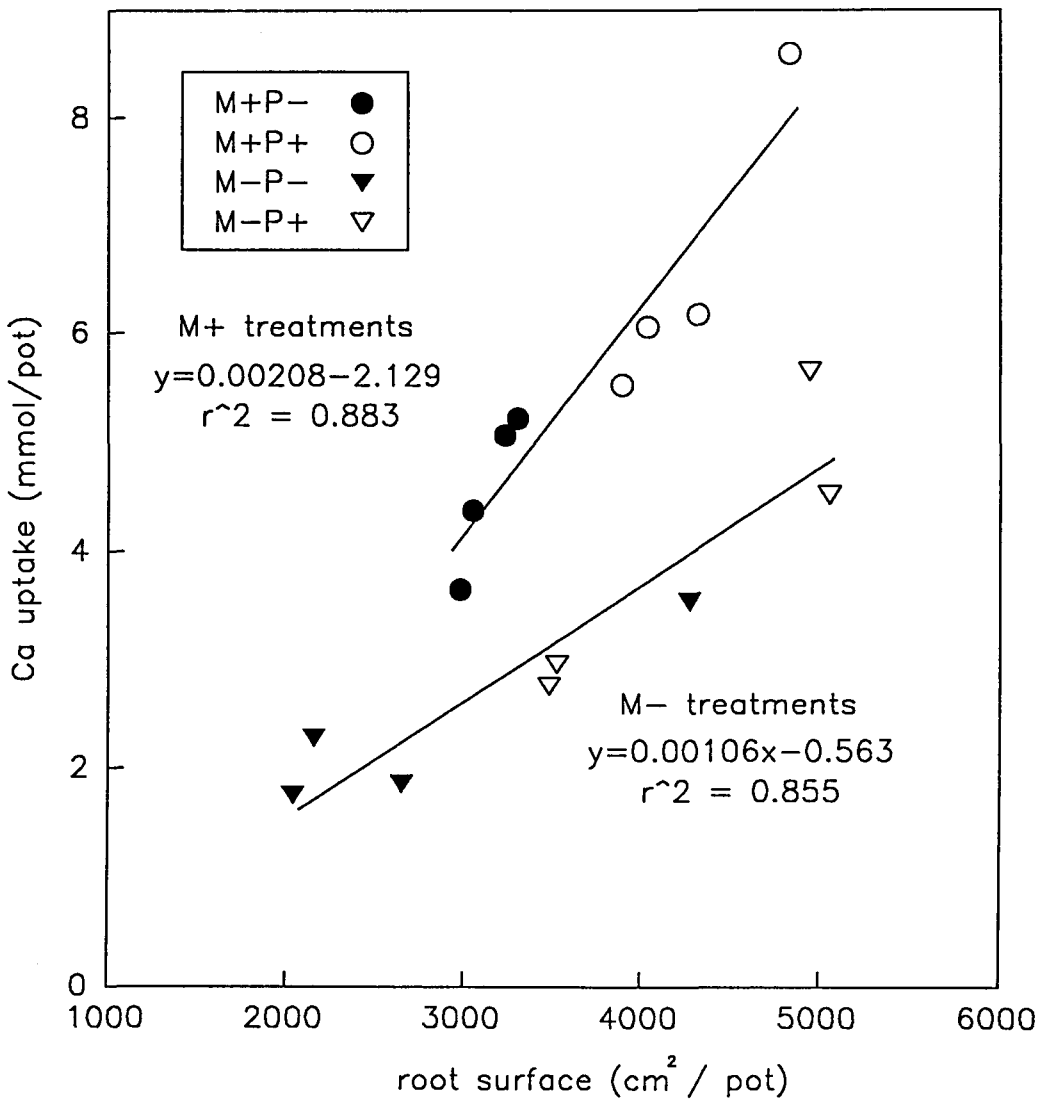


Fig. 2.9. Linear regressions between root surface and Ca uptake in mycorrhizal (M+) and mycorrhiza suppressed treatments (M-). Treatments were: M+P- = Mycorrhizal seedlings grown in unfertilized soil; M+P+ = Mycorrhizal seedlings grown in fertilized soil; M-P- = Non-mycorrhizal seedlings grown in unfertilized soil; M-P+ = Non-mycorrhizal seedlings grown in fertilized soil.

CONCLUSIONS

Mycorrhizae suppresion always decreased seedling growth, regardless soil phosphorus availability conditions. Changes in root diameter and configuration observed after mycorrhiza suppression maintained root length and surface despite the decrease in root biomass. Mycorrhizae increased the specific uptake rate for all nutrient. The ability of mycorrhizae in increasing P uptake depended upon soil P availability. Under low P availability conditions, P uptake was the one most decreased by mycorrhizae suppression. However, N shortages in M- treatments were not overcome by the mycorrhizae. Increases in Ca uptake in M+ treatments when related to root surface suggested that mycorrhizal seedlings took up water at rates twice as high as M- seedlings.

Despite superphosphate addition did not largely affect seedling growth, in M- treatments it increased the average needle weight and needle number to similar levels than in M+ treatments. While seedlings in the first harvest responded to nutritional shortages by delaying needle development, in the second harvest they controlled the number of needles. At that time, needle size was not affected by treatments. The delay in needle development could explain why seedlings showing the same foliar characteristics at the end of the experiment showed a different total growth. Root length and surface increased after superphosphate fertilization. This

increase was mostly originated by the raise of the proportion on roots from 0.5 to 1 mm thick.

CHAPTER 3

MODELLING P AND Mg UPTAKE BY *PINUS RADIATA* D. DON SEEDLINGS AS AFFECTED BY SUPERPHOSPHATE ADDITION AND BY MYCORRHIZA SUPPRESSION.

INTRODUCTION

The Barber-Cushman nutrient uptake mechanistic model, applies the theory of diffusion and mass flow to nutrient uptake. This model integrates the processes influencing soil nutrient supply with the nutrient absorption kinetics at the root surface (Barber, 1979). The Barber-Cushman model has been used, many times successfully, to simulate uptake of P, K, Mg and some heavy metals by agricultural crops (Schenk & Barber, 1980; Silberbush & Barber, 1983, 1984; Mullins et al. 1986; Rengel & Robinson, 1990; Ernani & Barber, 1984). However, for forest species, nutrient uptake modelling has received less attention. Van Rees et al. (1990a) and Kelly et al. (1992) quite successfully predicted P, K and Mg uptake using the Barber-Cushman model to slash pine (*Pinus elliotii* Engelm. var. *elliotii*) and loblolly pine (*Pinus taeda* L.) seedlings respectively. Gillespie & Pope (1990) successfully predicted P uptake for black locust (*Robinia pseudoacacia* L.), when the effect of pH was incorporated to the model. All these studies applied the model to either low or high soil nutrient supply characteristics but no research has been conducted to apply this model to forest species under contrasted soil fertility conditions.

It is now widely accepted that mycorrhizal associations can improve P uptake through several mechanisms (Bolan, 1990). The solubilization of inorganic P forms as well as P mineralization can be enhanced by mycorrhizal associations

(Hetrick, 1989). Mycorrhiza can also increase the physical exploration of the soil and the surface area for nutrient absorption (Sanders & Tinker, 1973; Tinker, 1978). Furthermore, nutrient affinity and threshold concentrations may be different for mycorrhizal and non mycorrhizal roots. Kothari et al. 1990b found that mycorrhizal infection caused an important uptake increase of all tested nutrient when referred to root surface. They suggested that the higher efficiency of mycorrhizal roots could be due to either lower Km values for mycorrhizal hyphae (Cress et al., 1979) or less overlapping of P depletion zones around external hyphae, due to the geometry of the hyphal system (Junk & Claasen, 1989). The Barber-Cushman nutrient uptake model does not consider any uptake processes associated with mycorrhizae because the surface area and absorption parameters of external hyphae are not known. However, the degree of agreement between model predicted and observed values for root systems infected and uninfected with mycorrhiza, have not been tested yet.

In the second chapter, it has been shown that P fertilization had a strong effect on the role of mycorrizae in taking up P, and that foliar levels of Mg were well under deficiency critical concentration. Therefore, the aims of the work presented here were, i) to test the ability of Barber-Cushman nutrient uptake model to predict P and Mg uptake under low and high P fertility conditions in mycorrhiza infected and uninfected radiata pine seedlings, and ii) to indetify the cause of Mg deficiency.

MATERIAL AND METHODS

Estimation of Nutrient Uptake

Nutrient uptake of *Pinus radiata* seedlings grown in pots for 120 days (from January 17 to May 17) was measured and compared to Barber-Cushman predictions of nutrient uptake. The experimental design used consisted of four replicates of a 2X2 two way of superphosphate fertilization, and mycorrhiza suppression arranged in a completely randomised design. This same experimental design was used in Chapters 1 & 2. The details of procedures used to measure nutrient uptake in seedlings grown from January to May are described in the Material and Methods section of Chapter 2.

In this way, the model was tested for high and low P fertility soils and for root systems with and without mycorrhizae. Four predictions per treatment were carried out, so, the model was run for each of the 16 pots.

Barber-Cushman Model

The Barber-Cushman model is a mathematical model that integrates different factors influencing nutrient uptake by roots. The model uses 4 different

parameters to describe soil nutrient supply characteristics: initial concentration of nutrient in soil solution (Cli), diffusion coefficient for nutrient movement through bulk soil (De), buffer power of the solid phase for nutrient in solution (b) and the water uptake rate (Vo) which describes the amount of water taken up per unit of root surface. To describe root morphology and growth pattern, the model uses 4 different parameters: initial root length (Lo), rate or constant of root growth (k), mean root radius (Ro), and half distance between root axes (R1). Finally, to describe nutrient absorption kinetics at the root surface, a Michalelis-Menten type equation is used. This equation uses 3 further parameters (Imax, Km & Cmin). Imax stands for the maximum nutrient intake rate, Km sets the concentration at the root surface for which uptake rate is half of its maximum value (Imax), and Cmin is the threshold concentration below which there is no net uptake. Following are listed the major assumptions of the model:

1. Roots are distributed in uniform and parallel pattern throughout the soil.

2. The soil is homogeneous in space and time apart from concentration profiles that develop around each root. Soil water content is constant in time and space.

3. Nutrient transport in the soil is by diffusion and mass flow in the radial direction only. Dispersion is neglected.

4. The root is cylindrical and root hairs, mycorrhizae and root exudates are not considered.

5. Adsorption at the root surface is described by a Michaelis-Menten type relationship that holds for the entire surface.

6. Nutrient influx is independent of the rate of water absorption.

7. Diffusion coefficient (De) and buffer power (b) are independent of ion solution concentration.

A detailed discussion of the mathematical equations and definitions of this model can be found in Barber and Cushman (1981) and Barber (1984). In this study uptake simulation was performed by running the version of the model published by Oates & Barber (1987) on an IBM compatible microcomputer.

Estimation of soil parameters

The three soil parameters used to describe soil nutrient supply capacity were estimated as follows: Cli was measured by extracting moist soil, collected at the begining of the experiment, with water (1/10) for 16 hours. Soil buffer power

was calculated from this equation:

$$b=\theta+\varrho Kd$$

(Van Rees et al. 1990a) were θ is the volumetric water content (θ=ϱ x soil moisture%/ 100; θ unities are ml water/ml soil), ϱ is the bulk density of the soil, and Kd is the partition constant between the liquid and the solid phase. Kd values were determined from the desorption isotherm estimated by water extraction at rates 1/10, 1/20, 1/40, 1/60, 1/80. The resulting isotherm was fit to a Freundlich function ($Cs=aCl^{1/c}$; where Cs is the nutrient concentration in the solid phase, and Cl the concentration in the liquid phase; a and c are the parameters to fit the function) for which the gradient of the slope was Kd. Kd was estimated at the initial soil solution concentration by using first derivative of the Freundlich equation.

Diffusion coefficient (De) was calculated from the equation (Nye & Tinker 1977):

$$De=D_l\theta f/b$$

where f the impedance factor was estimated by using the equation $f=3.1\theta^{1.9}$ (Van Rees et al.1990b). The impedance factor accounts for the tortuous pathway of ions moving through sequences of soil pores. And D_l is the diffusion of the nutrient in free solution (D_l= 8.9 X 10^{-10} m^2 s^{-1} for P, and as 7.1 X 10^{-10} m^2 s^{-1} for Mg).

Because the soil in which the experiment was carried out was rich in Ca (more than 75% of exchangeable cations were Ca), and because of Ca is mostly supplied by mass flow (Ulrich & Mayer, 1972; Ballard & Cole, 1974; Barber, 1984), Ca supply was assumed to be only by mass flow. By assuming that, the uptake of water was calculated from the equation:

$$Vo=(tCa/[Ca]_i)/ 2\pi \; Ro \; Lm \; T$$

where tCa is the total uptake of Ca by radiata pine seedlings (µmol Ca/pot), $[Ca]_i$ is bulk soil solution Ca concentration (umol Ca /ml), Ro is the average of root radius (cm), T is the time the seedlings grew (s) and Lm is the mean root length per pot (cm) considering the exponential root growth rate of roots. Therefore, Lm was calculated by dividing root growth period into 100 equally distant periods of time. Root length in each period was computed by using the exponential root growth equation described in the following page. Lm was then calculated as the average of the 100 different periods of time. Water uptake rates resulting from this equation were similar to the rates presented by Van Rees et al. (1990) for *Pinus elliotii* Engelm. var. *elliotii* seedlings.

Phosphorus in soil extracts was determined by the method of Murphy and Riley (1962). Magnesium and Calcium were determined using ICP spectroscopy. Soil moisture was determined at the begining and at the end of the experiment by

weight difference after drying the moist soil at 105° C.

Estimation of plant parameters

Root length at the end of the experiment was measured as described in the previous chapter. Root length at the begining of the experiment (Lo; root length in January) was estimated by two methods. The first method used the single treatment root : shoot ratio worked out at the end of the experiment (May) applied to the measured aerial biomass in January to calculate root biomass at this stage. Root biomass was then transformed to root length by using single treatment root length ratios worked out in May. The second method for estimating root length in January was by fitting an exponential growth equation to the measured root length values at the very beginning L (July, 1992) and at the end of the experiment L1 (May, 1991) ($L1=L\ e^{kT}$). The two methods gave very similar estimates of Lo, but only the latter was used for the model. The k of the exponential equation used to describe root growth during the simulation was estimated as follows:

$$k=(\ln L-\ln Lo)/T.$$

Average root radius (Ro) was estimated separately for each treatment as described in the previous chapter. The half distance between root axes (R1) was

determined using the following equation (Barber, 1984):

$$R1=1/(\pi Lv)$$

where Lv is the root-length density (cm root cm^{-3} soil).

Due to the lack of information on Michaelis-Menten uptake parameters for forest species, parameters for Mg and P were taken from Kelly et al. (1992), despite they measured these parameters for another pine species (*Pinus taeda*).

Sensitivity analysis

Sensitivity analysis was conducted in order to evaluate the effect of each parameter on P or Mg uptake. This analysis assumes all parameters are independent to each other. Each parameter was varied by factors between 0.25 and 2.0. Only one parameter was changed at a time. Results are expressed relative to predicted P and Mg uptake under initial conditions.

Statistics

Student's T tests were used to compare observed with predicted observations (prob.< 0.05 %). This same test was used to determine if slopes between predicted and observed values were different from one.

RESULTS

Modelling P and Mg uptake

The values of the parameters used to simulate P and Mg uptake processes are listed in tables 1 & 2.

The degree of agreement between measured and predicted uptake for P and Mg is shown in figure 1. The model was able to predict P uptake only for non-mycorrhizal seedlings under low P availability conditions (M-P-). Under fertilized conditions (P+) the model consistently overpredicted P uptake, whereas for M+P- treatment P uptake was largely underpredicted (Fig. 3.1 & Table 3.3). However, the model was able to predict the relative variations in P uptake observed across

M- treatments (Fig. 3.1).

In M+ treatments, predicted Mg uptake was significantly lower than the observed values (Table 3.3), whereas in M- treatments the difference was not significant. Nevertheless, in unfertilized conditions the observed uptake was a 26 % overestimated whereas in fertilized pots it was 3% underestimated (Table 3.3). Thus the slope of the curve fitted to M- treatments was significantly lower than one (slope=0.61, R^2=0.80).

P	Pot	De	b	Cli	Vo	R1	Ro	Lo	k	Imax	Km	Cmin
		cm2/s	Unitless	umol/cm3	cm/s	cm	cm	cm	cm/s	umol/cm2/s	umol/cm3	umol/cm3
M+P-	1	1.44E-08	3.72	0.0085	2.22E-06	1.37	0.054	6019	1.13E-07	2.68E-07	0.016	0.0006
	2	1.44E-08	3.72	0.0085	2.15E-06	1.66	0.054	4118	1.48E-07	2.68E-07	0.016	0.0006
	3	1.44E-08	3.72	0.0085	1.96E-06	1.71	0.054	3871	1.46E-07	2.68E-07	0.016	0.0006
	4	1.44E-08	3.72	0.0085	2.28E-06	1.68	0.054	4037	1.44E-07	2.68E-07	0.016	0.0006
M+P+	5	7.99E-09	6.70	0.0667	1.95E-06	1.57	0.058	4610	1.51E-07	2.68E-07	0.016	0.0006
	6	7.99E-09	6.70	0.0667	2.55E-06	1.65	0.058	4163	1.79E-07	2.68E-07	0.016	0.0006
	7	7.99E-09	6.70	0.0667	1.90E-06	1.57	0.058	4638	1.57E-07	2.68E-07	0.016	0.0006
	8	7.99E-09	6.70	0.0667	1.93E-06	1.71	0.058	3898	1.64E-07	2.68E-07	0.016	0.0006
M-P-	9	2.49E-08	2.15	0.0093	1.48E-06	1.43	0.040	5536	1.75E-07	2.68E-07	0.016	0.0006
	10	2.49E-08	2.15	0.0093	9.95E-07	1.34	0.040	6360	1.16E-07	2.68E-07	0.016	0.0006
	11	2.49E-08	2.15	0.0093	1.16E-06	1.42	0.040	5637	1.02E-07	2.68E-07	0.016	0.0006
	12	2.49E-08	2.15	0.0093	1.52E-06	1.50	0.040	5029	1.18E-07	2.68E-07	0.016	0.0006
M-P+	13	7.11E-09	7.53	0.0582	1.04E-06	1.44	0.048	5499	1.40E-07	2.68E-07	0.016	0.0006
	14	7.11E-09	7.53	0.0582	1.30E-06	1.51	0.048	4989	1.84E-07	2.68E-07	0.016	0.0006
	15	7.11E-09	7.53	0.0582	1.49E-06	1.30	0.048	6705	1.53E-07	2.68E-07	0.016	0.0006
	16	7.11E-09	7.53	0.0582	1.11E-06	1.71	0.048	3877	1.72E-07	2.68E-07	0.016	0.0006

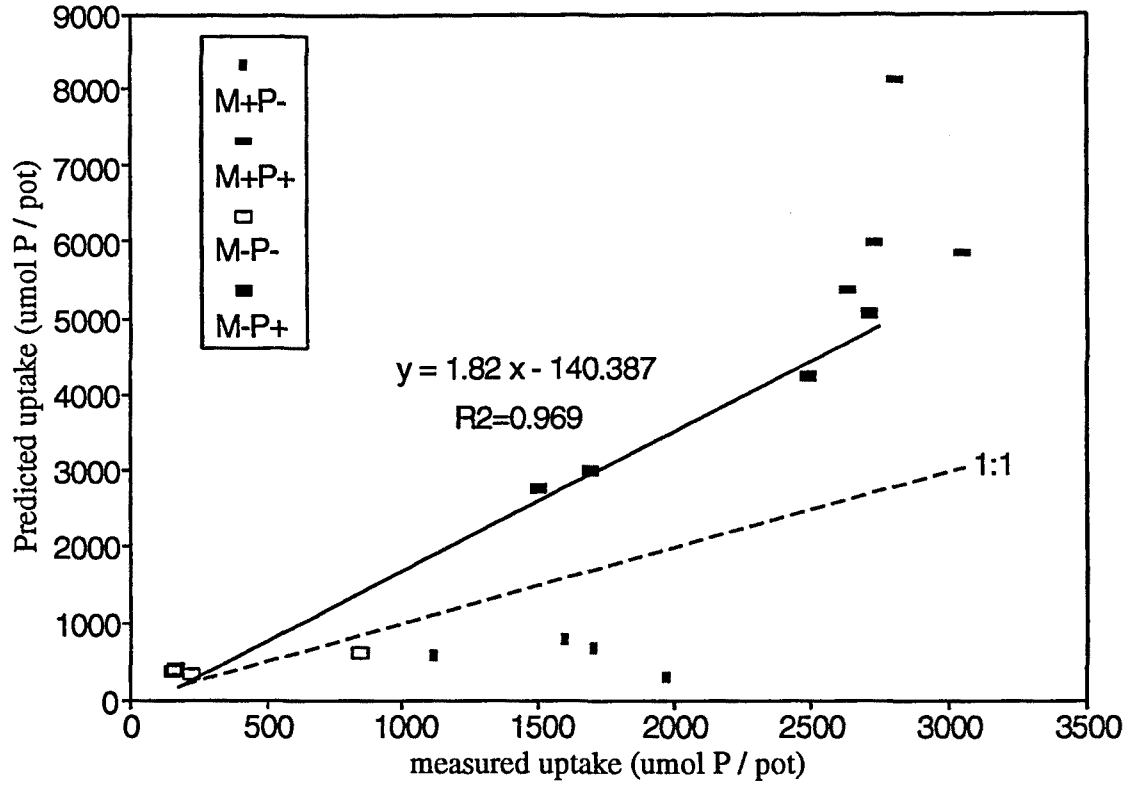
Table 3.1. Input parameters for each pot to simulate P uptake by using the Barber-Cushman model. De is the diffusion coefficient for nutrient movement through bulk soil; b buffer power; Cli initial concentration of nutrient in soil solution; Vo water uptake at root surface; R1 half-distance between roots; Ro mean root radius; Lo initial root length; k rate of root growth; Imax maximum influx of P at high concentrations; Km nutrient concentration in solution where net influx is 0.5 Imax; Cmin nutrient concentration in solution where nutrient influx is zero.

Mg	Pot	De cm2/s	b unitless	Cli umol/cm3	Vo cm/s	R1 cm	Ro cm	Lo cm	k cm/s	Imax umol/cm2/s	Km umol/cm3	Cmin umol/cm3
M+P-	1	1.9E-07	0.23	0.078	2.22E-06	1.37	0.054	6019	1.13E-07	1.29E-07	0.0098	0.001
	2	1.9E-07	0.23	0.078	2.15E-06	1.66	0.054	4118	1.48E-07	1.29E-07	0.0098	0.001
	3	1.9E-07	0.23	0.078	1.96E-06	1.71	0.054	3871	1.46E-07	1.29E-07	0.0098	0.001
	4	1.9E-07	0.23	0.078	2.28E-06	1.68	0.054	4037	1.44E-07	1.29E-07	0.0098	0.001
M+P+	5	1.9E-07	0.23	0.068	1.95E-06	1.57	0.058	4610	1.51E-07	1.29E-07	0.0098	0.001
	6	1.9E-07	0.23	0.068	2.55E-06	1.65	0.058	4163	1.79E-07	1.29E-07	0.0098	0.001
	7	1.9E-07	0.23	0.068	1.90E-06	1.57	0.058	4638	1.57E-07	1.29E-07	0.0098	0.001
	8	1.9E-07	0.23	0.068	1.93E-06	1.71	0.058	3898	1.64E-07	1.29E-07	0.0098	0.001
M-P-	9	1.9E-07	0.23	0.078	1.48E-06	1.43	0.040	5536	1.75E-07	1.29E-07	0.0098	0.001
	10	1.9E-07	0.23	0.078	9.95E-07	1.34	0.040	6360	1.16E-07	1.29E-07	0.0098	0.001
	11	1.9E-07	0.23	0.078	1.16E-06	1.42	0.040	5637	1.02E-07	1.29E-07	0.0098	0.001
	12	1.9E-07	0.23	0.078	1.52E-06	1.5	0.040	5029	1.18E-07	1.29E-07	0.0098	0.001
M-P+	13	1.9E-07	0.23	0.068	1.04E-06	1.44	0.048	5499	1.40E-07	1.29E-07	0.0098	0.001
	14	1.9E-07	0.23	0.068	1.30E-06	1.51	0.048	4989	1.84E-07	1.29E-07	0.0098	0.001
	15	1.9E-07	0.23	0.068	1.49E-06	1.3	0.048	6705	1.53E-07	1.29E-07	0.0098	0.001
	16	1.9E-07	0.23	0.068	1.11E-06	1.71	0.048	3877	1.72E-07	1.29E-07	0.0098	0.001

Table 3.2. Input parameters for each treatment to simulate Mg uptake by using the Barber-Cushman mechanistic model. De is the diffusion coefficient for nutrient movement through bulk soil; b buffer power; Cli initial concentration of nutrient in soil solution; Vo water uptake at root surface; R1 half-distance between roots; Ro mean root radius; Lo initial root length; k rate of root growth; Imax maximum influx of Mg at high concentrations; Km nutrient concentration in solution where net influx is 0.5 Imax; Cmin nutrient concentration in solution where nutrient influx is zero.

