

RHIZOSPHERE pH AND PHOSPHATASE ACTIVITY IN ORTHIC ALLOPHANIC SOIL UNDER *Pinus radiata* SEEDLINGS GROWN WITH BROOM AND RYEGRASS

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ABSTRACT

Under *Pinus radiata* plantations where the tree spacing is wider and most soils are phosphorus (P) deficient, the radiata tree response to P fertilizer is expected to be more influenced by the interaction between the applied P fertilizer, the tree and understorey vegetation. Therefore, a better understanding of the soil P chemistry under radiata pine trees in association with other plants is required. We investigated the effect of broom (*Cytisus scoparius* L.) and ryegrass (*Lolium multiflorum*) grown with radiata seedlings in Orthic Allophanic Soil treated with 0, 50, and 100 $\mu\text{g P g}^{-1}$ soil of TSP on the pH and phosphatase activity in the rhizosphere soils under glasshouse condition. The pHs of radiata rhizosphere soils either grown with broom or grass were lower than those in the bulk soils and the bulk and rhizosphere soils of grass and broom, whether they were grown alone or grown with radiata at the applications of 50 and 100 $\mu\text{g P g}^{-1}$ soil. These results suggest that P application enhanced root induced acidification in a P-deficient Allophanic Soil under radiata. The soils in the rhizosphere of grass and broom, grown in association with radiata, were also acidified by the effect of radiata roots. Acid phosphatase activity in soils under radiata, grass and broom decreased with an increased rate of P application. At all P rates, acid phosphatase activity was higher in the rhizosphere of radiata grown with broom than in the bulk soils. The phosphatase activity in the rhizosphere soil of radiata grown with broom was also higher than that of radiata grown with grass, but it was slightly lower than that in the rhizosphere of broom grown alone. These results suggest that broom may have also contributed to the higher phosphatase activity in the rhizosphere soils than in the bulk soils of broom and radiata when they were grown together.

Keywords: Phosphorus, rhizosphere deficiency, understorey vegetation, *Cytisus scoparius*, *Lolium multiflorum*, acidification

I. INTRODUCTION

Nowadays, radiata pine (*Pinus radiata*) silvicultural regimes have tended to be intensive, with understorey vegetation management, wider initial tree spacing and lower initial stocking rate of *P. radiata*, and routine application of P fertilizer to the forest plantations, where necessary. The wider initial tree spacing creates potential for increased weed growth in forest stands through increased light and greater

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nutrient resources (Gadgil *et al.*, 1988; Payn *et al.*, 1998). Understorey vegetation control operations using herbicides are common in the establishment of *P. radiata* plantations in New Zealand, and these operations usually result in a considerable increase in tree growth rate and survival of the trees (Nambiar and Zed, 1980; Gadgil *et al.*, 1