	Observed Uptake (μmol pot^{-1})	Predicted Uptake (μmol pot^{-1})	% of observed
P			
M+P-	1594.69 (311.12)	580.93 (214.05)	36.42
M+P+	2809.54 (150.80)	6341.39 (1219.82)	225.71
M-P-	345.06 (284.88)	419.62 (133.13)	121.60 (ns)
M-P+	2100.50 (515.16)	3755.76 (1089.03)	178.80
Mg			
M+P-	2548.31 (144.94)	1572.00 (148.73)	61.69
M+P+	3421.06 (898.62)	1913.85 (80.06)	55.54
M-P-	1237.23 (436.75)	1568.30 (439.16)	126.76 (ns)
M-P+	1917.57 (468.33)	1814.35 (275.59)	94.62 (ns)

Table 3.3. Comparison of observed P and Mg uptake with model predictions for radiata pine seedlings grown for 120 days period. Each figure represents a pot containing 10 seedlings. Figures in parenthesis refer to standard deviation and ns to percentages non-significantly different to 100% (prob. <0.05).



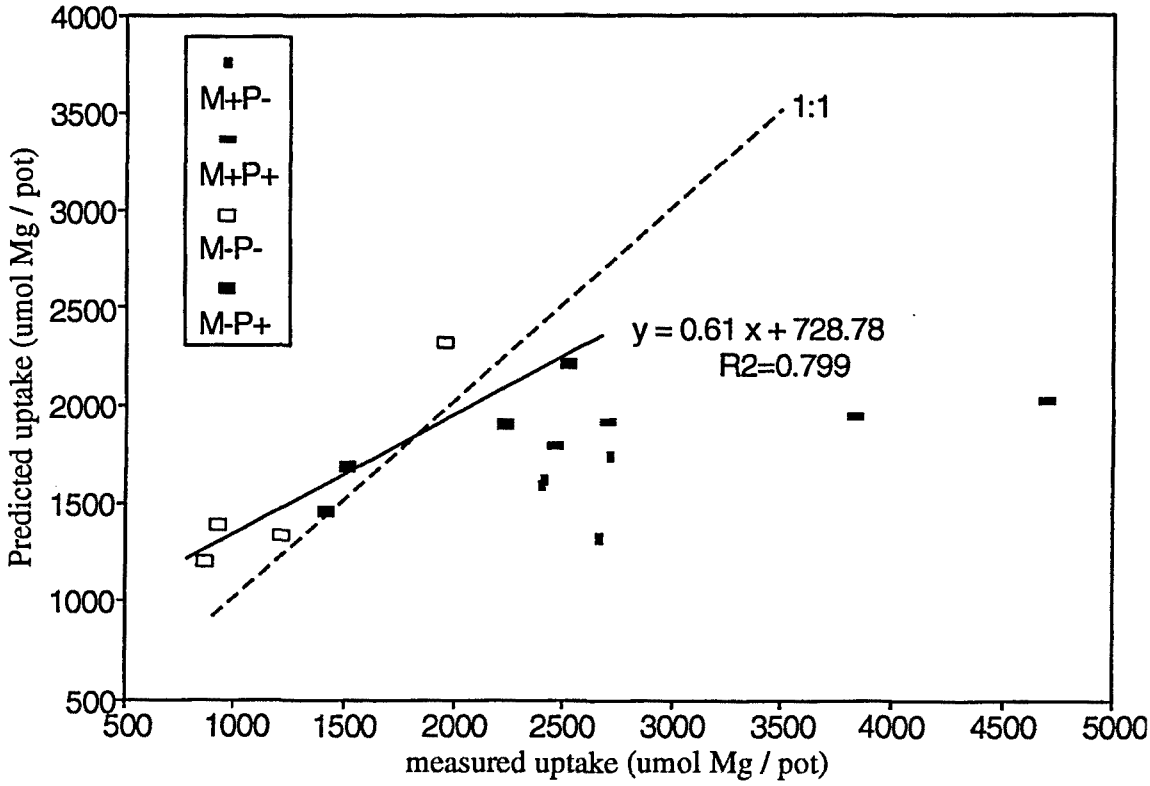


Fig. 3.1. Comparison of predicted by the Barber -Cushman model and observed P and Mg uptake for radiata pine seedlings grown in pots. Each point represents a pot containing 10 seedlings. The regression of uptake with fertilization includes only data from non-mycorrhizal pots.

Estimated concentration profile around root surface at the day 96 of the simulation period indicated a depletion zone for P and Mg in any treatment. While for P+ treatments concentration in the bulk soil solution at day 96 was still the same than the initial values (Cli), for P+ treatments it was slightly reduced (Fig. 3.2). At the same time, Mg concentration in the bulk soil was reduced to about 0.6 of its initial values (Cli).

Sensitivity analysis

General trends of the sensitivity analysis for the M+ treatments were similar to those of M- treatments, therefore, only the results from M- treatments will be presented. Sensitivity analysis for P revealed that the change of both diffusion (De) coefficient and buffer power (b) did not have a big impact on P uptake in any treatment (Fig. 3.3). Increases in initial soil solution concentration (Cli) and water uptake rate (Vo) increased P uptake. The change of the Michaelis Menten root kinetic parameters (Imax, Km and Cmin) did not show any big effect on P uptake in any treatment. Only the reduction of Imax in unfertilized treatments (P-) caused a decrease in P uptake (Fig. 3.4). Changing Ro and k (root growth parameters) corresponded to the biggest change on P uptake across all treatments. While the increase of k had a much larger effect on P uptake than increasing Ro, especially in P- treatments, when they were decreased their effects on P uptake were very

similar. The increase of R1 did not have any effect on P uptake whereas its reduction corresponded to a decrease of predicted uptake, especially in P-treatments.

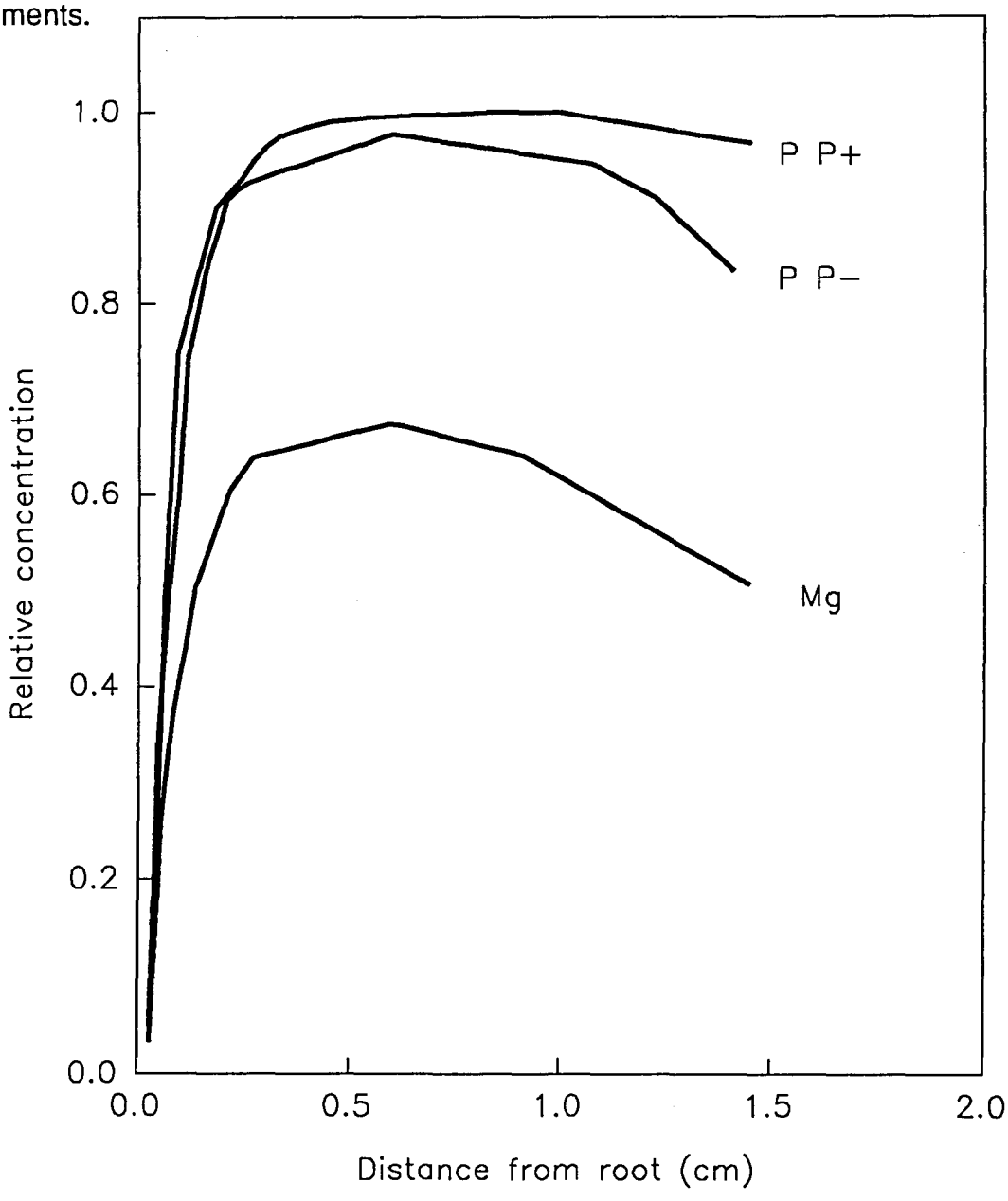
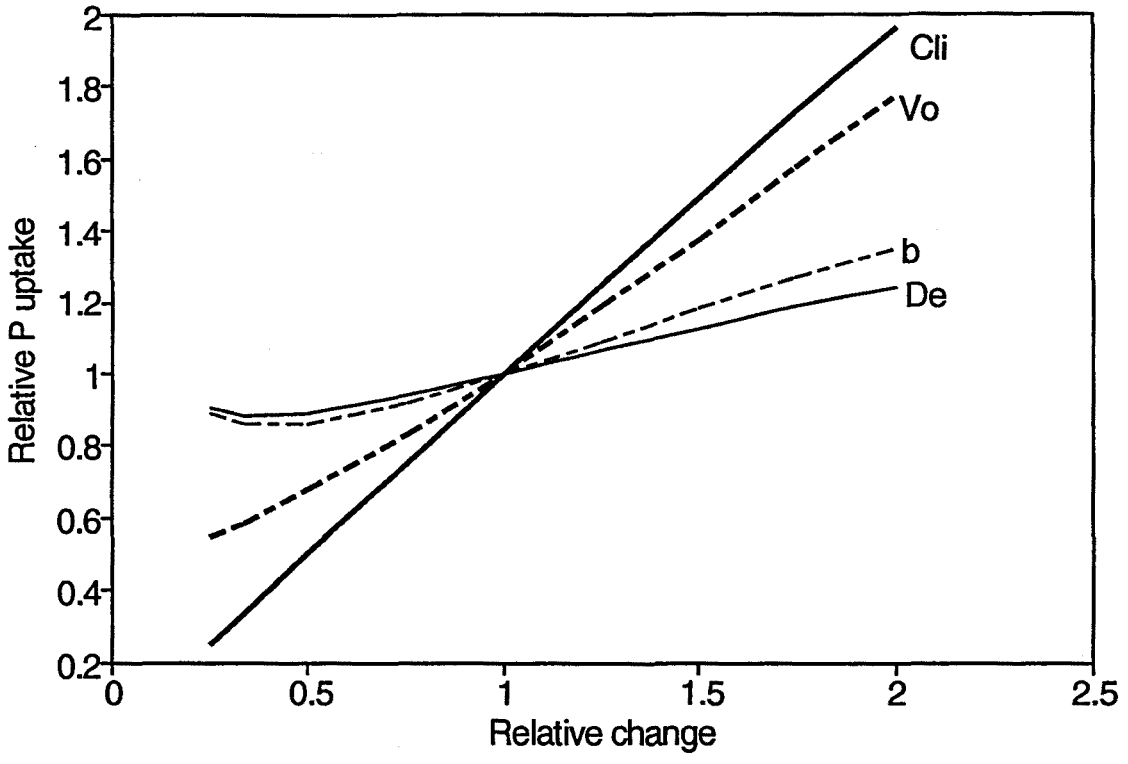


Fig. 3.2. Concentration of P and Mg relative to its respective initial concentration in soil solution increasing the distance from the root at the day 96 of the growth period. The curves presented are based on the ratio of the calculated concentration at increasing distances from the root divided by the initial soil solution concentration (*Cli*) of the respective element.



M-P-

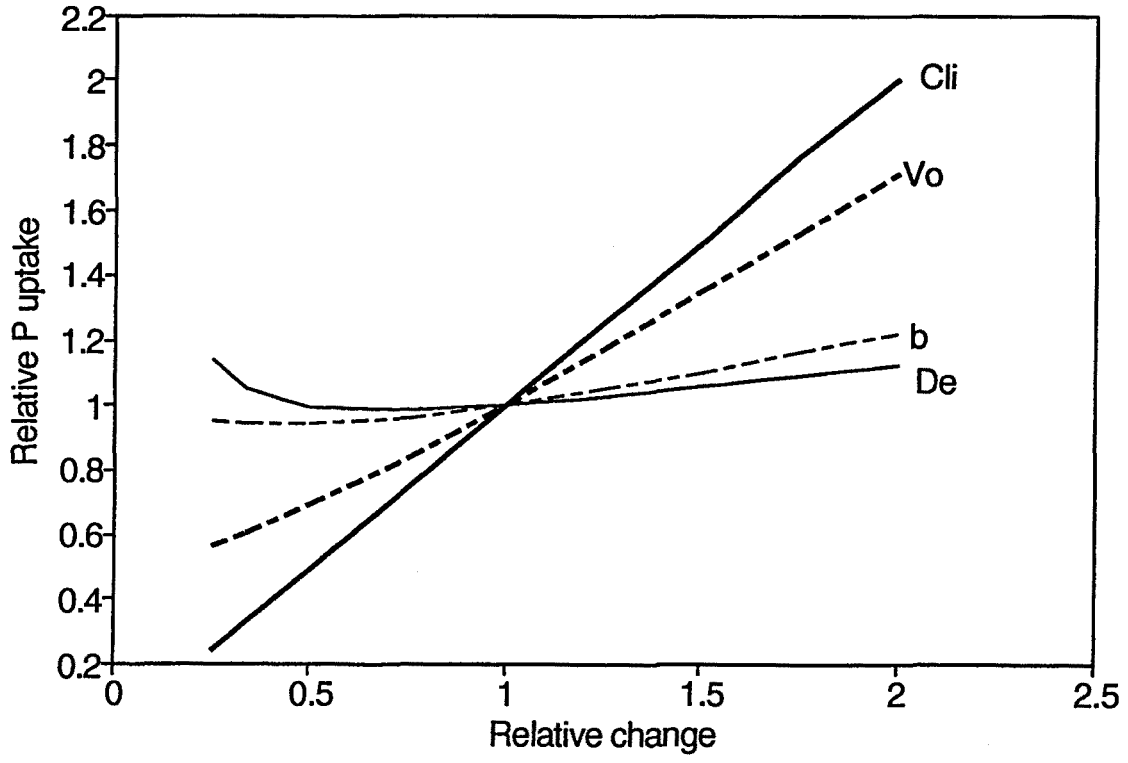
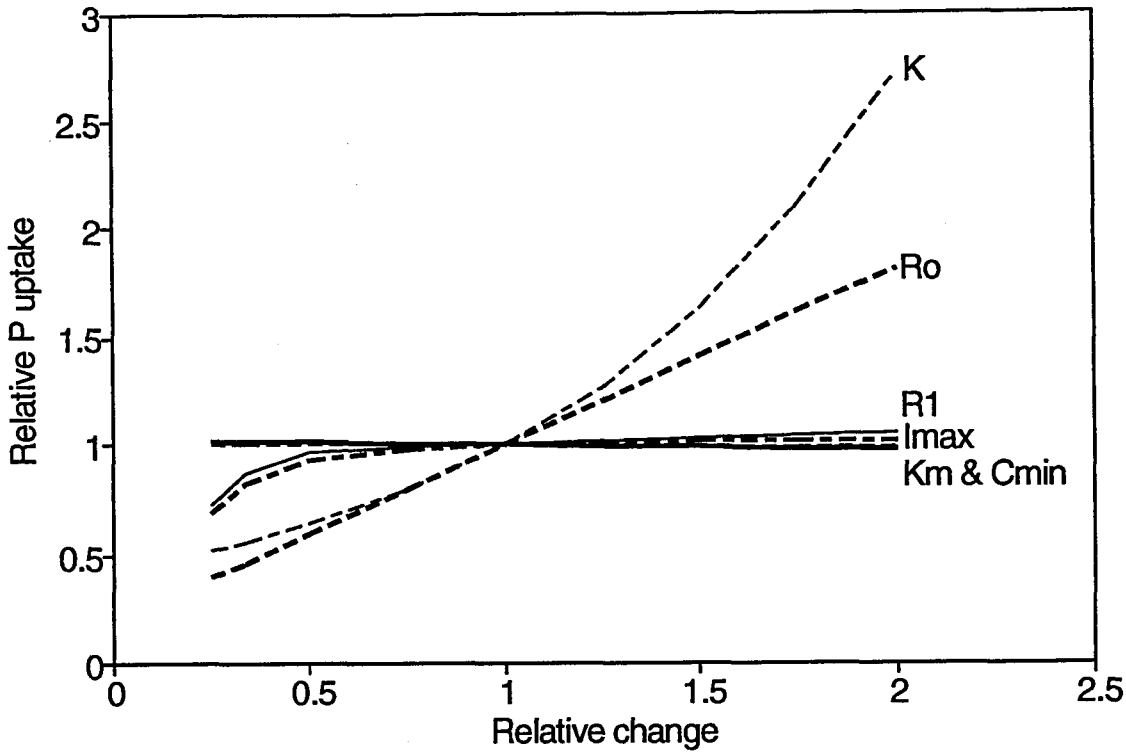


Fig. 3.3. Soil parameters sensitivity analysis of predicted P in non-mycorrhizal treatments in response to changing the initial parameter. Each parameter was varied individually while the others where held constant. Results are expressed relative to the initial conditions uptake.



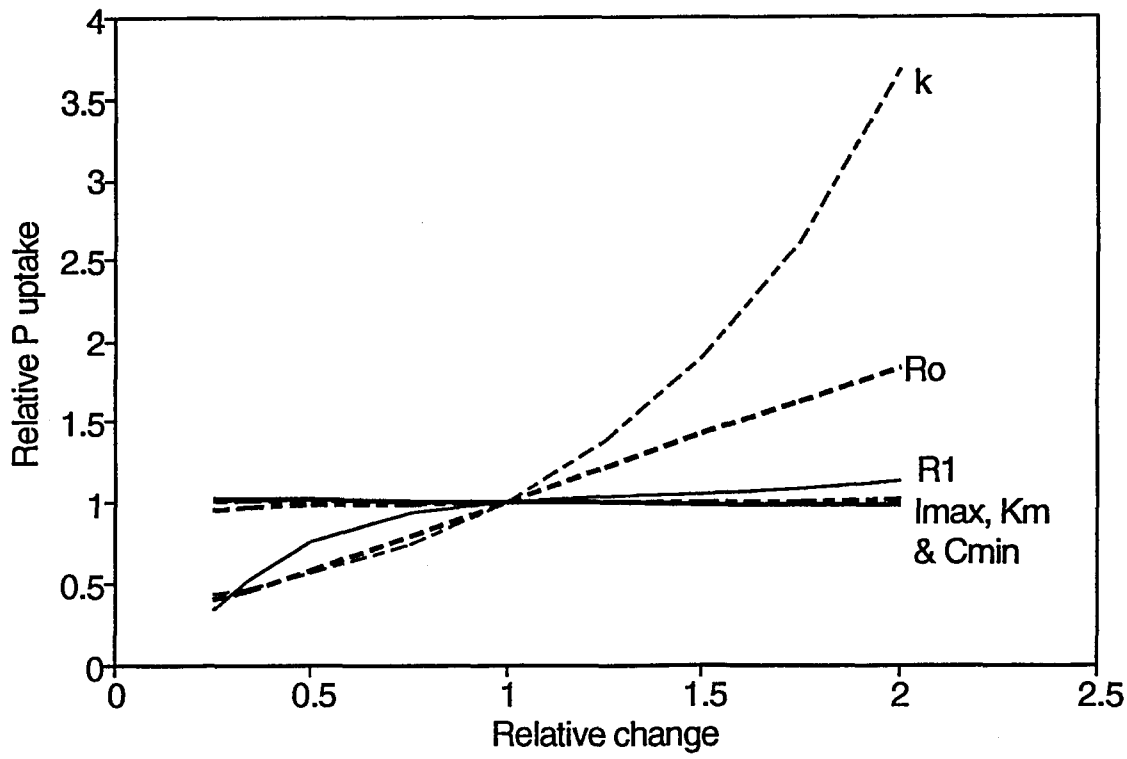


Fig. 3.4. Plant parameters sensitivity analysis of predicted P in non-mycorrhizal treatments in response to changing the initial parameter. Each parameter was varied individually while the others where held constant. Results are expressed relative to the initial conditions uptake.

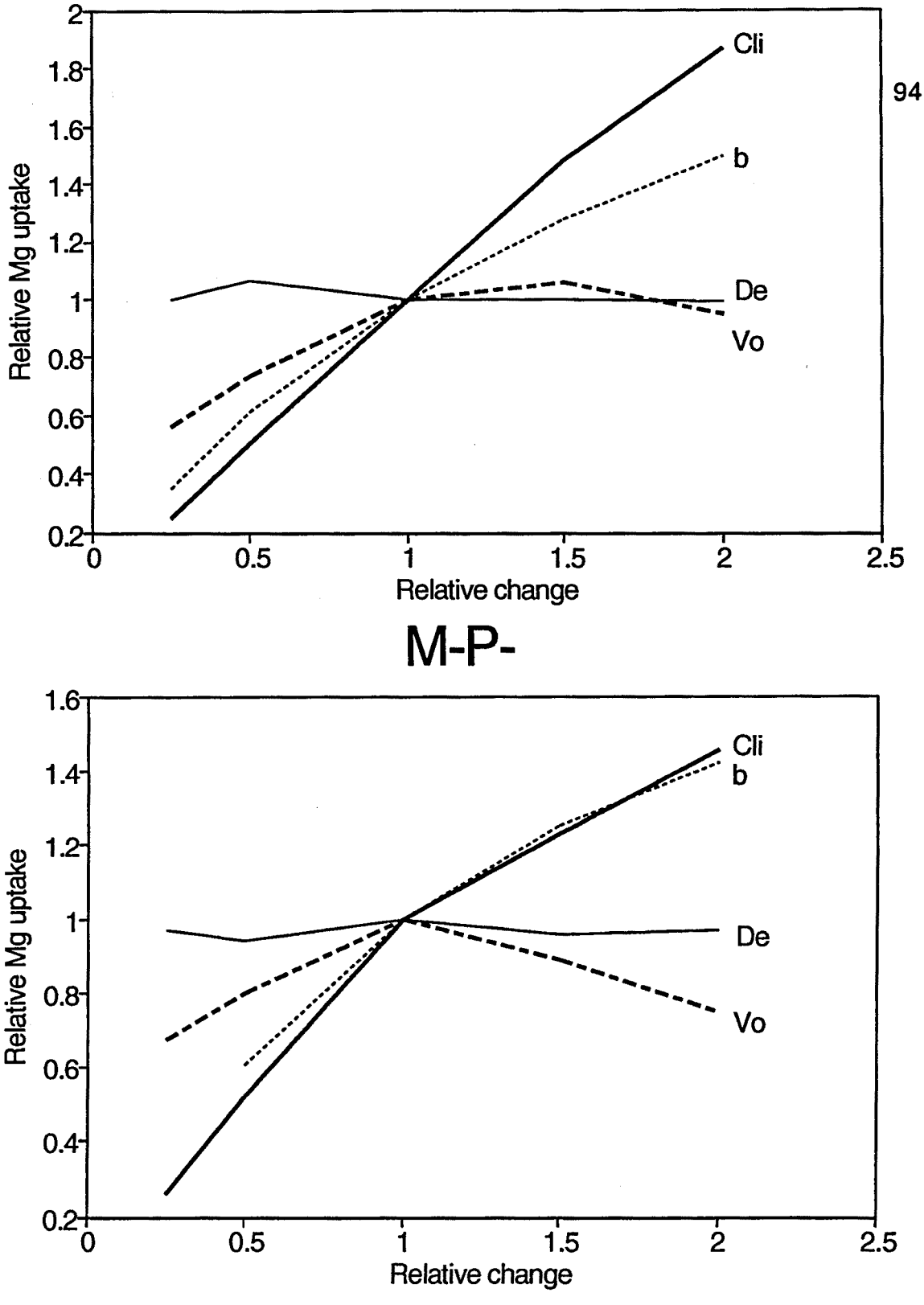


Fig. 3.5. Soil parameters sensitivity analysis of predicted Mg in non-mycorrhizal treatments in response to changing the initial parameter. Each parameter was varied individually while the others where held constant. Results are expressed relative to the initial conditions uptake.

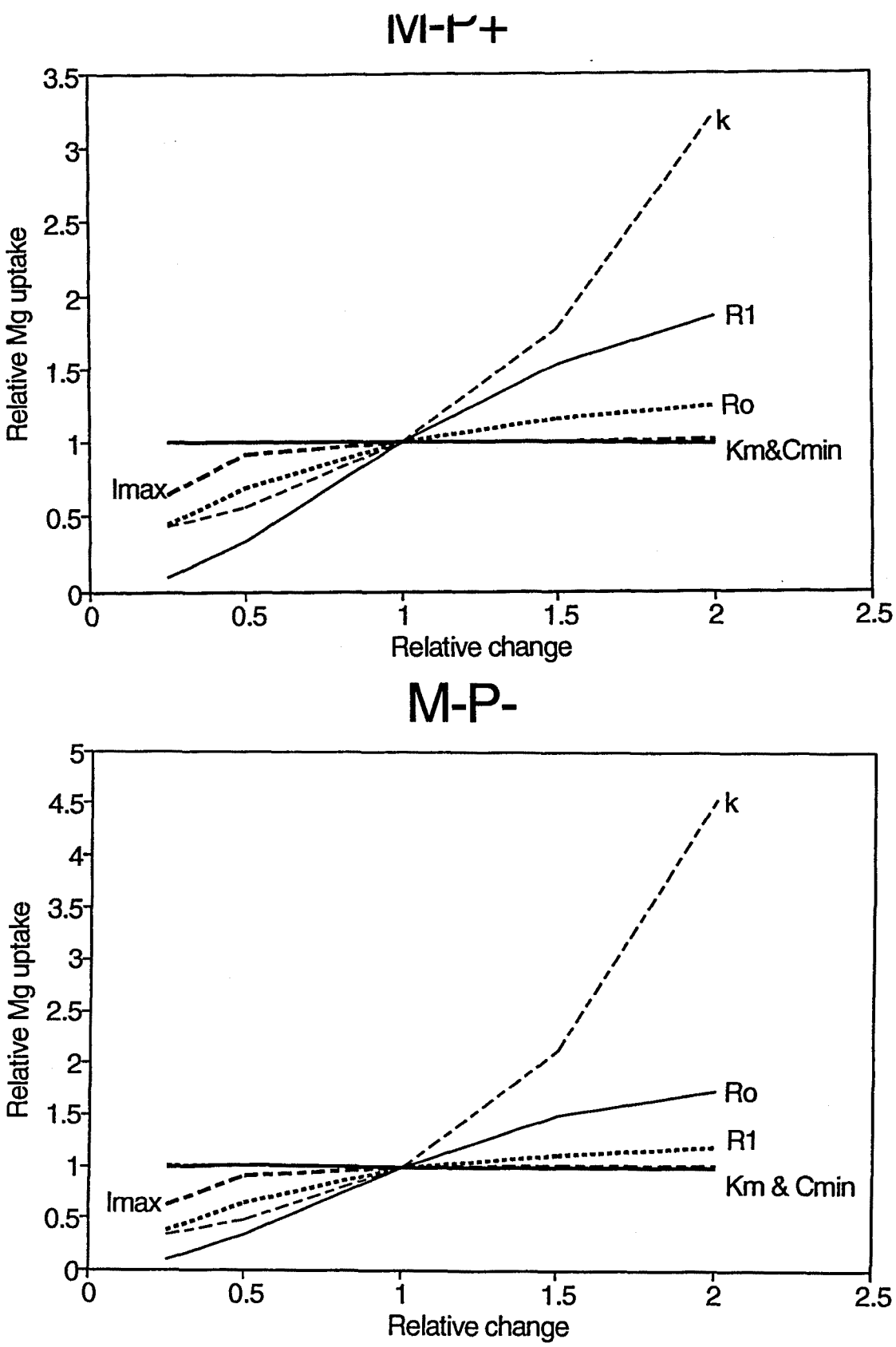


Fig. 3.6. Plant parameters sensitivity analysis of predicted Mg in non-mycorrhizal treatments in response to changing the initial parameter. Each parameter was varied individually while the others where held constant. Results are expressed relative to the initial conditions uptake.

The soil parameters that mostly controlled Mg uptake in any treatment were soil buffer power (b) and Mg initial soil concentration (Cli) (Fig. 3.5). Increases of b or Cli corresponded with increases of Mg uptake. On the other hand, the effective diffusion coefficient (De) did not have any affect on Mg uptake and changes in water uptake rate in P+ treatments (Vo) were only sensitives when it was reduced. In P- treatments increases in Vo resulted in decreases in Mg uptake.

Michaelis Menten parameters for Mg did not have any effect on Mg uptake. Only decreases in Imax caused a slight reduction of Mg uptake in all cases (Fig. 3.6). Increases in root exponential growth rate (k) caused the largest increase of Mg uptake. Increasing the initial distance between root axis (R1) also increased Mg uptake in any treatment. On the other hand, the effect of decreasing Ro was larger than when decreasing k.

DISCUSSION

Sensitivity analysis

The increase of both Cli and Vo led to an increase in P uptake in any treatment, suggesting that they were the parameters controlling the supply of P from soil to roots. Because the reduction in Imax caused a reduction in P uptake for fertilized treatments and Km and Cmin did not show any effect on P uptake, most of P uptake in fertilized treatments probably took place at the higher zone of Michaelis-Menten curve. Parameters describing root surface (Ro and k) were the most important plant parameters to change P uptake. In P- treatments they appeared to be even more important (Fig. 3.4), indicating that the increase of root surface provides a higher uptake increase when soil nutrient availability is low. Likewise, the larger decrease of P uptake observed in P- treatments when R1 was decreased, suggested that growing roots into new soil volumes was more advantageous in low than in high P availability conditions. The non significance of increasing R1, parameter describing the half-distance between roots, indicated lack of competition between roots.

Because root growth constant (k) and half distance between root axes (R1)

were the most important parameters increasing Mg uptake in any treatment, and because the change of mean root radius Ro did not change very much Mg uptake, it is suggested that roots took better advantage of exploring new soil volumes than of increasing its surface by growing in thickness (Ro). This further suggests that roots competed with each other. The fact that root kinetic parameters had little importance in affecting Mg uptake, match with the high sensitivity of soil parameters (Cli and b) and agrees with the large decrease of Mg concentration in soil solution during the simulation (Fig. 3.2). This fact indicates that Mg root uptake capacity was greater than soil supply capacity and is in concordance with the deficiency of Mg diagnosed by low foliar concentration of Mg across all treatments (see previous chapter).

Model predictions

In M-P- treatment P uptake predicted by the model was very similar to the observed values. Kelly et al. (1992) and Gillespie and Pope (1990) obtained similar levels of agreement by predicting P uptake in forests species. Although in most treatments the model did not predict the observed uptake of P, the regression between predicted and observed values in non-mycorrhizal treatments was highly significant. This fact suggested that the parameters the model used were able to describe the relative P uptake only in non-mycorrhizal conditions.

Fertilized treatments were always overpredicted by the model. Robinson (1991) reported that in plants other than very young ones, modelling usually overpredicted nutrient uptake. He suggested that in those plants not all root surface was equally effective in taking up nutrients and proposed the effective root length as the length at which the model fit to the observed data. Van Rees (1989) in *Pinus elliotii* seedlings showed that thicker roots were less able to take up K from a nutrient solution than thinner roots. In our experiment, because fertilized treatments showed an increase in root diameter (See previous chapter) it is reasonable to think that root efficiency in taking up P was lower in fertilized pots. The Robinson's effective root length in M-P+ and M+P+ treatments corresponded to a 64 % and 31 % of the total root length respectively. On the other hand, Michaelis Menten root kinetic parameters also could be responsible for the overprediction of P uptake under high P availability conditions. These parameters are normally worked out from nutrient-solution depletion studies using plants in particular conditions. Therefore, the resulting parameters are probably only applicable to the particular conditions in which they have been determined (Kelly et al. 1992). The parameters we used in our simulation may have not matched the actual conditions in the high P fertility conditions.

Barber-Cushman simulation did not consider nutrient uptake as affected by mycorrhizae (Barber, 1984). Because of the fact that mycorrhizal roots have been observed to take up P and other nutrients at higher rates than non-mycorrhizal

roots (Kothari et al., 1990a, see previous chapter), underpredicition of Mg and P under mycorrhizal conditions could be attributed to the effects of mycorrhizae. Many authors have obtained reasonable Barber-Cushman predictions of nutrient uptake without considering mycorrhiza (Gillespie & Pope, 1990; Van Rees et al., 1990a; Kelly et al., 1992). The lack of prediction observed in our experiment in M+ treatments indicated that, under these conditions, mycorrhiza played an important role in supplying P and Mg to the plant. Kothari et al. (1990b) stated that the increase of nutrient uptake per unit of root length in mycorrhizal plants, did not necessarily reflect the main contribution of hyphae in nutrient uptake, as they observed an increase of water uptake per unit of root length in mycorrhizal plants. In our case estimated water uptake was 1.67 and 1.74 times higher in unfertilized and fertilized mycorrhiza infected treatments respectively than in non-infected treatments. Therefore, in spite the model did not take into account the direct uptake by hyphae, it did consider this increase of water uptake per unit of root length observed under mycorrhizal plants.

The agreement between observed and predicted values for Mg was only achieved in M- treatments, by using Kelly and Barber (1991) root uptake kinetic parameters. Kelly et al. (1992) used Barber-Cushman model for loblolly pine seedlings and predicted only a 38% of the observed uptake. They attributed this to the fact that the Imax they used, was estimated under concentrations 100 times lower than the initial concentration they measured in the soil where seedlings grew

(Cli). According to this, Mg concentration in the soil solution used in our experiment was only about 5 times lower than the concentration where Imax was estimated. Hence, in our case the Imax from Kelly and Barber (1991) despite of being measured for a different pine species (*Pinus taeda* instead of *Pinus radiata*), was found to be useful to predict Mg uptake under non-mycorrhizal conditions. Mg uptake by mycorrhizal seedlings was largely underpredicted by the model (Table 3.1), suggesting that mycorrhizal hyphae had supplied Mg to the infected roots to increase the amount of Mg taken up per unit of root surface.

Thomson et al. (1990) discussed that there were some evidences suggesting that Km and Imax values for P transport systems in the germ-tubes of the fungi *Gigaspora margarita* are similar to those observed in plant roots (Bever & Burns, 1980). This fact is further supported by Li et al. (1991) experiment, where they found that soil P depletion caused by VA mycorrhiza infected roots was similar to the one caused by uninfected roots. Thus, it is possible to think that the increase of soil exploration caused by mycorrhizal hyphae is the main factor by which mycorrhiza increase nutrient uptake. In our experiment, changing Imax did not get the Barber-Cushman to fit to the observed values in M+ treatments for Mg nor for P pots, whereas when changing the exponential root growth constant (k), the model was able to predict uptake for P and for Mg even in M+ treatments. The increase of k required to fit the model was bigger for P in P- treatments (from 1.8 times the measured value) than for Mg (from 1.3 to 1.5), suggesting that under low

P availability conditions hyphae were more efficient taking up P than Mg. This fact is in agreement with the general lower mobility of soil P than Mg. According to this, and considering that in this simulation roots grew at exponential rates, to take up the same amount of Mg than a mycorrhizal infected root a root system without mycorrhizae would have needed to grow between about 1.4 to 2.3 times longer than it did whereas for P it would have needed to growth about 3.5 times longer. However, under high P fertility conditions, hyphae were more efficient taking up Mg than P. The estimated variations in root growth required to overcoming the lack of mycorrhiza are within the normal range of root growth in pine seedlings (Van Rees *et al.*, 1990a).

CONCLUSIONS

Soil parameters (b, Cli and Vo) and root growth parameters (R1, Ro and k) were the prime factors to which the model was more sensitive. Because of the soil capacity to supply Mg was much lower than for P, R1 became more important over Ro. This fact emphasizes that when roots depleted the soil, or in other words, in conditions where competition between roots took place, it was more advantageous for roots to grow in length than in thickness. Under low P availability, the effects of mycorrhiza in taking up P were greater than for Mg. Barber-Cushman model

was able to predict Mg uptake under non-mycorrhizal conditions and P uptake only in M-P- treatment. A better understanding of the nonuniformity of root nutrient uptake kinetics, would help in improving the modelling of nutrient uptake.

Part II

CHAPTER 4

CHEMICAL CHARACTERISTICS OF LITTER LEACHATES AND TRANSFER OF NUTRIENT FROM FOREST FLOOR TO MINERAL SOIL IN A MATURE *PINUS RADIATA* D.DON STAND.

INTRODUCTION

The forest floor contains large amounts of the ecosystem nutrient reserves. As a great part of stand nutrient uptake is returned by litterfall to the soil organic horizons (Binkley, 1986), forest floor dynamics may play an important role in nutrient cycling in forest ecosystems. Large quantities of nutrients are stored in forest floor as organic components that eventually become available for further cycling by decomposition processes. Berg & Staaf (1981) discussed that nutrient contained in litter could be leached, immobilized or mineralized depending mostly upon the degree of decomposition of the organic matter. In the forest floor, there is a gradient from fresh organic matter (0 year old) to humified organic matter. Therefore products exported on mineralization of fresh organic layers (Oi horizon) might be immobilized in the underlaying horizons which probably will be subsequently mineralized or leached. Polglase et al. (1992b) presented evidences that inorganic P leached from Oi horizon was found in organic forms at the Oe horizon. On the other hand, forest floor horizons have a great potential to modify the chemistry of incident throughfall (Raison & Khanna, 1982), and retain part of the inorganic N and P in the incident throughfall (Qualls et al. 1991). In some cases organic matter can be ratained in the forest floor as much as 64 years, in some others it can be decomposed in less than one year (Olson, 1963). Because the forest floor capacity in retaining and releasing nutrients, it may be viewed as

a buffer for nutrient transfer to the mineral soil and supply to plant roots. Modifications of forest floor have been used especially in intensive silviculture practices, in order to provide adequate nutrition to the stand. Consequently, several studies have been addressed to understand the effects of such practices on nutrient availability and forest growth (Flinn et al., 1980; Attiwill et al. 1985; Smethurst & Nambiar, 1990). However, few studies have measured the amounts and fractions of nutrient being exported from the forest floor.

In the study reported here, the amounts and forms of nutrient and organic matter exported from the forest floor were measured by using zero tension lysimeters placed underneath forest floor Oa horizon. Seasonal and spatial patterns of nutrient leaching from forest floor were also studied.

MATERIAL AND METHODS

Study site

The study site, located 100 km N from Barcelona (close to Santa Coloma de Farners; 41° 52' N, 02° 38' E), is a 18-year old *Pinus radiata* D.Don stand

growing on a Dystric Xerochrept (US Soil Taxonomy) derived from granitic parent material. The forest floor consists of an Oi (1.947 Mg C/ha), an Oe (1.938 Mg C/ha), and an Oa (4.978 Mg C/ha) and averages 7 cm in depth (Cortina, 1992). Some characteristics of forest floor and surface mineral soil are summarised in Table 4.1. Prior to its planting to radiata pine, the site supported a plantation of chestnut (*Castanea sativa* Tourn.). Climate of the area is mediterranean, annual precipitation is about 789 mm, and mean annual temperature is 15.6°C.

Lysimeter description and collection of litter leachates and soil solution

The forest floor leachates were collected on a monthly basis, starting from 5 March 1990 to 5 September 1991. Twenty zero tension lysimeters randomly placed between the forest floor and the surface mineral soil in a 20 by 20 m plot. Lysimeters were made of 30X18.5 cm trays. About 4 g of boric acid per lysimeter (approximately 0.06 M in collected solution) were used each time as a preservative. The sample collection bottles were buried in pits and covered to avoid light. Water fluxes were estimated from the amount of water measured in bottles when leachates were collected. At the end of the study, material lying on each lysimeter was separated into Oi, Oe and Oa horizons, oven dried (60°C) weighed and analyzed for C, N and P content.

Forest floor

	C (%)	N (%)	P mg Kg^{-1}	C/N	C/P	N/P
Oi	47.8 (0.7)	0.705 (0.10)	530 (90)	67.80	901.89	13.30
Oe	41.0 (2.1)	1.083 (0.12)	750 (60)	37.85	546.67	14.44
Oa	20.22 (9.4)	0.820 (0.34)	474 (159)	24.23	411.59	16.94

Mineral soil

	Bulk density g cm^{-3}	pH (H_2O)	pH (KCl)	C (%)	N (%)	P mg Kg^{-1}
0-5 cm	1.32	4.7	3.6	2.76	0.15	135
5-15 cm	1.65	5.3	3.6	1.06	0.10	97
15-30 cm	1.77	5.6	3.8	0.66	0.05	75

Exchangeable cations [cmol(+) Kg^{-1}]

	Ca	Mg	K	Na	Al
0-5 cm	1.78	0.35	0.18	0.38	0.92
5-15 cm	0.55	0.17	0.12	0.17	1.25
15-30 cm	0.48	0.33	0.08	0.24	1.26

	Sand (%)		Silt (%)		(%)
	Coarse	Fine	Coarse	Fine	Clay
5-15 cm	62.46	14.47	7.23	7.93	7.89
15-30 cm	61.76	13.73	8.36	8.04	8.09

Table 4.1. Some characteristics of the forest floor and underlying mineral soil. Figures in brackets refer to standard deviation.

Leachates were stored at 4°C till analysed. Soluble reactive phosphorus (SRP) was determined by Murphy and Riley (1962) method. NH_4^+-N and NO_3^--N were determined by Technicon Autoanalyzer Technique. A subsample of the leachates was taken for digestion with H_2SO_4 and H_2O_2. After digestion total P, and NH_4^+-N were determined in the same way. Non-reactive soluble P was operationally defined as the difference between total P and SRP, and organic N as the difference between total NH_4^+-N and inorganic NH_4^+-N. Cl^- and $SO_4^=$ were analyzed by ion chromatography. Carbon in leachates and C and N in ground forest floor were determined by combustion using a CHNS Carlo Erba 1500 elemental analyzer. To analyze for the total P content in forest floor, a subsample of each different layer was wet digested with HNO_3 and $HClO_4$ (2:1). P in the digest was determined by ICP spectrometry. Amounts of nutrient exports in forest floor leachates were determined by multiplying nutrient concentration with the respective amount of leachate collected. All monthly nutrient flows presented in this chapter are corrected to natural months.

Statistical analysis

Analysis of variance (ANOVA) was used to test differences over time in nutrient concentrations and leached amounts (SAS institute 1982). The Duncan multiple range test was used to test differences between months. Pearson

correlations between the amount of nutrient contained in every lysimeter and the nutrient amount and concentration in the litter leachates were also computed by using SAS.

RESULTS

Litter leachates composition

Chemical composition of the litter leachates was highly variable depending upon the lysimeter, especially for SRP, Cl^- and $SO_4^=$. Some lysimeters in the plot gave consistently higher concentrations at all times. Qualls et al. (1991) reported similarly high variability for inorganic P in the throughfall. Despite this high spatial variation in nutrient concentrations, there were clear seasonal changes to note for all measured nutrients and fractions (Fig. 4.1). Feller (1978) and Raison & Khanna (1982) found a strong seasonal pattern in litter leachates concentrations. Similarly to the chemical composition of the litter leachates, the total amount and concentration of carbon and nutrients contained in each lysimeter were highly variable in the space (Table 4.1). Amounts of carbon and nutrients contained per lysimeter ranged from 34.29 g to 88.85 g C $lysimeter^{-1}$; from 1.214g to 10.297 g

N lysimeter^{-1} and from 0.23 to 0.70 g P lysimeter^{-1}. However, single lysimeter amount or concentration of nutrients showed very weak positive correlations with its respective leachate concentration. R^2 was always lower than 0.25. P fractions showed even weaker relationships than for N. Oa horizon C/N, C/P and N/P ratios did not show good relationships with forest floor leachates concentrations either.

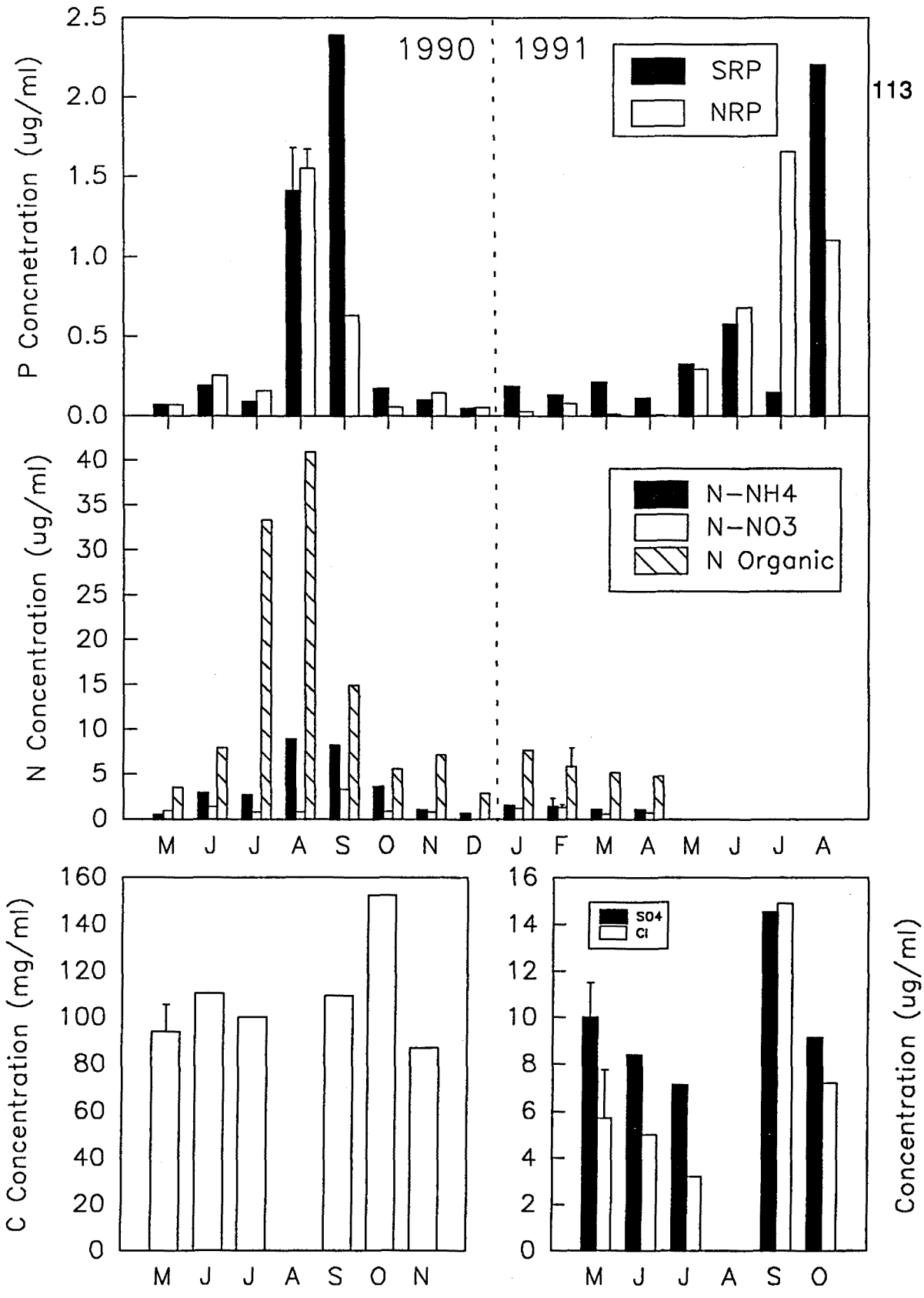


Fig. 4.1. Seasonally change on element concentrations in forest floor leachates. Bars refer to ANOVA standard error.

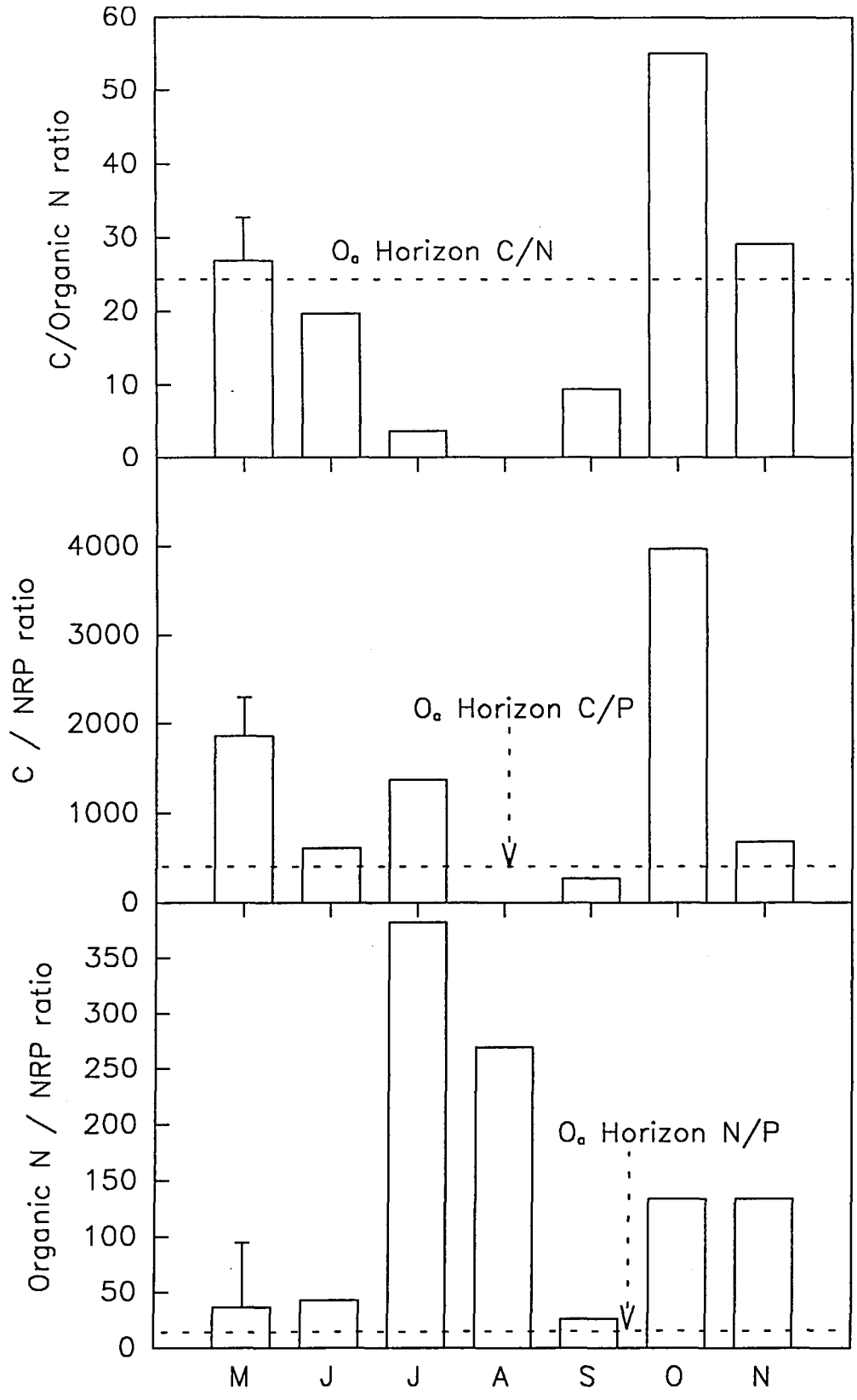


Fig. 4.2. Seasonal changes of C/organic N, C/NRP and Organic N/NRP ratios in litter leachates. Dashed line indicates the respective ratio in bulk Oa horizon. Bars refer to ANOVA standard error.

SRP concentration ranged between 0.02 to 2.5 mgP l^{-1}, and it was consistently high during summer time (Fig. 4.1). While in August 1990 SRP increased suddenly, in 1991 it started to increase gradually in May. The large increase in SRP concentration observed in September 1991 corresponded to the significant increase in Cl^- and $SO_4^=$ concentrations. Non reactive P (NRP) concentration ranged between virtually zero to 1.5 mgP l^{-1}. Its high concentration was always associated with that of SRP (August, September 1990; July, August 1991). In 1990 the pattern of NRP concentration was suddenly increased in August, wereas in 1991 it increased gradually from March on.

Concentrations of organic N and NH_4^+-N increased during summer. High organic N lasted for 3 months and, as for P, was associated with NH_4^+-N increase which however lasted for only two months. Nitrate concentration in the litter leachates remained low except in September. The largest N fraction in the leachates was organic N. In October, when the concentration of N and P fractions dropped, C concentration showed a slight peak (Fig. 4.1).

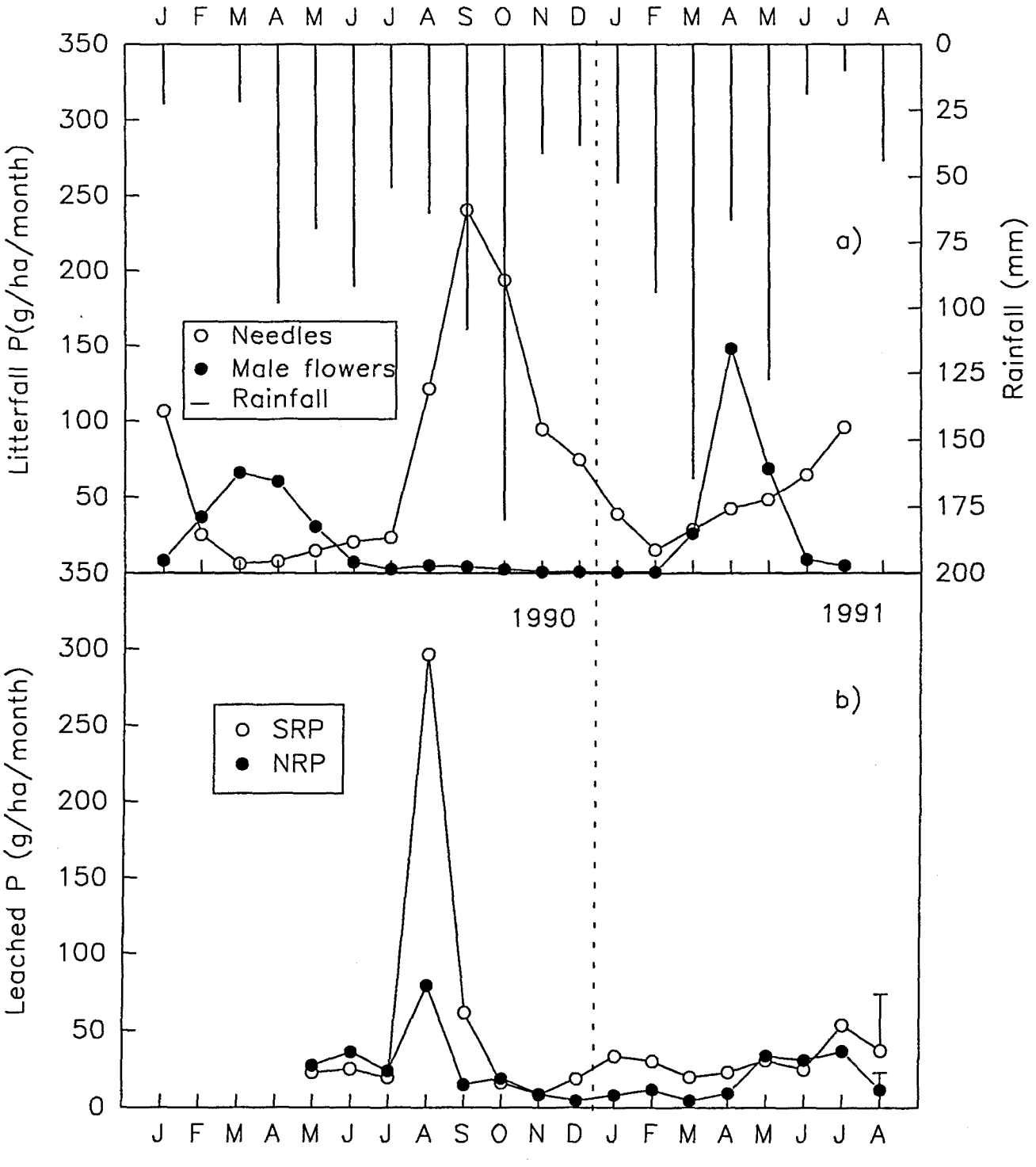


Fig. 4.3. Rainfall and P inputs in litterfall compared to the amounts of P forms leached from the forest floor. Bars refer to the ANOVA standard error.

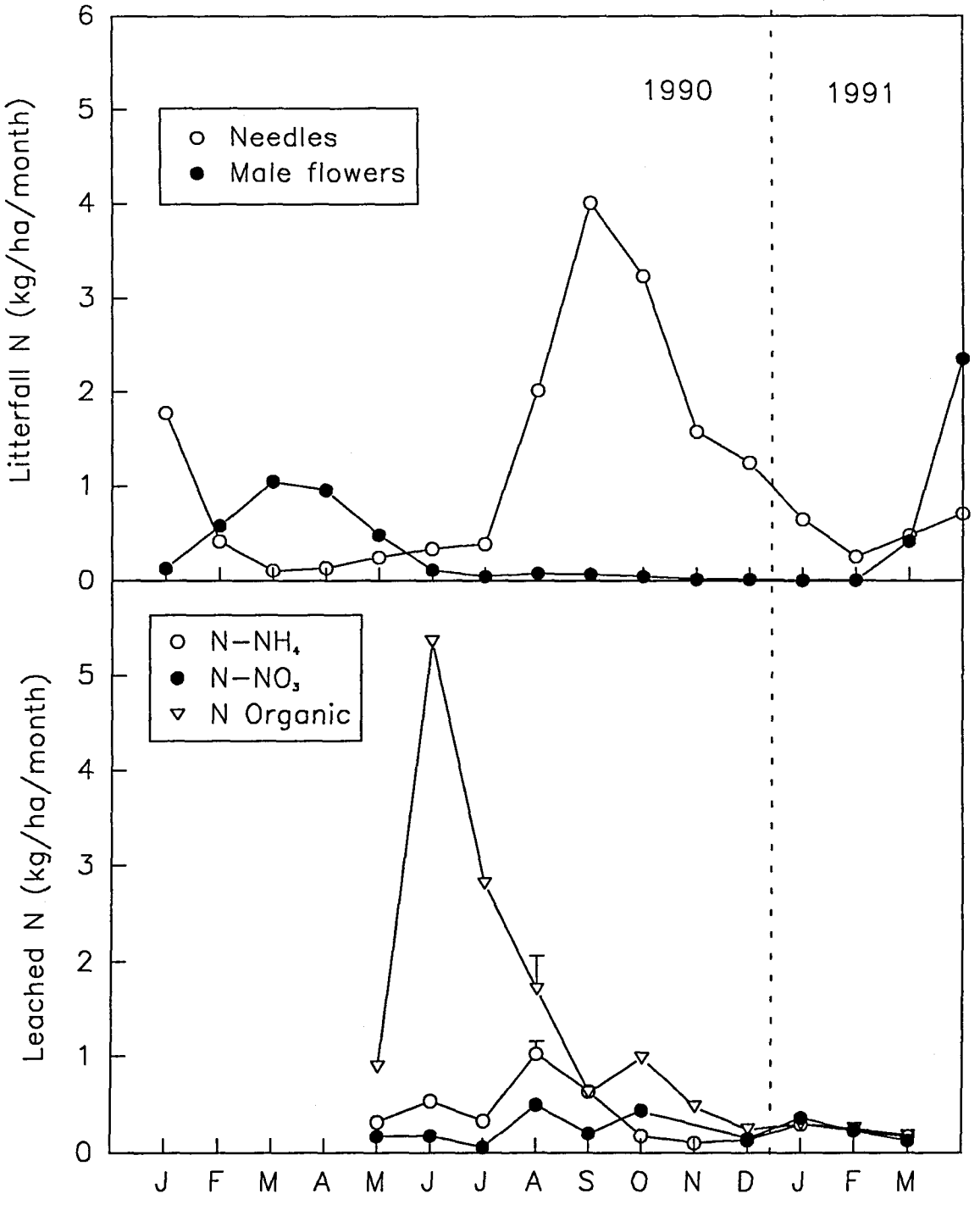


Fig. 4.4. N inputs in litterfall compared to the amounts of N forms leached from the forest floor. Bars refer to ANOVA standard error.

C/Organic N and C/NRP ratios were low when N and P concentration in litter leachates were high (Fig. 4.2). On the other hand, the high carbon concentration observed right after maximun litterfall (October 1990) increased both ratios. High organic N/NRP ratio during July and August indicated that the amount of organic N was higher than the NRP concentration. In Fig. 4.2 are also shown C/N, C/P and N/P ratios in bulk Oa horizon. While the average of C/Organic N ratio in the litter leachates was very close to that of Oa horizon (C/N Oa = 24), the average of C/NRP and organic N/NRP ratios in litter leachates were in most cases much higher than those in Oa horizon.

Amounts of nutrient leached from forest floor

The amount of SRP leached from forest floor was quite steady throughout the year, except for the August-September (1990) peak which represented a 65 % of the total SRP annually leached (Fig. 4.3). SRP peak in summer 91 represented only a slight increase over the normal levels. This fact might be partly attributed to the lower rainfall recorded in July 91 (55 mm July 1990 vs 10 mm July 1991). The amount of NRP leached during spring was always significantly higher than in the autumn or winter and showed its maximum in August 1990 that accounted for a 33.21 % of the total NRP annually leached. N leached was mostly in organic form (Fig. 4.4). Similar results have been shown by several authors

(Yavitt & Fahey, 1986; Rosen & Lundmark-Thelin, 1987; Stevens & Wannop, 1987 & Qualls et al. 1991). Maximum amount of organic N leached was in June and amounted for 39 % of total organic N annually leached. This maximum decreased gradually later. Leaching of inorganic N forms, NO_3^--N and NH_4^+-N, had their maximum in August 1990 as were for SRP and NRP maximums. NO_3^--N and NH_4^+-N leached in August 1990 amounted for 21% and 26 % of the total annually leached respectively.

Nutrient concentration in different forest floor horizons laying on the lysimeters (Oi, Oe, Oa) did not show strong relationships with the amount of nutrient leached in each lysimeter. Percolated NH_4^+-N positively correlated with C and N concentrations in Oa horizon (R^2 = 0.31 p=0.0135 & R^2 = 0.30 p=0.0150 respectively). On the other hand, lysimeter total C pools showed slight and positive correlations to all measured nutrients except for leached C (Table 4.2). While total N pools in whole forest floor laying on the lysimeters related only to leached P forms and organic N, the amounts of N only in Oa horizon showed weak relations with the nitrogen forms. Likewise, total pools of P in Oa horizon also showed better relations to the leached amounts that the total amounts of P in the forest floor. C/N, C/P and N/P in Oa horizon did not relate to the amounts of nutrient leached from litter, only N/P ratio showed a weak relationship to leached NH_4^+-N (R^2 = 0.26 p=0.0252).

Pearson correlation coefficient (r)						
(n=19)	Forest floor content (Kg ha^{-1})			Oa horizon content (Kg ha^{-1})		
Amount leached (kg ha^{-1})	C	N	P	C	N	P
SRP	0.54 (0.0182)	0.47 (0.0429)	ns	ns	0.54 (0.0176)	0.49 (0.0328)
NRP	0.50 (0.0286)	0.45 (0.0480)	ns	ns	ns	ns
NH_4^+-N	0.63 (0.0039)	ns	ns	0.60 (0.0062)	0.57 (0.0118)	ns
NO_3^--N	0.54 (0.0163)	ns	ns	0.53 (0.0196)	0.51 (0.0254)	0.48 (0.0399)
Organic N	0.69 (0.0011)	0.59 (0.0073)	0.56 (0.0125)	0.59 (0.0082)	0.61 (0.0054)	0.54 (0.0181)
C	ns	ns	ns	ns	ns	ns
$SO_4^=$	0.48 (0.0376)	ns	ns	ns	ns	ns
Cl^-	0.58 (0.0089)	ns	ns	0.61 (0.0054)	0.60 (0.0067)	0.47 (0.0437)

Table 4.2. Pearson correlation coefficients between the amount of nutrient leached in each lysimeter and total nutrient content in the whole forest floor laying on lysimeters (Oi, Oe and Oa horizons) or in only the Oa horizon. In brackets is shown the probability of the significance of the correlation ($p>0.05$). ns; non significant. SRP refers to soluble-reactive P and NRP to non-reactive soluble P.

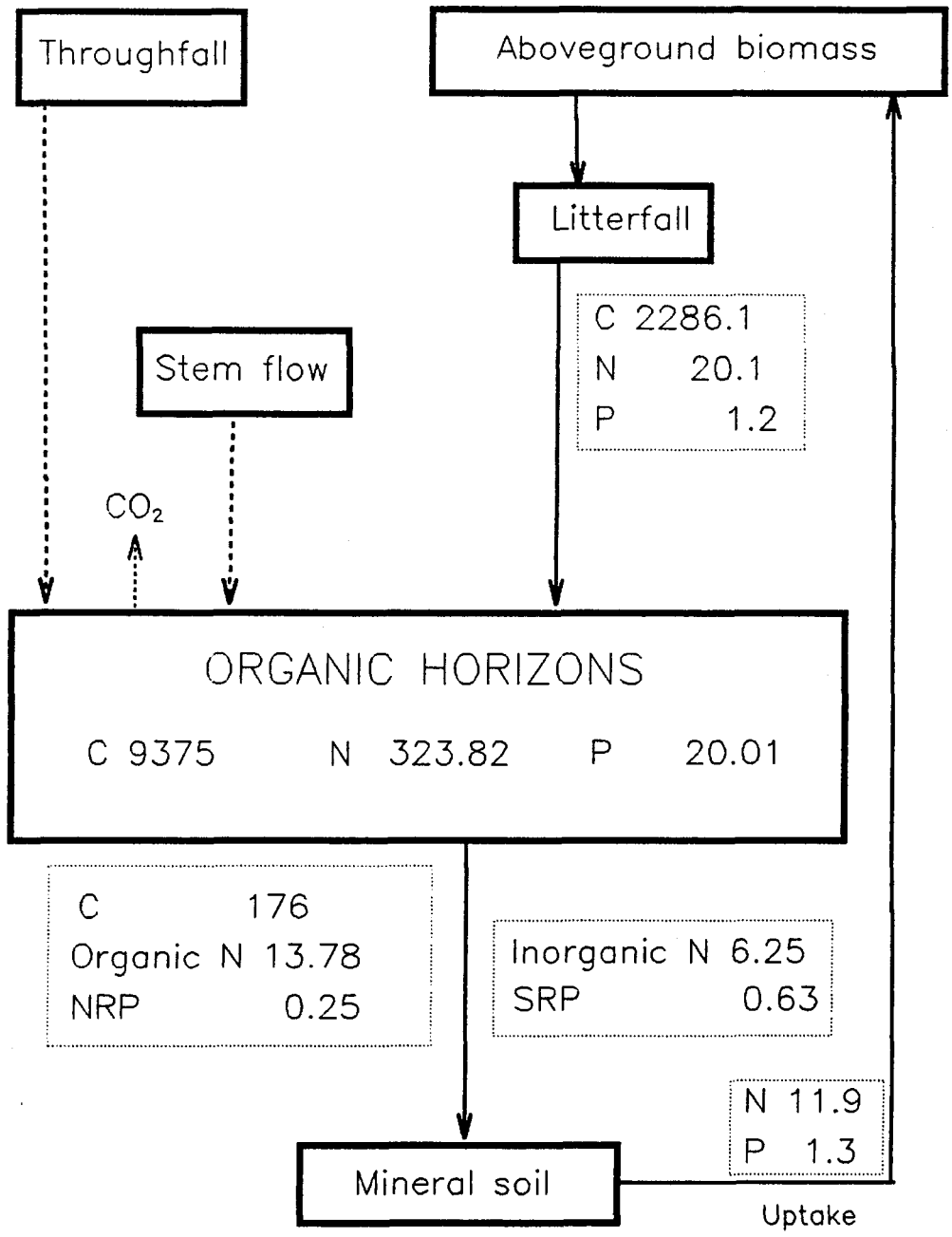


Fig. 4.5. Annual nutrient exports from the forest floor compared to forest floor nutrient inputs and to the forest aboveground biomass annual increment. Figures next to arrows represent nutrient flux and are expressed in kg ha^{-1} yr^{-1}. Figures inside the box represent nutrient content and are expressed in kg ha^{-1}. Throughfall and stem flow values for N and P are from Escarre et al. (1984) while C is from Qualls et al. (1991).

DISCUSSION

Mechanisms of nutrient transfer

The largest increase in leaching of SRP occurred just one month before the maximum litterfall in September 1990. In the senescent needles collected from tree crowns in the same plot, Cortina (1992) found that up to 38 % of the original P content of the needles was solubilized when they were immersed in water for 24 h. These results agree with those given by Bogatirev et al., 1983; Qualls et al. (1991) & Polglase et al. (1992c). Moreover, Cortina (1992) found that senescent needles collected from tree crown had much higher P content than needles collected from litter traps during the maximum litterfall (1113 mg P Kg^{-1} vs. 270 mgP Kg^{-1}). Therefore, it appears that the high amount of P leached in August resulted from direct loss from brown needles recently fallen or still attached to the crown, following the summer drought. Further support to this is given by Bellot & Escarré (1991) who in an evergreen mediterranean Holm oak (*Quercus ilex L.*) forest, observed a summer maximum leaching for most analyzed nutrients in throughfall. Thus, the supposed high P in throughfall that occurred in mid summer at our plot would not had been completely retained by litter layers. On the other hand, the large increase in SRP leaching agreed with the maximum obtained for Cl^- indicating that these high values in August 1990 originated mostly by leaching

from the dead needles. The increase in the NRP observed in spring was preceded by the fall of male flowers (Fig. 4.3) suggesting that pine male flowers form a direct or an indirect source of NRP to the mineral soil. The large amounts of organic P compounds contained in pine pollen could have accounted for this NRP increase. The low rainfall observed in winter-srping 1990 contrasted to the high winter-spring raifall in 1990 (Fig. 4.3 a). This fact could have accounted for the delay of the increase in NRP leaching observed in 1990 (Fig. 4.1). The NRP maximum observed in August may have been partly the result of the throughfall. Qualls et al. (1991) showed that NRP in throughfall represented about 40 % of the total. The peak concentration of both inorganic N forms (NO_3^--N and NH_4^+-N) agreed with those of P forms, Cl^- and $SO_4^=$, suggesting that they had the same source. Cortina (1992) reported that large quantities of nitrogen (NO_3^--N, NH_4^+-N and organic N) forms were also made soluble after soaking senescent pine needles in distilled water for 150 hours. Most of this N was released during the first 24 h. The large amounts of nutrients leached from the forest floor during summer (August-September) represented a high proportion of the nutrients leached throughout the year. Although the amounts of P leached during summer appeared to depend upon the rainfall regime of the year, the concentration peaks observed during the two subsequent years were very similar (Fig. 4.1 & 4.3).

Organic N leaching peak did not agree with that of any other element. This fact suggested that its source was different and was possibly related to biotic

immobilization-mineralization processes in the forest floor. Qualls et al. (1991) showed that organic N leached from the forest floor was nearly three fold in amount to that found in throughfall. In our experiment this fraction of nitrogen represented 68.7 % of the total N leached. Further studies are needed to elucidate the role of this large labile organic N fraction on tree nutrition.

Total N and P concentrations related very well with C concentration in Oa horizon (R^2 = 0.96 and 0.92 respectively) suggesting that most of N and P contained in the forest floor was in the organic form. Because of that, C/N and C/P ratios can be used to describe the organic matter quality in the forest floor. Qualls et al. (1991) reported similar C/N ratios in decomposing needles as observed in the litter leachates and similar or lower C/P ratios. In our experiment when C/N and C/P ratios of Oa horizon were compared to the respective ratios in the litter leachates, the same values were shown for C/N (Fig. 4.2), whereas C/P ratio on the average was much high in litter leachates. Thus, the organic matter leached in the leachates form from the forest floor was as rich in N as the solid organic mater in the Oa horizon but much poorer in P. This fact suggested that higher retention of NRP relative to organic N was taking place in the organic layers (Fig. 4.2). The large seasonal fluctuation in the C/organic N, C/NRP and organic N/NRP ratios in litter leachates indicated that the quality of organic mater leached varied with season. Specifically, the organic N/NRP ratio indicated that organic mater bond to P behaved differently to the organic mater bond to N.

Similar to the results shown by Qualls et al. (1991) the amount of litter and nutrients contained in our lysimeters or its C, N and P concentration and C/N, C/P and N/P ratios did not relate very well with the litter leachates concentration nor with the amount of nutrient leached per lysimeter. Thus, the spatial variability in litter mass or quality defined as C/N C/P and N/P ratios did not account for most of the variability of the transfer of nutrient between forest floor to mineral soil, nor for the litter leachates chemistry. Nevertheless, the amounts of N and P in Oa horizon showed slightly better relation to the amount of nutrients leached than the total amount in the forest floor, suggesting that Oa horizon composition affected the leaching of forest floor nutrients more than the other forest floor horizons. Because the studied organic matter quality ratios only represented forest floor bulk organic matter properties, studying the quality of labile organic compounds in the forest floor layers would probably give valuable information in understanding the forest floor leachates dynamism.

Amount of nutrients leached from the forest floor

Total N leached from the forest floor was 19.8 kg N ha^{-1} $year^{-1}$, 70 % as organic N, 20% as NH_4^+-N and the rest (10%) as NO_3^--N. On the contrary 70 % of the 0.87 kg P ha^{-1} $year^{-1}$ leached was in the soluble reactive forms (SRP). The amounts of P and N leached annually from the organic layers represented a 4 and

6 % of the respective amounts in the forest floor, whereas C represented only a 1 %. Atmospheric outputs of C may fold many times C exports through leaching. Tewary et al (1982) reported that the respiration rates of forest floor ranged from 0.05 to 0.52 kgC ha^{-1} $hour^{-1}$.

According to Escarré et al. (1984), the inorganic nutrient input in throughfall and stem flow in Holm oak forest from a nearby area (Montseny), represented a large proportion of the amount of inorganic N leached in our experiment, and it was greater than SRP leached from the litter layers (Fig. 4.5). Qualls et al. (1991) observed that forest floor was not a source of dissolved inorganic N and P for the mineral soil but was a sink for these nutrients added as throughfall. The same authors reported an increase in the organic forms of these nutrients leached from the forest floor when compared to nutrients in throughfall. Inorganic N and P leached from the forest floor in our experiment were very close to the amount as throughfall estimated by Escarré et al. (1984).

Cortina (1992) measured nutrient inputs in the litterfall at the same time the forest floor leachates were collected, and estimated the annual increase of nutrient content in the aboveground biomass (Fig. 4.5). Nutrient inputs in litterfall where similar to the total amounts of nutrient exported by leaching. The annual amount of P leached from forest floor accounted for 66 % of the annual increase of P in stand aboveground biomass, whereas N leached nearly two-folded N increase in

aboveground biomass (Fig. 4.5).

CONCLUSIONS

A large proportion of the total nutrient leached throughout the year took place during short periods of time. This peaks represented a 61 % (August-September 1991) and 51 % (June-July 1991) of total P and N leached respectively. N and P leached from the forest floor were largely in the organic forms. Organic N was leached from the forest floor at a much higher rate than NRP (averaged organic N/NRP ratio in litter leachates 10 folded N/P ratio in Oa horizon).

The amount of nutrient transferred from the forest floor to the mineral soil accounted for most of the nutrient requirement for growth of the forest, especially for N. However, there were evidences suggesting major content part of this nutrient flow originated in forest canopy. Total inputs of nutrient in the forest floor exceeded that leached (Fig. 4.5). Except for the intense summer peaks, nutrient released from the forest floor followed a constant rate throughout the year. Peaks in nutrient concentration and leaching observed during summer may, for most

cases, be attributed to large increases in throughfall that were not completely retained by the organic layers. These high peaks did not coincide with high rainfall periods, suggesting that nutrient leaching was mostly ragulated by the availability of soluble compounds. The quality and quantity of litter did not account for spatial variability in litter leachates, which might be because the forest floor leachates were greatly influenced by throughfall characteristics. Nevertheless, the forest floor produced large seasonal variations in the leaching of organic N.

CHAPTER 5

ORGANIC AND INORGANIC PHOSPHORUS SEPARATION IN LITTER LEACHATES AND SURFACE MINERAL SOIL SOLUTION USING ANION EXCHANGE RESINS.

INTRODUCTION

Organic phosphorus (Po) can be a significant proportion of total soil P in both the solid (Cosgrove, 1967, Harrison, 1987) and solution phases. Fox et al., (1990) reported solution phase organic P from 10 to 70 % of the total solution P in some forest soils while others (Bogatirev et al. 1983; Qualls et al. 1991; Cortina & Romanyà, 1992; Polglase et al. 1992b) have reported organic P comprising between 0 to 50 % of total P in forest floor leachates.

Organic P has also been shown important in the P nutrition of plants (Basak & Bhattacharia, 1962; Adepetu & Corey, 1977; Kadeba & Boyle, 1978). Yet, while it can be absorbed directly by plant roots (Hemleben et al. 1975; Islam, et al. 1979), inorganic orthophosphate is still considered the form primarily available to soil organisms. Therefore, the importance of organic P in plant nutrition is mainly dependent on its mineralization to inorganic orthophosphate (Hedley et al. 1982).

Inositol hexaphosphate (phytate) has been reported as the most common identifiable form of soil organic P. These esters may account for as much as half the organic P in the soil (Anderson, 1967). A variety of methods have been used to investigate soil solution P composition (Martin 1964b; Martin & Wicken, 1967; McKercher & Anderson, 1968; Omotoso & Wild, 1970; Lim, 1977; Tate, 1979;

Jones & Bromfield, 1982; McKercher & Tinsley, 1982; Hawkes et al. 1984; Adams & Byrne, 1989), but the complexity of the soil solution components usually leave much of the organic P unidentified. However, resin chromatographic techniques have been used with some degree of success in soil science. Martin (1964 a,b), using a resin technique, obtained a good separation between organic and inorganic P. He was able to obtain a separation between inositol hexaphosphate, RNA nucleotide, and glycerophosphate when added to soil extracts and was able to partially describe organic P composition in several soils.

McKercher & Anderson, (1968) used a slightly different resin batch elution chromatographic technique to separate inositol penta- and hexaphosphate from the lower phosphate esters of myo-inositol. In the field of food science, similar resin method has been successful in separating inositol hexaphosphate from inorganic orthophosphate in foodstuff extracts (Harland & Oberleas, 1977 & Ellis and Morris, 1983).

Deciphering the cycling of P in soils depends on the ability to identify the P compounds present and their potential or susceptibility to transform into available or mobile forms. Polglase et al. (1992b) work has attempted to look at the organic and inorganic partioning of P in litter and soil by using a chemical fractionation procedure. Since exchange resins have been used to separate P compounds with some success, we have chosen to employ a modified batch resin elution technique

in order to (i) identify the possibilities of this method for separation of inorganic and organic P in soil solutions, and (ii) apply the method in order to contrast the inorganic and organic pools of P in the forest floor leachates and the underlying mineral soil solution under a mature *Pinus radiata* D.Don stand.

MATERIAL AND METHODS

Forest floor leachates and surface soil solution were added to an anion exchange resin AG1X8 (Cl-from). In order to fractionate different P compounds, the resin was eluted by sequential batch extractions with LiCl solutions.

Collection of soil solution and forest floor leachates

From the same site used in the previous chapter (see site description in material and methods section of chapter 4), forest floor and surface mineral soil solutions were collected. The forest floor leachates were collected over a 30 day period (ending 5 August, 1991) with 20 zero tension lysimeters used in the previous chapter. This period was chosen in order to obtain high concentrations

of P in solution. A detailed explanation of lysimeter characteristics and forest floor leachates preparation procedures can be found in the previous chapter. The lysimeters collected a total of 4.3 mm and all leachates were bulked to give one composite sample for the plot. Rainfall during the thirty days was 9.9 mm.

Mineral soil solutions were procured by sampling the 0 to 5-cm depth of mineral soil from 12 regularly spaced locations within the same 20 by 20 m plot from which the litter leachates were collected. All soil samples were combined into one sample. The soil was collected at the same time the litter leachates were removed from the field. The soil sample, still field moist, was shaken for 16 h with a 1:10 soil to solution ratio. The extracting solution was deionized water (pH = 7). Soil water content at the time of sampling was 9.5% by weight.

Resin Method

BioRad [Richmond, CA] AG1X8 anion resin, in the formate-form (100-200 mesh), was converted to the chloride-form by shaking with 1N LiCl (4 bed volumes) for 1 hour. Afterwards, it was rinsed 3 times with 1N LiCl then twice with deionized water.

For both, leachate and soil solution samples, two grams of the moist resin

were placed in 20 ml syringe, then 40 ml of leachate were added to the resin by successively shaking two batches of 20 ml of sample for one hour. Next, they were displaced from the resin by inserting the syringe plunger. The effluent was passed through a Nalgene cellulose acetate 0.45 micron membrane filter attached to the end of the syringe. This filtered solution was saved for P analysis.

The binding energy between soil P forms and the anion exchange resin depends upon the different physico-chemical characteristics of these forms. In order to separate these fractions sequential batch extractions of the resin followed. Ten ml of 0.1, 0.15, 0.2, 0.25, and 0.3 M LiCl solutions (pH=4.5) were added to the resin column, shaken for 1 hour, and filtered as before. The range of LiCL concentrations utilized was chosen according to a previous experimental work, which showed that this sequence left all phytate P in resin and eluted all orthophosphate and some simple organic P compounds (Guanosin monophosphate and Glucose 6P). All solutions removed from the resin were saved for P analysis.

As an internal check of orthophosphate and inositol hexaphosphate recovery and elution patterns, the soil solution samples were each spiked with 1 ml of a 20 ug P ml^{-1} solution of orthophosphate (as KHPO4) or phytic acid (as sodium phytate) and handled in a similar manner. To check for P contamination of resin and extracting solutions, the procedure was also run with just deionized water. All

procedures were replicated four times.

The original solutions and all resin extracts were analyzed for soluble reactive phosphate (SRP) by the method of Murphy and Riley (1962) and total P. Total P analysis also used the method of Murphy and Riley (1962) following digestion with H_2SO_4 and H_2O_2. All SRP determinations were accomplished the same day of the extractions. As in the previous chapter, Non-reactive soluble P was estimated by the difference between total P and SRP.

Statistical analysis

Analysis of variance (ANOVA) was used to test differences in P levels between forest floor and mineral soil, as well as differences between a horizon between sequential resin extracts (SAS Institute, 1982). When interactions between elution steps and solution were identified, ANOVA was used to compare each solution separately. The Duncan multiple range test was used to compare resin extracts of soil solution with those that were spiked with orthophosphate or inositol hexaphosphate.

RESULTS

The main objective of this research was to contrast the signatures of soluble P in litter leachates to that in mineral soil solution. In order to directly compare samples the amount of P in each resin extract was expressed as a portion of the total P of that form that was applied to the resin. For example, if 1 ug of SRP was applied to the resin, the SRP in a LiCl extract of the resin extract was expressed as a percent of the total SRP applied to the resin.

Total P

There were large statistically significant differences between the total P (TP) fractions in the forest floor leachates and mineral soil solution (Fig. 5.1). In fact, all LiCl extractions of the resin were statistically different. Over 10 % of the TP in the mineral soil solution did not sorb to the resin, compared to only about 2.5% of the TP in the forest floor leachates. When 0.10 and 0.15 M LiCl solutions were used to extract TP from the resin, the forest floor sample had the higher portion of TP released (36 and 26 % for forest floor leachates versus 16 and 14 % for mineral soil solution; respectively).

Extracting the resin with 0.2 and 0.25 M LiCl continued to show statistical differences between the forest floor and mineral soil solutions. Considering just the mineral soil samples there was a significant trend for more TP removal as the extracting solution concentration went from 0.2 to 0.3 M LiCl. The amount of total P removed under these conditions went from 2% with the 0.2 M solution to 10% of the initial TP with the 0.3 M solution. In this same range the TP extracted from the forest floor leachates decreased from around 7 to 1%.

One of the greatest P fractions in both studied solutions were those P compounds that could not be removed from the resin. In the mineral soil this fraction represented 45% of the initial TP to the resin, while it accounted for 25% of the TP leached from the forest floor.

SRP

Considering the forest floor leachates, over 90 % of the SRP added to the resin was removed with the 0.10 to 0.20 M LiCl extractions (Fig. 5.2). The amount removed by the other extractions were small and, when totalled, accounted for 100% of the initial SRP.

In contrast, just 64 % of the SRP in the mineral soil solution was recovered

with all the LiCl extracts while as much as 8% of the SRP in the solution did not sorb to the resin at all.

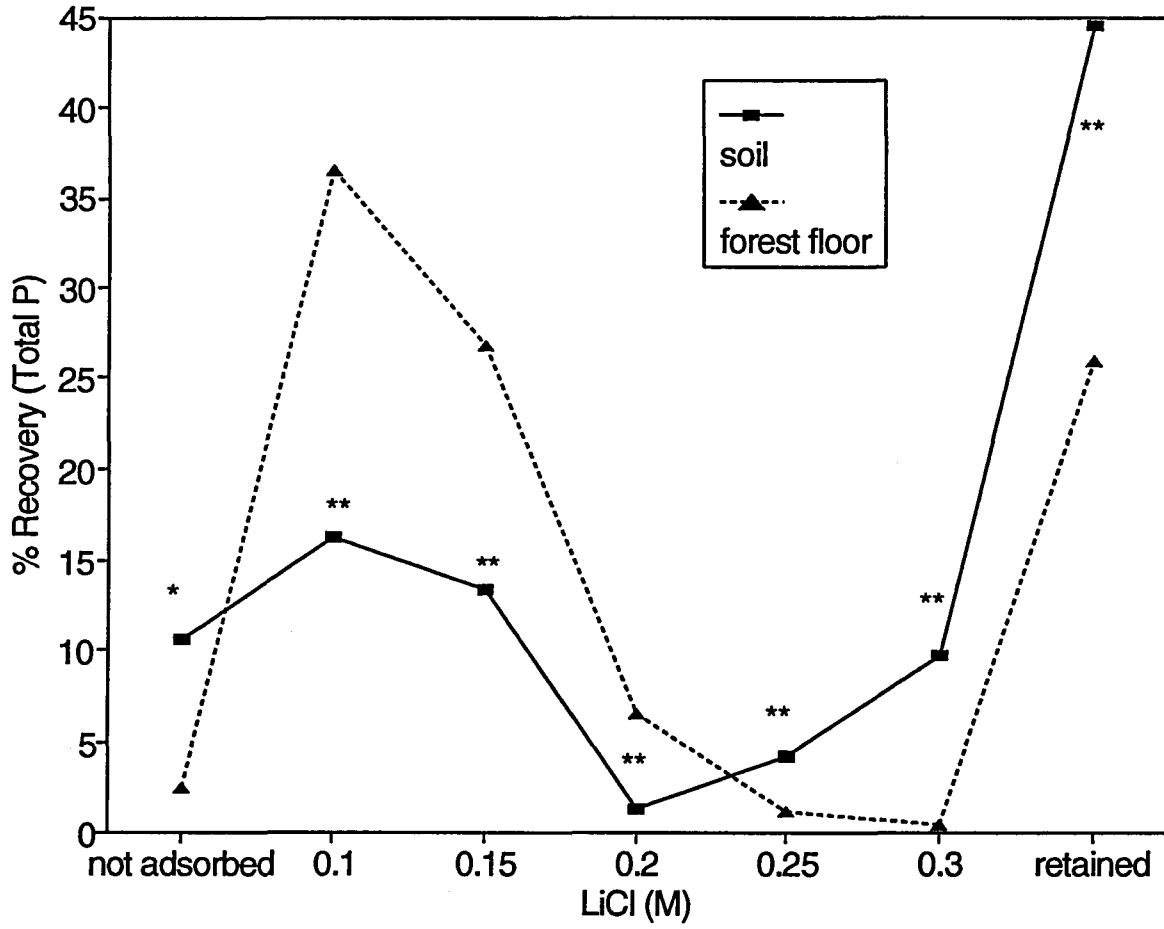


Fig. 5.1. Total P in forest floor and underlying mineral soil solutions in sequential extracts of an AG1X8 100-200 mesh (Cl-form) resin. Each extract of the resin is made with and increasing change in the LiCl concentration of the extracting solution. Results are expressed as percentage of the initial total P added to the resin. * significant differences between the two types of samples 0.05 level while ** represents the 0.01 level of significance.

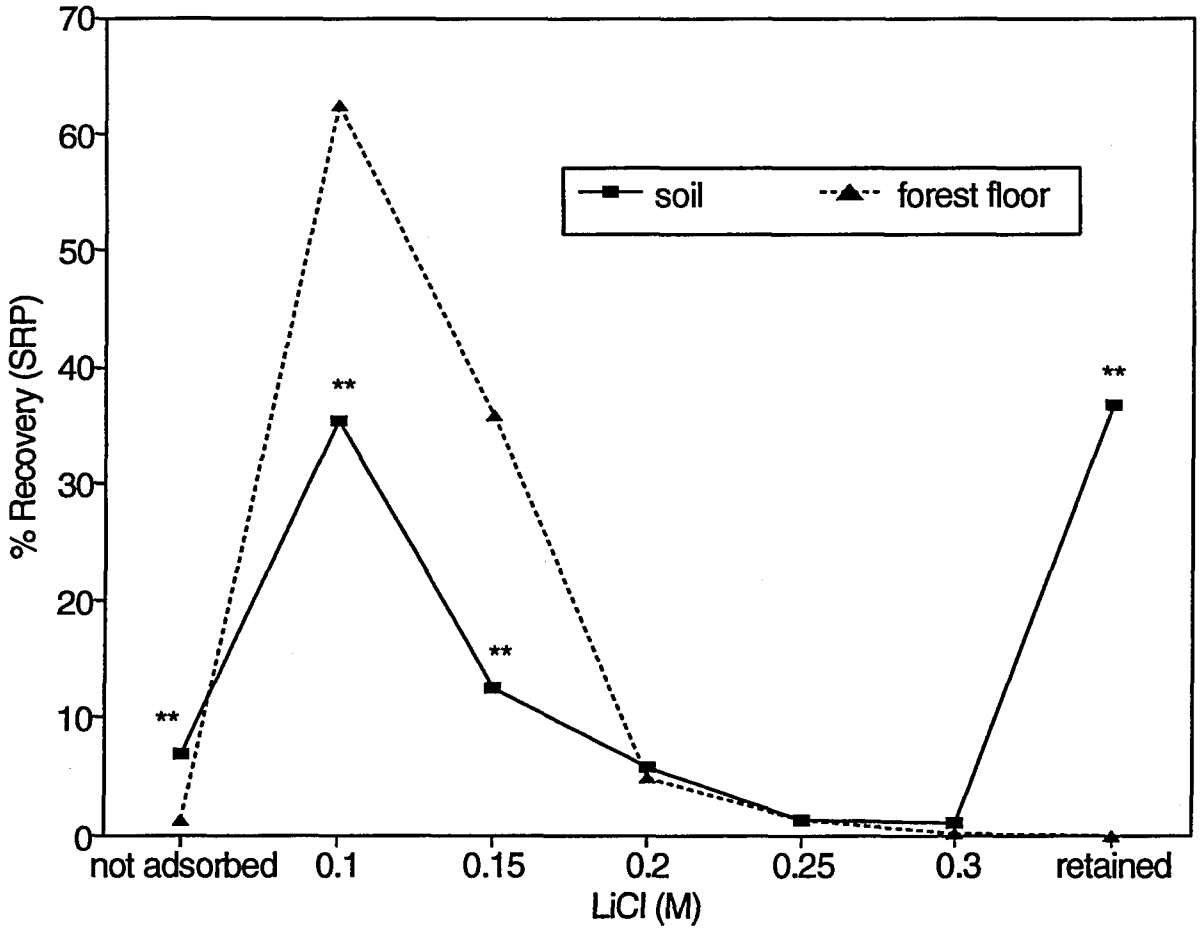


Fig. 5.2. Soluble reactive phosphorus (SRP) in forest floor leachates and underlying mineral soil solution in sequential extracs of an AG1X8 100-200 mesh (Cl-form) resin. Each extract of the resin is made with and increasing change in the LiCl content of the extracting solution. Results are expressed as percentage of total P added to the resin. * significant differences between the two types of samples 0.05 level while ** represents the 0.01 level of significance.

NRP

Of the total NRP added to the resin, the largest category for both solutions was the NRP retained by the resin after elution (73% for forest floor leachates and 46% for mineral soil solution; Fig. 5.3). The NRP of the mineral soil solution was eluted at low and approximately equal levels from 0 to 0.15 M LiCl (i.e., about 8-16% of NRP added). The forest floor leachates remained at low levels for extracts 0 and 0.10 M LiCl but increased to a comparable level with the 0.15 M LiCl extract. Beyond 0.20 M LiCl, the NRP in the mineral soil solution increased to around 12%, while the forest floor leachates remained close to zero throughout this range.

Elution of Orthophosphate and Phytate Standards

Orthophosphate or phytate was added to the mineral soil solution, the solution was added to the resin, and the resin was eluted as above (Fig. 5.4). All the orthophosphate was retained by the resin, with the vaste majority eluted with the 0.10 and 0.15 M LiCl extractions. A small amount of orthophosphate was removed with the 0.20 M extraction. One-hundred percent of the added orthophosphate was recovered from the resin with the LiCl extractions. In comparison, after the elution virtually all the phytate was retained on the resin

withthe exception of 10-15% that was not sorbed on the resin at all.

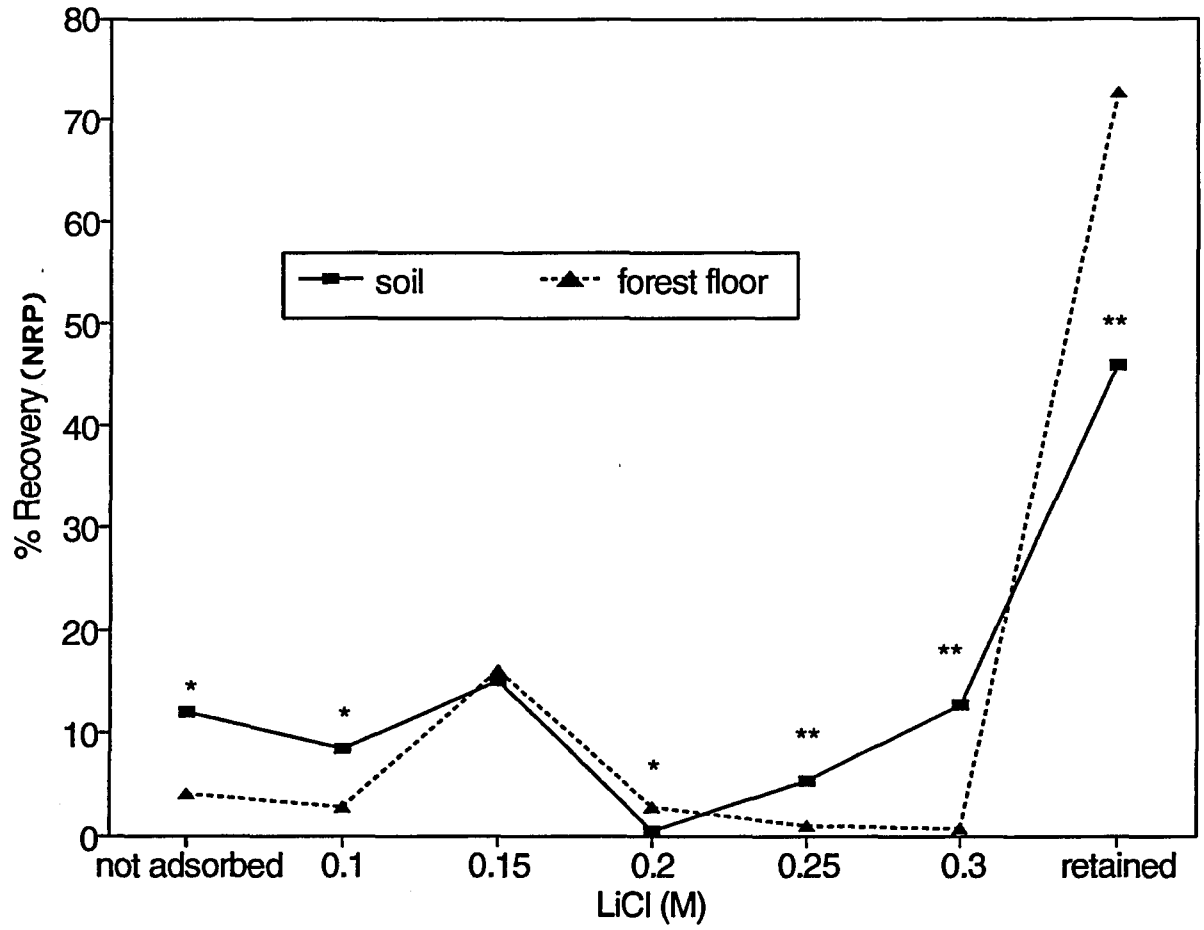


Fig. 5.3. Non-reactive soluble P (NRP) in forest floor leachates and underlaying mineral soil solution in sequential extraxcts of an AG1X8 100-200 mesh (Cl-form) resin. Each extract of the resin is made with increasing LiCl concentration of the extracting solution. Results are expressed as percentage of total P added to the resin. * significant differences between the two types of samples 0.05 level while ** represents the 0.01 level of significance.

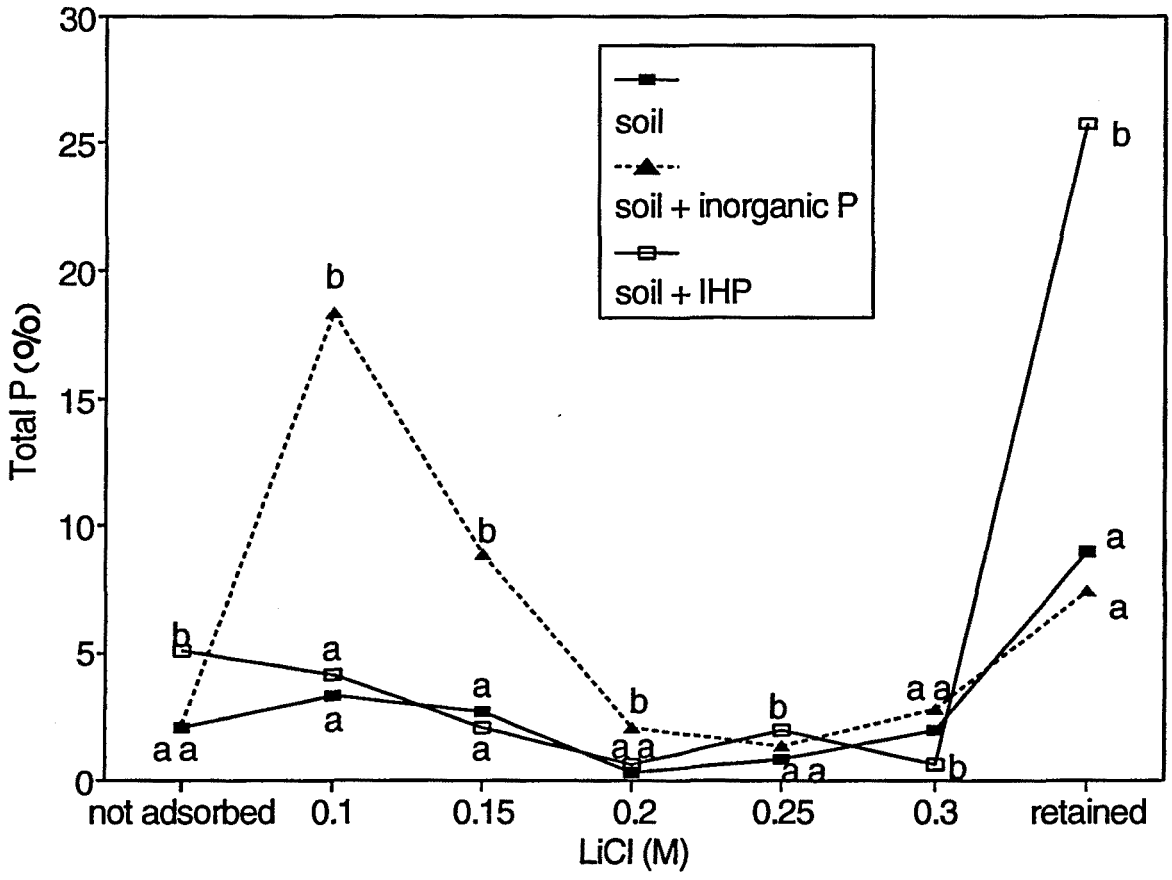


Fig. 5.4. Effects of spiking the mineral soil solution with inorganic orthophosphate and inositol hexaphosphate on the P elution pattern from AG1X8 100-200 (Cl-form) resin. Each extract of the resin is made with and increasing change in the LiCl content of the extracting solution. Results are expressed as percentage of total P added to the resin. Different letters show significant differences 0.05 level.

Total Amounts of P

Fig. 5.5 compares estimates of the absolute amount of SRP and NRP in the mineral soil (surface 5 cm) and forest floor leachates on a m^2 soil surface basis. In general, the amount of soluble P in the mineral soil is a greater mass of P, with the difference being dominated by the NRP resistent to removal from the resin. This particular fraction of P accounts for over 130 mg P m^{-2} at this one-time comparison. It exceeds any other form of P in both studied solutions by at least a factor of 4. Only the SRP and NRP retained fractions from forest floor leachates appear to have the potential to make a important contribution of the total soluble P relative to the mineral soil.

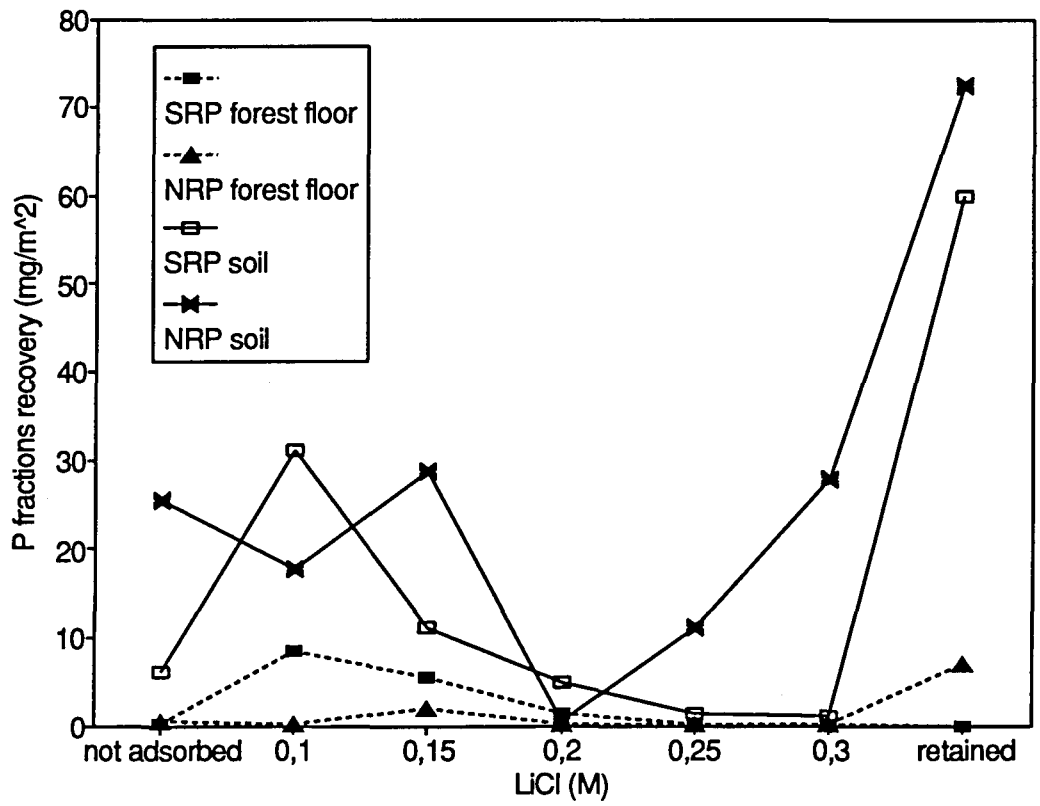


Fig. 5.5. Estimates of SRP and NRP fractions in the forest floor leachates and underlying mineral soil solution expresed in mg m^{-2}.

DISCUSSION

Comparison of forest floor and mineral soil

Several interesting points can be made using these data. First, it again brings into question what one is measuring with the Murphy and Riley (1962) method. It has previously been shown by Dick and Tabatabai (1977) that the Murphy-Riley method has the potential to cause organic P hydrolysis, but that this effect depends on the type of organic P compound. Our results showed that SRP was detectable in the fraction that did not sorb onto the resin and was not entirely recoverable from the resin once applied. However, when orthophosphate was added to the sample, all of it was sorbed to the resin and all was recoverable. These results suggest that there existed in the mineral soil extract forms of P detectable by the Murphy-Riley reagent, yet with a higher or lower affinity for the resin than had orthophosphate. This group of P compounds represents over one-third of the SRP measured in the mineral soil solution. Therefore, over one-third of what we would operationally call orthophosphate appears to be organic P in a readily hydrolyzable form. It can also be inferred that a significant portion of the P that is not recovered from the resin is not phytate since associated work (unpublished data) has shown that little, if any, phytate is hydrolyzable by the Murphy-Riley method. It further suggests that these P forms need to be better

understood before detailed studies of P transformations and cycling in soils are undertaken.

The solution of the mineral soil and the forest floor leachates had different and distinct signatures. The forest floor solution was more dominated by orthophosphate than was the mineral soil solution. This conforms with recent work on *Pinus elliotii* var. *elliotii* which showed significant levels of inorganic P in litter and its early mobility in the decomposition process (Polglase et al. 1992). Likewise the mineral soil was more dominated by what appeared to be organic phosphates that were strongly sorbed on the resin. Therefore, on a relative basis the largest change from the forest floor to the mineral soil is the reduction in the SRP fraction and an increase in the fraction not recovered from the resin, probably dominated by phytate. It is also interesting to note that the gradual increase of NRP observed after 0.2 M LiCl extract in soil solution was not observed in litter leachates at all. These data further suggest that plant roots growing in the forest floor or the mineral soil would find very different soluble P forms. These data do not describe the plant availability of the P forms and these data do not describe the processes responsible for the shift in the leachage signatures, but it is reasonable to assume that immobilization processes (biotic or abiotic) have resulted in the reduction in total concentration across most fractions as well as the shift in the signature. As said before, P not recovered from the resin makes up the largest organic P fraction in both solutions, suggesting that phytate or similar compounds are the major

organic P compound in both contrasted cases.

The resin separation method first proposed by Martin & Wicked (1967) and adapted here, appears to be useful in qualitatively and quantitatively describing differences in the soluble P forms between different soil conditions. One limitation of the method is that it is not a clean separation of inorganic and organic P forms. In all extracts that had SRP, NRP was also generally present. However, the method does appear to be useful for documenting relative changes in P forms when samples are contrasted. When combined with a range of phosphate analytical techniques we suggest that this technique will prove useful in describing P forms and function in soil environments.

Part III

CHAPTER 6

EFFECTS OF SLASH BURNING ON SURFACE SOIL PHOSPHORUS FRACTIONS AND SORPTION AND DESORPTION OF PHOSPHORUS.

INTRODUCTION

Burning of logging residues prior to planting or seeding is a common practice in many different forests (Ewel et al. 1981; Stewart & Flinn, 1985). This practice produces some of the most intensive fires described in natural systems (Walker et al., 1986). P is lost by volatilization during fires at temperatures higher than 360°C (Cotton and Wilkinson, 1988) and also by particulate transfer in smoke. Raison et al. (1985) showed even a low intensity prescribed burn in eucalypt forest caused exports of P to the atmosphere of up to 50 % of the total P contained in the combusted fuels. Further losses of P may occur during the subsequent months after fire because of export of ash by erosion processes (wind or rain). Despite P loss due to fire, many authors have reported large increases of labile P pools in surface soil immediately after burning (Ellis & Graley, 1983; Wilbur & Christensen, 1985; Simms, 1987). In the long term, however, P losses as a result of fire may diminish plant productivity (Binkley & Christensen 1992).

Although losses of P during fire may be significant relative to natural rates of replacement (eg. Raison et al. 1985), the increase of P pools in the surface mineral soil are normally much larger than exported

P. Ferran et al. (1991) reported a net increase of 36 kg P ha^{-1} in the 0-5 cm of soil after a wild fire in a *Pinus halepensis* forest. Soil-ash interactions and the effect of heat in the surface soil clearly affect P fertility in the short term after the fire. Kwari

& Batey (1991) studying agricultural soils found an increase of P sorption capacity after fire. However, few studies have examined the effects of fire on soil P interactions in forest soils and little is known about fire effects on P cycling processes.

Establishing the effects of fire on soil P chemistry is important because forest felling and burning activities are occurring in the world at unprecedented levels (Goldammer & Jenkins, 1990; Kauffman et al, 1992), and P is one of the mineral nutrients that is commonly deficient in forest soils (Pritchett & Fisher, 1987). This study describes the effects of fire intensity on phosphorus forms and sorption-desorption chemistry in a extremely low P availability soil from a harvested mixed Eucalyptus forest. Changes in labile inorganic P after incubating the soil are also reported.

MATERIAL AND METHODS

Experimental site

The study site was a low elevation mixed forest in East Gippsland (37° 42' S, 148° 43' E) in Southeast Australia. The dominant species are Silvertop Ash (Eucalyptus sieberi), White stringybark (E. globoidea) and Brown stringybark (E. baxteri). Mean annual rainfall is 1113 mm and this is quite evenly distributed throughout the year. Mean temperatures range between 19°C in February to 9°C in July. The soil is duplex (red podzolic) (Stace et al. 1968) and derived from granite. According to fertilization trials and nutrient cycling studies (Raison et al., 1991) P is the most limiting nutrient for plant growth on this site. The texture of surface mineral soil (0 to 5 cm) was dominated by sand (86 %). Other characteristics of the soil are summarized in Table 6.1.

Depth (cm)	Bulk density (g cm^{-3})	pH (KCl)	Total C (%)	Total N (mg Kg^{-1})	Total P (mg Kg^{-1})	Bray I (mgKg^{-1})
			Unburnt			
0-2.5	0.97	4.28	5.26	1107	36	0.37
2.5-5	1.12	4.31	3.28	922	33	0.20
5-10	1.19	4.49	2.62	708	27	0.23
			Burnt			
0-2.5	0.97	6.42	4.50	1066	64	11.79
2.5-5	1.12	4.54	3.15	820	38	2.40
5-10	1.19	4.54	2.47	676	29	0.39
			Ashbed			
0-2.5	1.11	7.82	3.53	981	106	13.13
2.5-5	1.12	6.90	3.33	826	51	12.89
5-10	1.19	5.16	2.63	765	39	4.87

	Exchangeable cations [mmol(+) Kg^{-1}]								
	Ca	Mg	K	Na	Mn	Al	Fe	H	Sum
Unburnt soil (0-5 cm)	23.4	8.69	1.13	3.11	0.39	7.62	0.27	4.87	49.5

Table 6.1. Selected characteristics of the studied soils. Concentrations of total C and N were measured 1 month after the fire. Total P, pH and Bray I measurements correspond to 6 months after the fire. Exchangeable cations are from the untreated soil.

Harvesting treatment and field sampling

A 10 ha area was clearfelled in March-April 1989, logs were removed and the remaining slash was burnt on 20 April 1989. Prior to burning, random sampling of the soil in the study area based on 48 cores of 5 cm of diameter cores indicated relatively little soil variation (eg Table 6.2). Soon after the fire, the site was surveyed using the line-transect method to define the proportion of area occupied by snig tracks, unburnt, moderately burnt areas and intensely burnt soil where accumulations of woody slash had been combusted. Surveys consisted of 16 linear transects each 100 m in length. The results of the transects showed that 31 % of the surface was unburnt, 18 % was burnt by a fire of moderate intensity, 19 % was intensely burnt (ashbed) as a result of combustion of piles of woody residues, and 32 % surface left was occupied by snig tracks (also unburnt). Twenty four h. after the fire was started, soil temperature at 10 cm depth in the intensely burnt area, was still higher than 50°C. Four randomly located areas within each of the unburnt, burnt (moderate intensity) and ashbed areas were selected for sampling. The unburnt soil served as a control. Seven months after burning, 32 soil cores were collected at random from each area by using 5 cm diameter cores. Another 32 cores per microsite were capped and left in the field to incubate (Raison et al. 1987) for about two months (from November 28th 1989 until January 30th 1990). All soil cores were divided into three layers (0-2.5 cm, 2.5-5 cm and 5-10 cm) and sieved through 5 mm mesh without drying. Soils were bulked to

provide 4 replicates per depth and kept field moist and cool (4°C) until further analysis.

	Soil horizons		
	0-2.5 cm	2.5-5 cm	5-10 cm
Bulk density ($g\ cm^{-3}$)	0.97 (0.026)	1.12 (0.022)	1.19 (0.021)
pH (KCl 2N)	3.85 (0.04)	3.92 (0.05)	4.01 (0.04)
Total C (%)	3.58 (0.28)	3.10 (0.19)	2.45 (0.19)
Total N ($mg\ kg^{-1}$)	981 (58)	926 (46)	765 (39)
Total P ($mg\ kg^{-1}$)	151 (8)	94 (3)	75 (3)

Table 6.2. Variability of several soil parameters in the site prior to burning. Means are were based on 48 5 cm-diameter soil cores. Figures in parenthesis refer to standart error.

Eucalyptus seedlings sampling

Eight months after fire, 8 naturally regenerated Eucalyptus sieberi seedlings growing on each unburnt, burnt and ashbed areas were harvested and its height was measured. Seedlings regenerated from coppicing. Following, four composite samples per treatment of roots, needles and stems, were ovendried at 60^{0}C and ground. P concentration was then determined using a continuous-flow autoanalyzer TRAACS 800, after digestion with concentrated H_2SO_4 and H_2O_2.

Laboratory incubation

Soils (50 g) from the November sampling were placed in 2 l jars and incubated for 67 days at 24°C in the dark (4 replicates for each soil area and depth). During the incubation period, a container of water was also placed in the jars in order to minimize soil drying. CO_2 evolution was measured using a 1N KOH trap. Blanks without soil were also monitored. The KOH solution was replaced after 1, 12, 22, 38, 52 and 67 days of incubation and titrated with HCl to determine the amount of CO_2 evolved. Jars were opened for about 5 min every four or five days to replace O_2.

Soil analysis.

Samples taken 7 months after burning were analyzed for total organic P using the ignition method (Olsen & Sommers, 1982). P extracted from unignited soil was used as a measure of inorganic P and the additional P extracted from ignited soil was taken as a measure of organic P. The addition of these two fractions was taken as total P. Less labile P was extracted by shaking the soil for 16 h with 0.5 N NaOH in 1:40 weight:volume. Labile organic P was extracted for 30 min with 0.5 N $NaHCO_3$ (1:20 weight:volume) and it was estimated by subtracting the inorganic fraction. Soluble P was extracted with 0.01 M $CaCl_2$ (1:10 weight:volume) by shaking for 24 h. pH was also measured in this extract using a glass electrode. All inorganic P fractions in solution were determined using Murphy & Riley (1962) method. Except for the ignition method and for the soluble P, total P was determined in all soil extracts by using the same method following digestion with concentrated H_2SO_4 and H_2O_2. Less labile and labile organic P were estimated as the difference between total P in the extract minus its respective inorganic P.

All initial soils (Nov. 1989) and field and laboratory incubated soils were analyzed for labile inorganic P using Bray I method (1:30 weight:volume) extracted for 5 min extraction. Mineral N was also extracted in these samples using 1N KCl (1:5 weight:volume) by shaking for 1 h. NH_4-N and NO_3-N were determined

colorometrically following distillation.

To obtain sorption isotherms, four replicates of initial soils were mixed to create a single bulk sample. Solutions of 0.01 M $CaCl_2$ containing increasing amounts of KH_2PO_4 were prepared which gave concentrations of 2 to 150 ugP ml^{-1}. Solutions were added to the soil in 1:10 weight:volume and allowed to equilibrate by shaking for 24 h. To separate the solid phase from the liquid phase, samples were centrifuged at 5000 rpm for 30 minutes. Phosphate in solution was determined by using Murphy & Riley (1962) procedure. The soil was kept cool (4^0C) and subsequently extracted with Bray I for recovery within one day. The amount of P sorbed was calculated from the difference between the initial and final P concentrations in solution. Resulting isotherms were fitted by using classical linearized Freunlich and Langmuir equations. A modified Langmuir equation (Kuo, 1988) which accounted for the decrease in the free energy of sorption with increasing phosphate sorption was also fitted to the data. The linear form of the Langmuir equation is:

$$C/X = (1 / K\,Xm) + C/Xm$$

where X is the amount of P sorbed to the soil (mgP Kg^{-1} soil) at the soil solution phosphate concentration (C), Xm is the maximum sorption capacity (mgP Kg^{-1}) and K is the binding constant or the affinity coefficient (l mg^{-1}). The linear form of the

modified Langmuir isotherm is :

$$X = Xm - K^{-1/2} Xm^{1/2} (X/C)^{1/2}$$

and the linear form of Freundlich equation is:

$$\log(X) = \log (b) + (1/m) \log (C)$$

The Freundlich equation is of empirical nature and the parameters do not have unities.

To measure P desorption, phosphate enriched soils were extracted for 5 minutes with Bray I extract (1/30 weight:volume for 5 min). The amount of added P that the soil samples held in solution previously to the Bray I extraction, was calculated by multiplying the volume of solution left in the sample (estimated by weight), by the solution concentration which had been previously determined by the Murphy and Riley (1962) method. The quantity of P extracted by the Bray I procedure was then estimated as the difference between that actually extracted minus that initially contained in the soil solution.

Statistical analysis

Differences in P fractions, N mineralization and C respiration between fire intensities were tested using analysis of variance techniques (SAS institute, 1982). Each depth was tested separately and means were compared by Duncan's multiple range test. The SAS linear regression procedure was used to fit straight lines, whereas non-linear curves were fitted using a Sigma Plot non-linear least squares program. Differences between the linear forms of the isotherms were tested using analysis of variance, using the concentration of P in the liquid phase as a covariate. In order to compare soils having different sorption capacity the Bray I recovery curves were expressed as a percentage of the amount of P previously sorbed in each case. Differences in these were also tested by using analysis of variance after transforming proportions to arcsin square root and linearizing by common logarithms.

RESULTS AND DISCUSSION

Changes in soil P fractions

Bulk density of the ashbed 0-2.5 cm soil increased by 14 % when compared with the unburnt soil (Table 6.1), probably because of the loss of organic matter.

The ashbed soil had a three fold higher values for total P concentration (Fig. 6.1) and total P content (referred to surface: not shown) in the 0-2.5 cm layer than unburnt soil from the same depth. In the 2.5-5 and 5-10 cm soils, total P concentrations were about 50 % higher under ashbed than in the unburnt soil. The burnt soil contained 78 % more total P than the control in the 0-2.5 cm sample, but no significant differences were observed for other soil depths. The total P increase observed after the fire, was mainly due to a large increase of inorganic P. In the ashbed and burnt 0-2.5 cm soils, total inorganic P was 14 and 4 fold higher respectively than in the unburnt soils. Likewise, NaOH extractable inorganic P showed a similar trend but the differences between fire affected soils and the control were smaller for this fraction (Fig. 6.1).

The increase in inorganic P fractions after fire in both ashbed and burnt soils was always greater in the surface horizon. For the burnt soil, an increase in inorganic P occurred only in the surface 0-2.5 cm soil. For the ashbed soil there were statistically significant increases, for all depths studied 7 months following the fire. Ellis & Graley (1983) found an increase in available P for only the upper 2 cm soil. Our results show that the depth at which fire effects are noticeable depends upon the intensity of the fire. The magnitude of the changes depended on the fraction of P considered. Labile inorganic P was the fraction most sensitive to changes introduced by fire. Whilst total inorganic P contents increased by about 6.5 and 2-fold in the 0-10 cm ashbed and burnt soils respectively, the NaOH

extractable inorganic P increased by 4 and 0.7-fold and the labile inorganic P increased by about 36-fold for the ashbed soil and 13-fold for the burnt soil. This large increase in inorganic labile P after the fire in surface horizons has been reported by several authors (Ellis & Graley, 1983; Marion & Black, 1988; Ferran et al., 1991; Polglase et al. 1992) and is usually considered to be of short duration (few years).

In figure 2 is shown that the increase of inorganic P due to the fire was high in the surface horizon especially for the total and less labile P fractions. Likewise, although in the ashbed surface soil labile inorganic P was high, it represented a low proportion of total P in this horizon (Table 6.3). Khanna et al. (1992) found that P contained in ash was relatively insoluble in water. Likewise, Ohno and Erich (1990) found little labile P increase after adding wood ash to the soil. It is likely that a great part of the phosphorus added to the surface soil in the ashbed treatment was not in labile form, and remained immobile at the soil surface even at the 7th month following fire. Labile inorganic P pools in the soils which did not receive high amounts of ash (subsurface ashbed 2.5-10 cm soils and surface burnt 0-5 cm soil) were increased relatively to the total inorganic P. In contrast, less labile inorganic P pools were relatively decreased (Table 6.3). Khanna et al. (1992) showed that the solubility of ash P increases when the ash is neutralized by the soil. This fact could explain the increase in labile inorganic P content in soils not holding high amounts of ash. Leaching processes from other horizons containing

high amounts of ash and the effects of heat could also account for labile inorganic P increases in subsurface horizons.

Depth (cm)	Fraction of total inorganic P extracted (%)		Fraction of total organic P extracted (%)	
	Bray I	Inorganic P-NaOH	Organic P-$NaHCO_3$	Organic P-NaOH
		Unburnt		
0-2.5	4.98	100.00	nd	60.53
2.5-5	3.27	95.02	nd	52.82
5-10	3.71	78.44	nd	49.42
		Burnt		
0-2.5	38.39	86.02	7.98	55.67
2.5-5	26.65	84.88	6.92	59.16
5-10	5.68	65.66	6.79	55.01
		Ashbed		
0-2.5	13.79	50.22	19.45	71.74
2.5-5	36.05	57.31	19.73	100.00
5-10	26.48	69.61	15.74	56.00

Table 6.3. Percentages of labile and less labile organic and inorganic P fractions, with respect of total inorganic and organic P respectively extracted with ignition method (H2SO4 1N). nd refers to non-detectable P fractions.

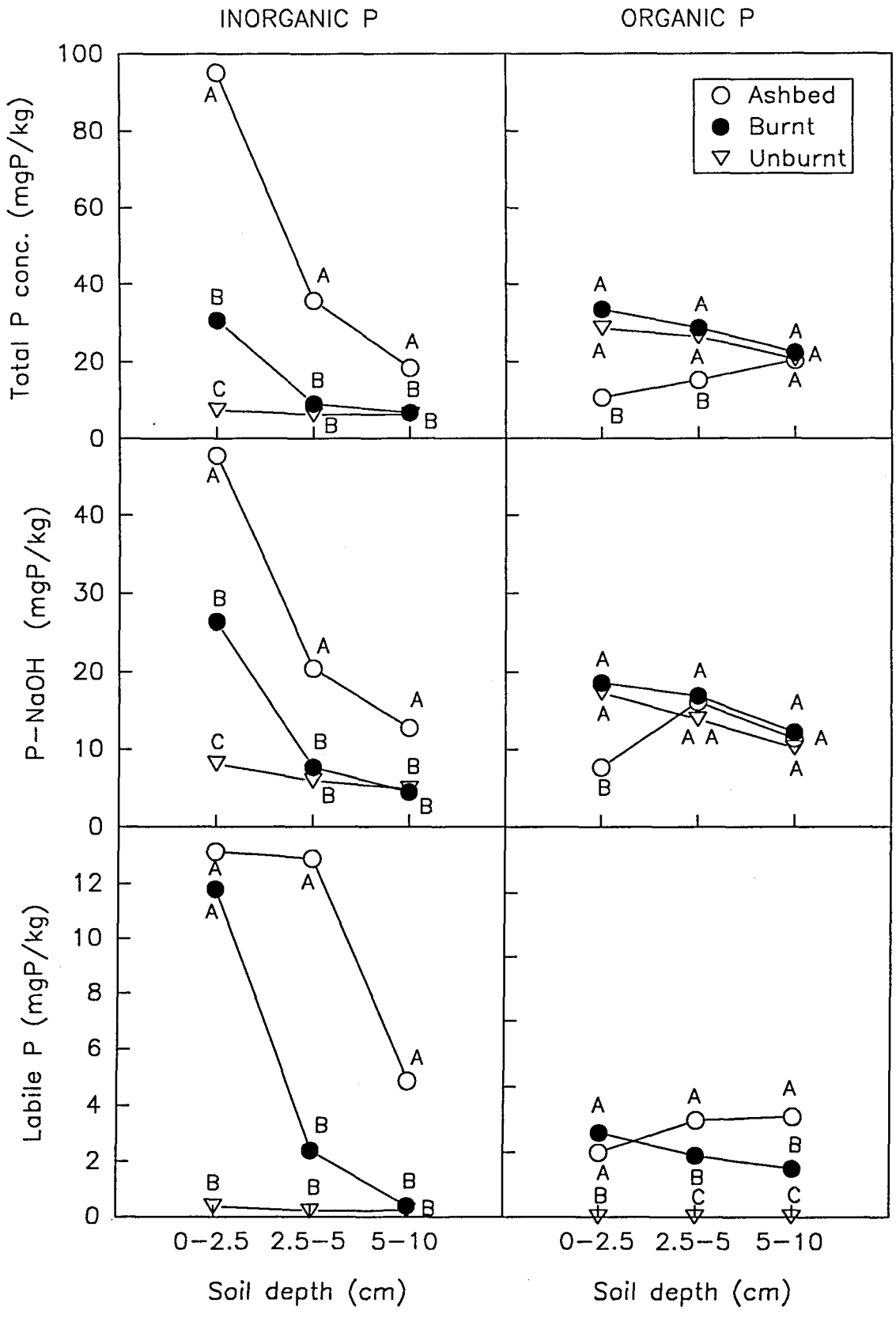


Fig. 6.1. Effects of fire intensity on P fractions in the duplex red podzolic soil. Three different intensities of fire were described: intensely burnt (ashbed), moderately bunrt (burnt) and unburnt. Different letters show significant differences ($p<0.05$) when compared at the same soil depth.

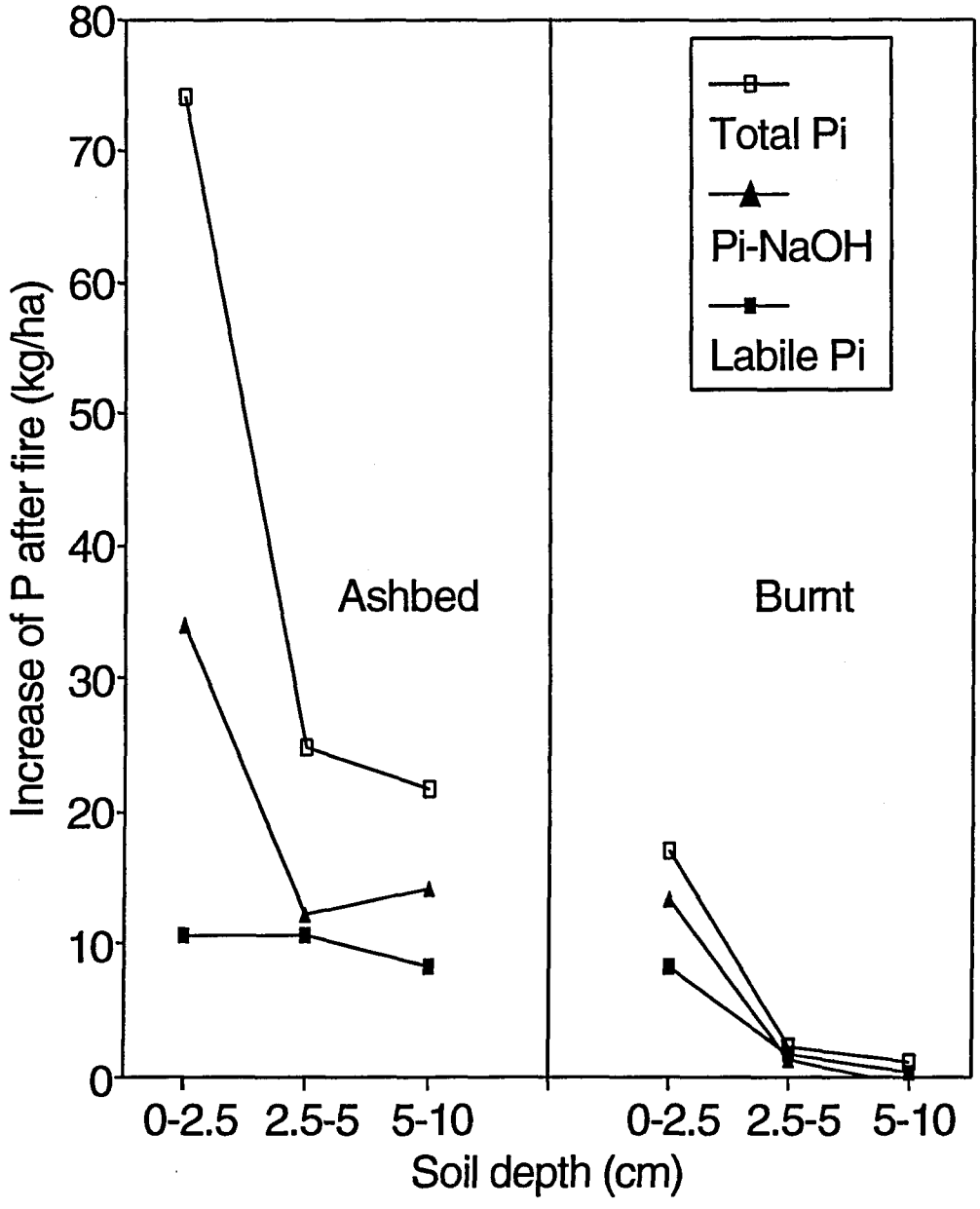


Fig. 6.2. Increase in inorganic P fractions (expressed as kg per unit area of surface soil) for the burnt and ashbed soils.

The concentration of total organic P was decreased by 63 and 43 % respectively in 0-2.5 cm and 2.5-5 cm of the ashbed soil respectively, while the burnt treatment did not show any difference from control (Fig. 6.1). Other authors have also reported organic P decreases after fire (Saa et al 1991; Kwari & Batey 1991). This decrease could be explained by two different processes: i) the combustion and volatilization of the organic matter as a result of the fire which would have released most of its P into inorganic forms, and ii) by the large pH increase recorded in the 0-5 cm of the ashbed soil which would have increased the solubility of the remaining organic matter causing some organic P to move to the lower horizons. Because the remaining organic P in ashbed 0-5 cm horizons was mostly NaOH extractable (Table 6.3), and because ashbed and burnt soil had significant amounts of labile organic P in all layers (Fig. 6.1), it is suggested that organic P pools in the surface of the ashbed soil (0-5 cm) were more labile and consequently proner to leaching than the organic P in other soils. It should be noted that most of the total P (80 %) in the unburnt soil was in the organic form. The decrease in total organic P resulting from the combustion of organic mater could have contributed in part to increase inorganic P pools in fire affected soils. This total organic P decrease, especially in ashbed 0-2.5 cm soil, was much greater than the increase in labile organic P forms. This fact indicated that, losses of organic P by combustion and volatilization were quantitatively more important than losses by leaching.

Changes in labile inorganic P after incubation.

Both, field and lab incubations showed similar results. Unburnt soil and 5-10 cm burnt layer showed an increase in labile inorganic P after incubation ranging from a 68% to a four fold increase, whereas for the ashbed and for the 0-2.5 cm burnt soil, there were large decreases (Fig. 6.3). Despite the large decrease in labile inorganic P observed in fire affected soils after both, lab or field incubation of 68 days, ashbed and surface burnt soils still showed higher labile inorganic P than the control. Similar results were obtained by Kutiel & Shaviv (1989) after incubating heated soil under lab conditions. If N and C mineralization rates are used as a measure of soil microbial activity (the mineralization of these elements showed a similar trend among treatments; Fig. 6.3), the large decrease in the pools of labile inorganic P in soils affected by fire was not likely to be caused by mineralization-immobilization processes but by P soil chemical fixation. Indeed, the control and 5-10 cm burnt soils showed a significant increase of labile inorganic P pools after incubation instead of the large decrease observed in the fire affected soils (Table 6.3). In the ashbed soil, the increase of P sorption capacity described later would have facilitated this large P fixation. The fact that in the ashbed 0-2.5 cm soil, N mineralization decreased (possibly because the decrease in organic matter observed in this horizon) would give further suport to the fact that P immobilization was not caused by soil biological processes.

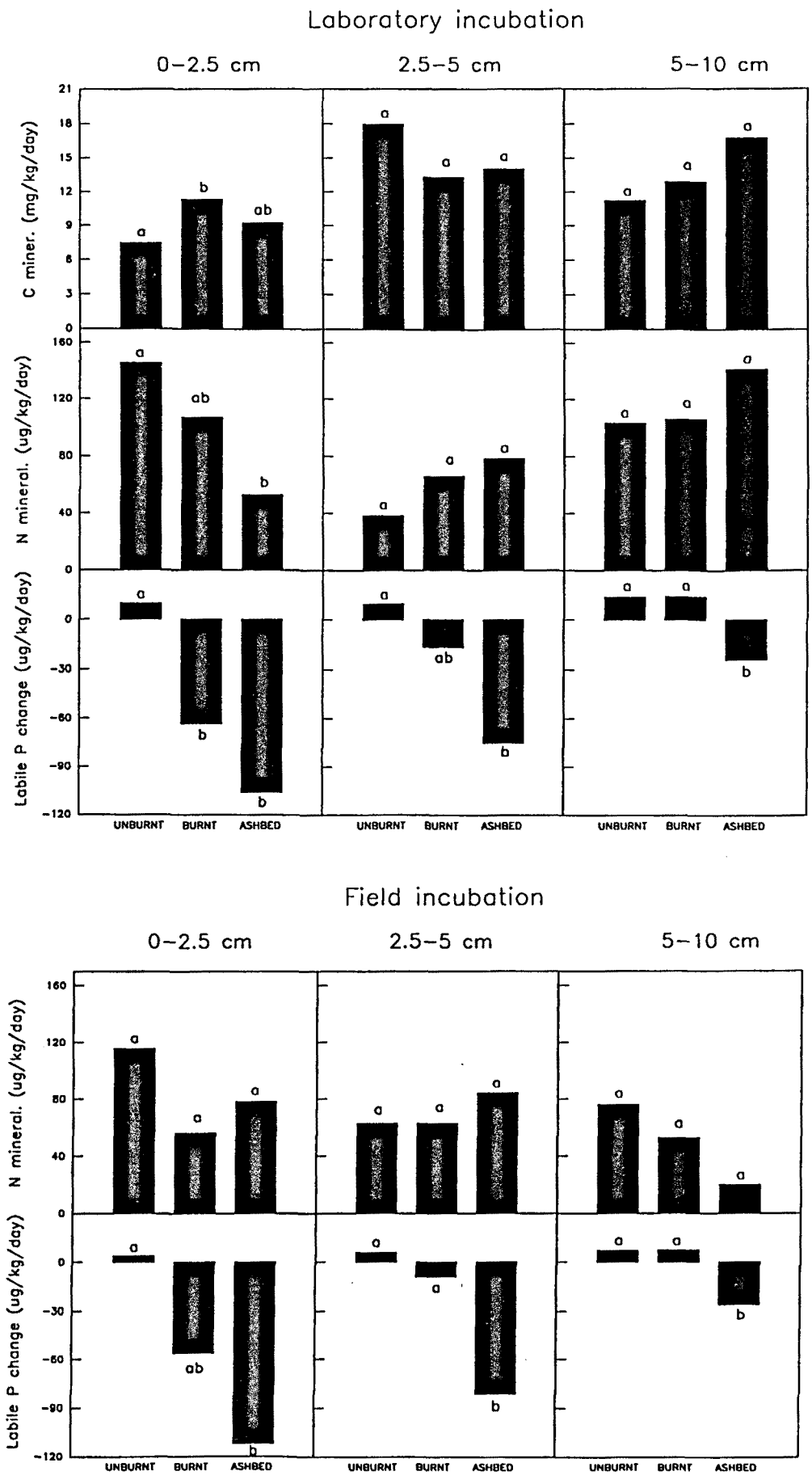


Fig. 6.3. Mineralization of C and N, and change of labile inorganic P (Bray I) after incubation. Different letters show statistically significant ($p<0.05$) differences within each soil depth using Ducan's multiple range test.

P sorption and desorption

The ashbed 0-2.5 cm and 2.5-5 cm soils showed a large increase in P sorption as compared to control and burnt soils (Fig. 6.4). The burnt soil did not show any difference in P sorption from control at any depth. Freundlich equation fitted the data for all soils between 0.01 to 10 mgP l^{-1} in soil solution with a regression coefficient (r^2) greater than 0.94 (Table 6.4). Outside this concentration the observed points did not follow the linearized Freunlich curve. The slope of Freundlich fit for the ashbed soil was significantly higher at all depths than the control or burnt soils, ranging from a two fold increase for the top layer to a 33% increase for 5-10 cm layer. Slopes of the Freundlich lines for the burnt soil did not differ from control treatment.

Both types of linearized Langmuir adsorption plots (C/X vs C and X/C vs X) were curvilinear across all studied soils. However, the former was closer to a straight line, similar in results reported by others (Veith & Sposito, 1977; Kuo, 1988). Therefore, C/X vs C plots were fitted by using two intersecting straight lines each one representing a different population of P sorption sites (region 1 and region 2) of contrasted affinity for P (Table 6.4) (Syers et al., 1973). When fitted to a modified Langmuir isotherm which accounted for the change in the slope of the plots (Kuo, 1988), the values obtained for Langmuir adsorption maximum (Xm) were similar to those obtained at the second segment of the linearized Langmuir

curve. Similar to the results obtained by Freunlich isotherms, Langmuir maxima where high for the ashbed soil, especially for 0-2.5 cm soil (Table 6.4). For the burnt soil, Langmuir maxima were slightly lower than for the control soil. Xm estimated for the first region also showed a clear increase in the ashbed soil, but only in 0-2.5 cm and 2.5-5 cm samples. All estimates of the Langmuir binding constant (K) were lowest for the 0-2.5 cm ashbed soil. Both regions of the Langmuir fit for the ashbed 2.5-5 cm soil also gave lower values for K as compared to the control soil.

An increase in P sorption capacity in the ashbed soil is similar to the results presented by Kwari & Batey (1991) and Polglase et al. (1992). As reported by Bar-Yosef et al. (1988), the increase in pH in the ashbed soil would cause a decrease in P sorption capacity because of changes in competition with OH^-, variations in the density of clay edge sites and the effect of pH on the relative proportions of P species. Therefore, it is likely that the observed high P sorption capacity in ashbed soil was caused by other changes in soil chemistry. Both, heating and ash addition can affect soil P sorption capacity. Kwari & Batey (1991) found that heat itself would increase soil P sorption capacity and when heat was combined with ash addition its effects were much larger. They interpreted these changes as caused by an increase in sesquioxide complexes and possibly by release of Al from organic matter complexes. Haynes and Swift (1989) investigated the adsorption of P by Al-peat and Al-humate, and found that upon raising pH the P adsorption

capacity was increased. They attributed this to increasing hydrolysis and polymerization of hydroxyl-Al associated with organic matter. The large increase of pH and the loss of organic matter in the ashbed soil could have caused high P sorption capacity as a result of the free Al left in the soil after organic matter burning or leaching. Further support for this hypothesis is given by Humphreys and Craig (1981), who after heating soils in the laboratory, noted an increase in the P fraction associated with aluminium (Al-P).

Depth (cm)	Freundlich fit slope (1/m)	b	r^2	Modified Langmuir fit Xm	K	Langmuir fit Rg	Xm	K	r^2
				Unburnt					
0-2.5	0.37	2.19	0.99	342.4	3.23	1	144.4	9.87	0.99
						2	405.0	0.51	0.99
2.5-5	0.35	2.25	0.94	379.2	2.37	1	128.7	61.07	0.97
						2	363.5	0.85	1.00
5-10	0.33	2.28	0.99	350.4	4.29	1	158.1	12.64	1.00
						2	376.8	1.00	1.00
				Burnt					
0-2.5	0.37	2.14	0.99	289.2	4.01	1	98.7	16.24	0.99
						2	402.9	0.41	1.00
2.5-5	0.36	2.18	0.98	308.9	3.67	1	93.2	49.69	0.91
						2	330.5	0.69	0.99
5-10	0.31	2.21	0.99	300.7	4.68	1	143.0	14.93	0.98
						2	330.0	0.68	0.99
				Ashbed					
0-2.5	0.81	2.30	0.99	1210.7	0.29	1	512.3	0.66	0.95
						2	1080.6	0.24	0.99
2.5-5	0.58	2.41	0.99	553.4	2.67	1	223.4	7.05	0.96
						2	505.3	2.84	1.00
5-10	0.43	2.33	0.98	426.4	2.90	1	152.4	19.54	0.92
						2	418.0	1.08	1.00

Table 6.4. Linearized Freundlich isotherms parameters, and Langmuir and modified Langmuir adsorption maximum (Xm) and binding constant (K) for the different soils. Rg. refers to each one of the two intersecting straight lines to which the linear Langmuir curve (C/X vs C) was fitted.

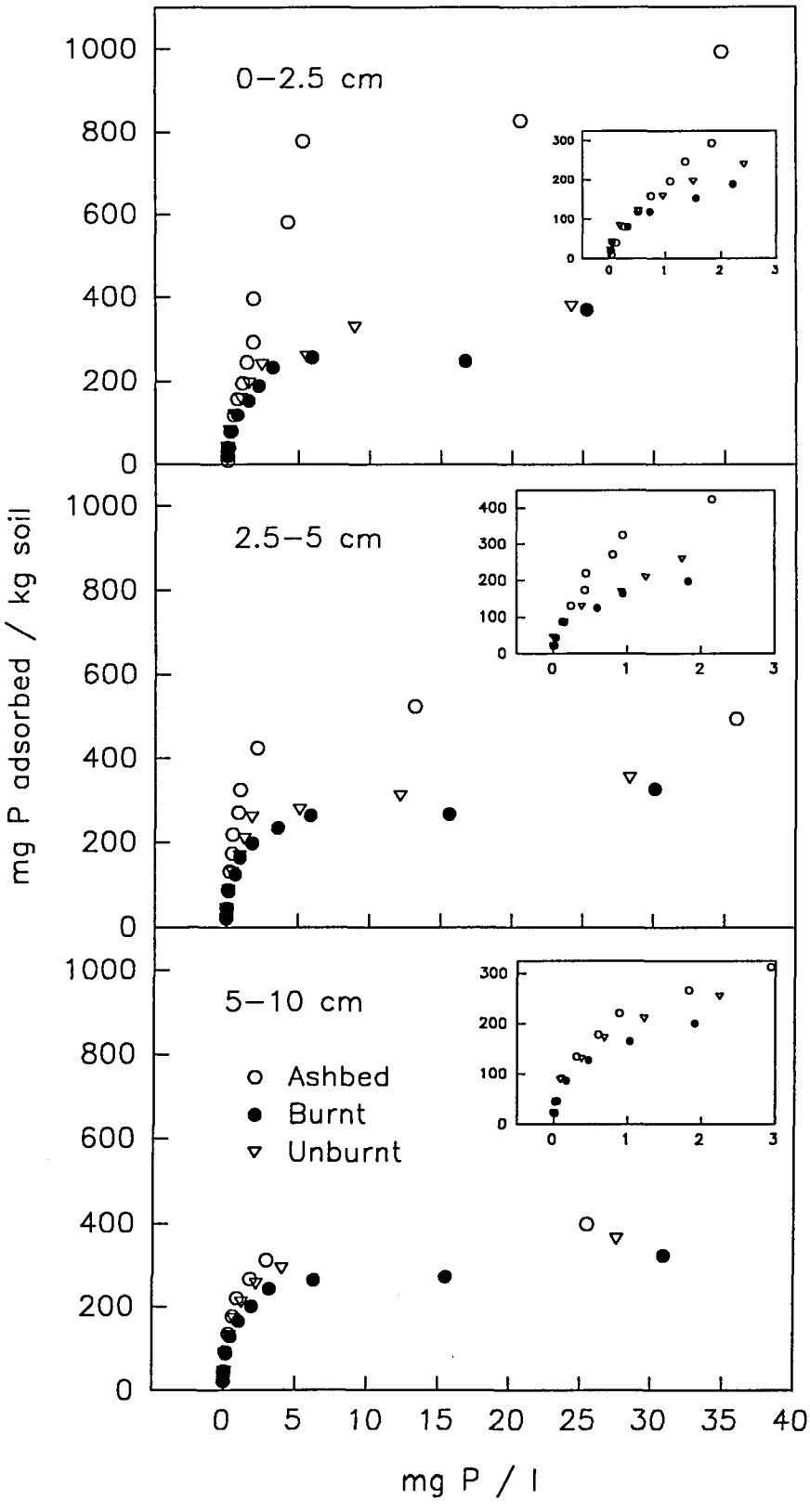


Fig. 6.4. Effects of fire intensity on P adsorption isotherms at three soil depths. A detail of the lower part of the curve is shown in the little graphs.

The increase in the maximum sorption capacity (Xm) for all ashbed soils as compared to the unburnt soil, was much higher than the respective increase in total inorganic P. On a m^2 soil surface basis for the surface soil (first 10 cm), the increase of Xm was 8.5 fold greater than the increase of total inorganic P. This suggest that in soils where P sorption has been increased by fire, surface sorbed P occupies only a small fraction of the available sorption sites. Further support for this is given by the fact that the Xm estimated with the traditional Langmuir equation agrees well with the Xm estimated by the modified Langmuir isotherm (which accounts for the decrease of free energy of sorption with increased phosphate sorption, Kuo, 1988) (Table 6.4). In soils which are far from the saturation point, this decrease of free energy of sorption may not be important. Thus, despite the presence of high amounts of inorganic P following fire, percolation losses of P are unlikely. Diaz-Fierros et al. (1990) did not detect any P loss in runoff solution during the first four months after the fire.

High soil P sorption capacity after the fire could possibly have affected soil P supply characteristics. The distribution coefficient (Kd) describes the propensity of the P in solution to react with the soil solid phase. If X/C is used as an estimation of Kd (Sposito, 1989), then for low P concentrations in soil solution the surface ashbed soil had lower Kd when compared with other depths and soils. Whereas, as soil solution P concentration increases, Kd for this same ashbed soil tended to stay at slightly higher levels than the others. This latter tendency was

also shown by the 2.5-5 cm ashbed horizon (Fig. 6.5). Van Rees et al. (1990), suggested that the distribution coefficient (Kd) provides an estimate of the nutrient supplying capacity of the soil. In our experiment the surface ashbed soil had higher or lower Kd and consequently higher or lower P supply capacity as compared to control, depending upon the concentration of phosphorus in soil solution (Fig. 6.5). If we use soluble P (extracted with 0.01 M $CaCl_2$) as an estimation of soil solution concentration in the studied soils, it was always lower than 0.035 mgP l^{-1}. At this concentration, ashbed 0-2.5 cm soil, would have a lower Kd and consequently lower buffer power than the control soil. However, since the ashbed soil had 3.5 fold concentration of P than control, P supply in this surface soil could still be higher than in the control horizon. Phosphorus supplying capacity in the ashbed 2.5-5 cm and 5-10 cm soils would be always higher than in the control soils because P concentration and Kd were higher. The higher slope value that was obtained by fitting the Freundlich equation to the data gave higher Kd for ashbed soil. This result agrees with the Kd values at higher soil solution concentrations. Soil solution P concentration for which Kd was lower for the ashbed 0-2.5 cm soil, was off the linear zone of the Freunlich fit.

When soils were enriched with high amounts of P, the percentage of P recovered by Bray I extractant, in soils not affected by fire, increased to an equilibrium. (Fig. 6.6). This fact indicated that low amounts of P added to the soil were more tightly retained than high amounts. This pattern, however, was altered

depending upon fire intensity. Curves for the ashbed and burnt 0-2.5 cm soils did not show significant differences among themselves but were different than control soil curves. In these cases the percentage of recovery did not depend on the amount of P recently adsorbed and was always higher than in the control soil. 5-10 cm ashbed soil also showed a similar trend. The ashbed 2.5-5 cm layer was the only soil showing a slight decrease in recovery as adsorbed P increased. The burnt soil, 2.5-5cm layer did not show any significant difference compared to the control treatment. The percentage of P desorbed by Bray I in ashbed soil was greater than in the control soil especially when low quantities of P had sorbed to the soil. This increase in percentage recovery for the ashbed soil corresponded with an increase of P sorption capacity (Xm) at all depths. Kuo et al. (1988) reported a significant correlation between Xm and the recovery of the added phosphate over a wide range of soils. This increase in P recovery as Xm becomes high, could be interpreted as a weaker binding of P adsorbed in the ashbed soil. The lower affinity constant (K values) obtained for these soils and, the decrease in Kd observed in 0-2.5 cm ash bed soil give further support for this interpretation.

Plant responses to changed P availability

Foliar concentrations in seedlings growing in fire affected soils were sensitive to the increased P availability observed in ashbed and burnt areas (Fig. 6.7). The large increase in P availability observed under ashbed areas corresponded to a 60 % increase in seedlings height 8 months after the fire, while seedlings growing under burnt areas did not show any growth response.

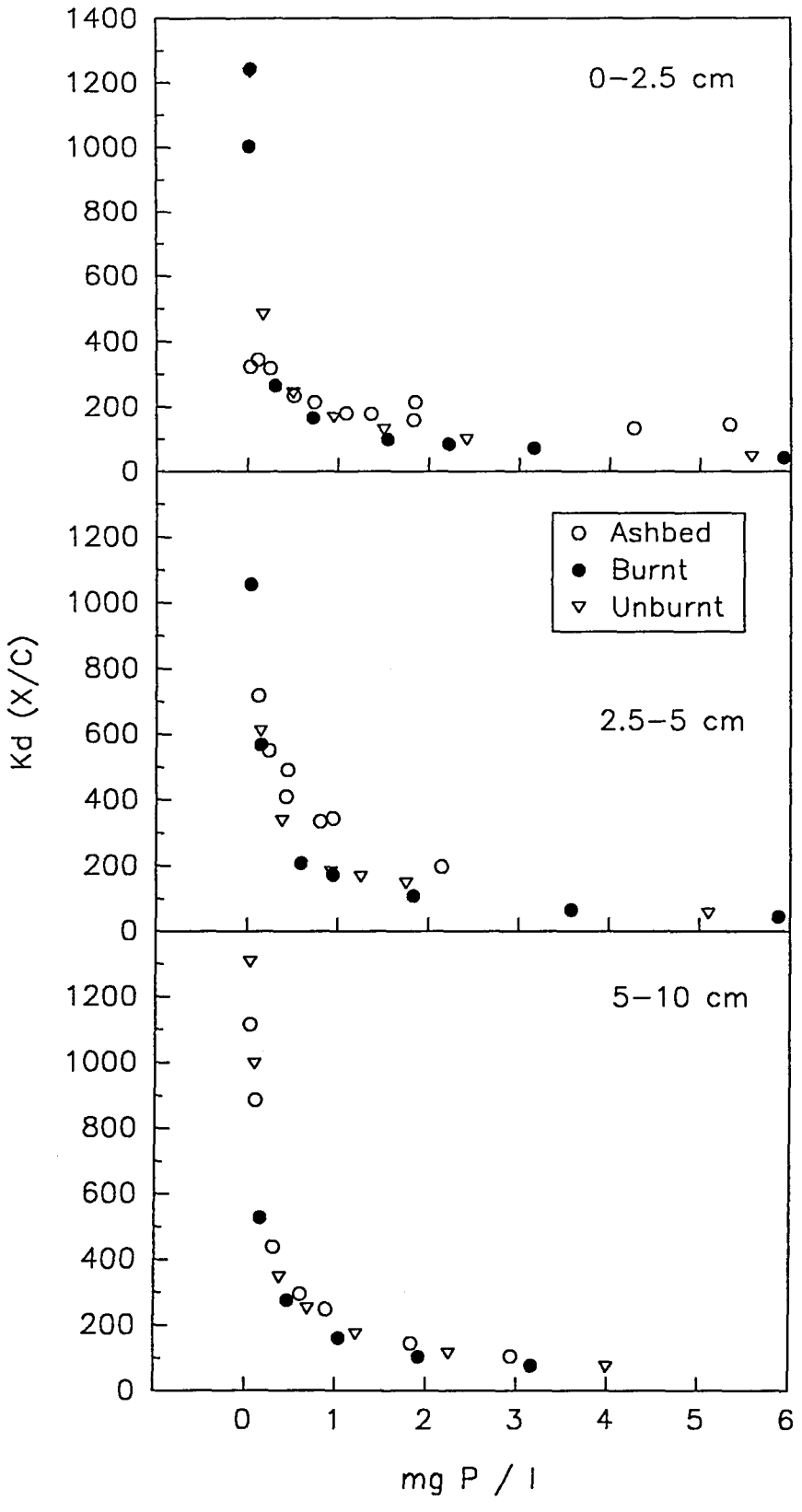


Fig. 6.5. Distribution coefficient (Kd; X/C) with increasing soil solution concentration; as affected by fire intensity and soil depth.

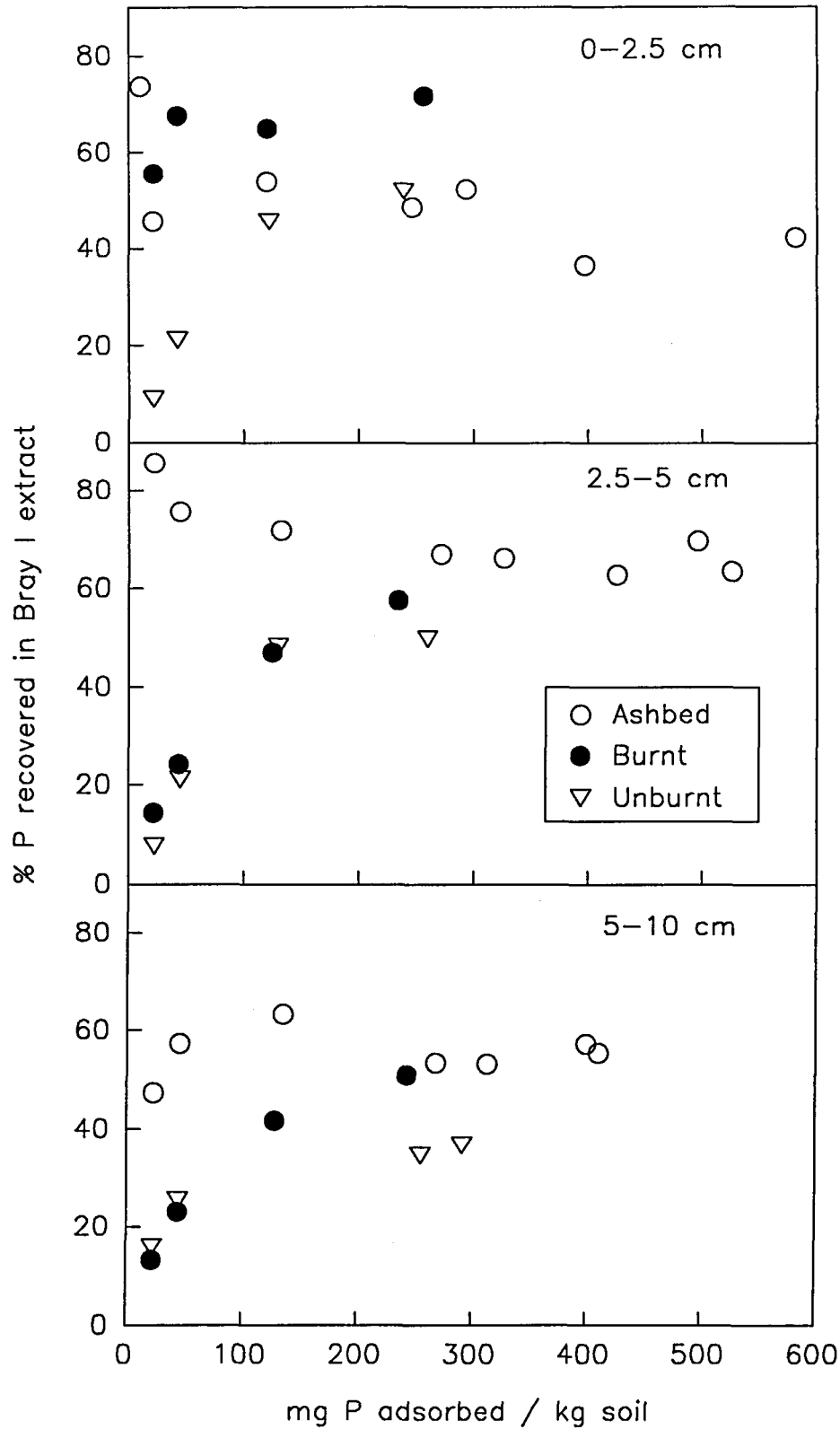


Fig. 6.6. Percentage of added P recovered in Bray I extract from soils subjected to three fire intensities and equilibrated with P as shown in Fig. 6.2.

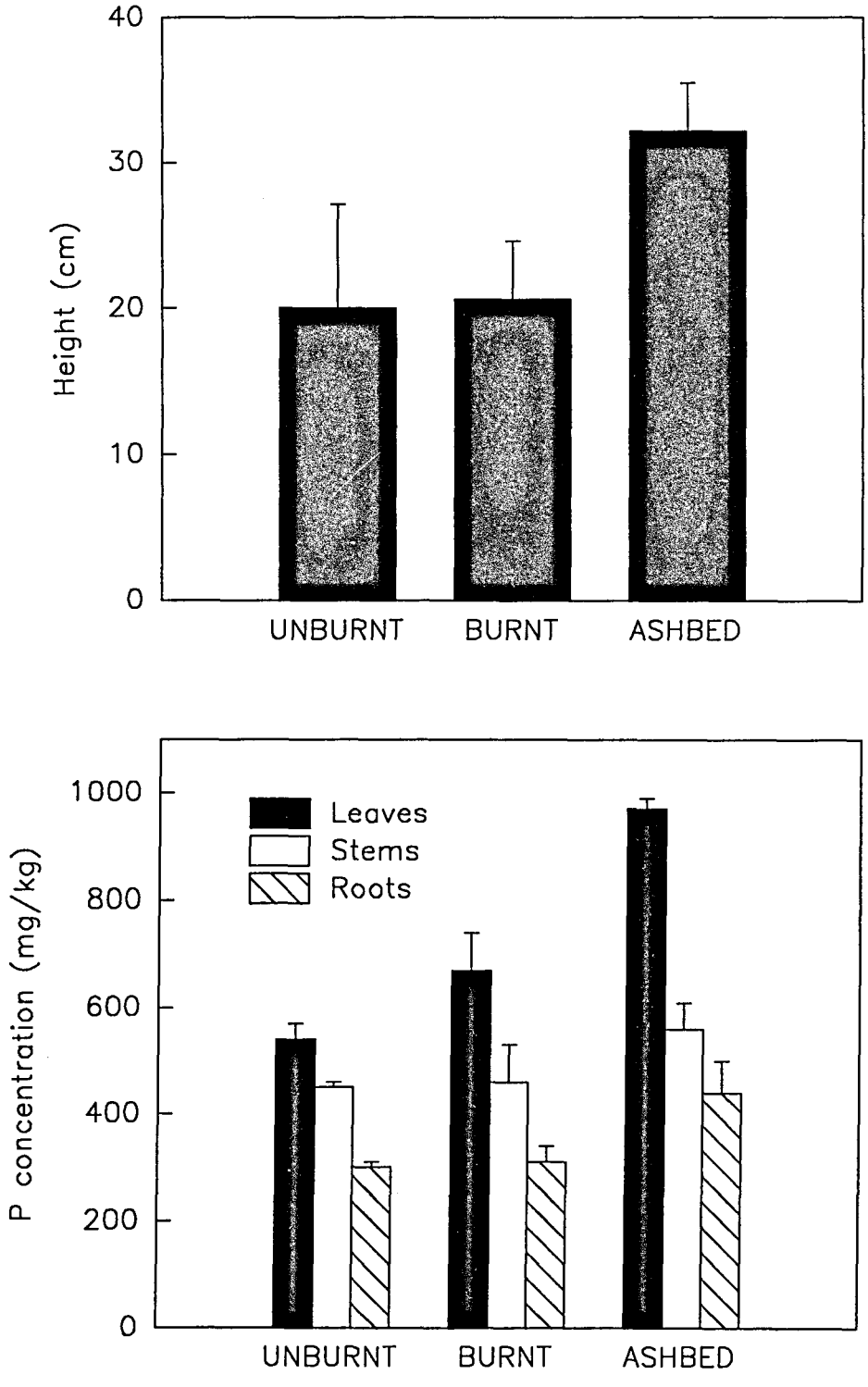


Fig. 6.7. Seedling height and element concentrations in components of *Eucalyptus sieberi* seedlings growing on different soil micorsites 8 months after the fire. Bars refer to standart error.

CONCLUSIONS

The effects of fire on phosphorus forms were primarily evident in the surface soils and depended upon fire intensity. Although fire largely increased P sorption capacity, the sorbed P was less tightly bound to the solid phase. As the increase of P sorption capacity as a result of the fire was much larger than the increase in total P, P losses in leaching are unlikely to occur in areas intensely burnt. Most of the changes induced by the fire increased soil P availability. Nevertheless, the decrease of the distribution coefficient (Kd) observed under certain conditions of burnt intensity, could be viewed as a negative effect on soil P supplying characteristics.

The increase in labile organic P observed after the fire contrasted with a decrease of total organic P and less labile organic P under ashbed conditions. The reason for this labile organic P increase is not known but it could be attributed to changes in organic matter structure as a result of the fire. Further research should be carried out in order to interpret labile organic P changes after the fire.

Harvesting and burning increased the spatial heterogeneity of soil P in the field. The ashbed and burnt microsites represented 19% and 18% respectively, of the surface area of the slash burnt coupe. Understanding of the long term effects

of the fire on soil fertility, and growth of regenerating vegetation is facilitated by stratifying the site based on fire intensity, and sampling randomly within areas of differing intensity.

OVERALL CONCLUSIONS

Mycorrhiza suppression did not show large effects on soil N and P availabilities. Changes were usually small and not always negative for plant growth. While mycorrhiza suppression increased autoclave N (fraction related to potentially mineralizable N), it decreased phosphatase activity. Seedling growth and nutrient uptake were greatly decreased after mycorrhiza suppression. In early stages of growth nutrient shortages caused a delay in single needle development whereas later on, they caused a decrease in needle number. This decrease was overcome after superphosphate fertilization. Mycorrhiza suppression largely increased root length and surface per unit of aboveground biomass. The increase of physical soil exploration in mycorrhizal root systems was one of the main factors responsible for the mycorrhiza increased nutrient uptake. Under low fertility conditions P uptake was extraordinarily increased by mycorrhiza, whereas under high fertility its increase was greatly reduced. Although mycorrhiza increased N and Mg specific uptake rates and increased seedling growth, they did not overcome N and Mg deficiencies. Barber-Cushman nutrient uptake mechanistic approach was able to predict P and Mg uptake only in mycorrhiza suppressed treatments. Under high P availability conditions the model consistently overpredicted P uptake. This fact was attributed to the nonuniformity of thick root surface in taking up P. A better understanding of root nutrient absorption kinetics

in relation to root development will help in simulating nutrient uptake. Likewise, to further comprehend nutrient uptake mechanisms as affected by mycorrhiza, descriptions over time of mycorrhizal short roots density and hyphae growth should be carried out.

Total amounts of nutrient released from the forest floor by leaching represented an important part of the nutritional yearly forest stand requirements. Leaching processes normally occurred at a rather steady rates but, occasionally high peaks of leaching represented a significant part of the total nutrient leached throughout the year. Interestingly, these peaks possibly originate in the forest canopy. The signature of P forms in litter solution is markedly different to the signature of P forms in surface mineral soil. Organic phosphates appeared to be more dominant in mineral soil than in forest floor solution. Organic P dynamism needs to be better understood in order to delimitate its importance in forest nutrition.

Slash burning stimulates P availability shortly after the fire. Ash inputs to the surface soil contain large amounts of inorganic P. Physical and chemical ash-soil interactions simultaneously with the effects of heat can also modify former P equilibrium conditions between soil solid and liquid phase. Increases in P sorption capacity resulting from these interactions can be greater than increases in inorganic P. The increase in soil spatial variability observed after the fire should be

considered especially to further investigate the effects of fire on nutrient availability over the long term.

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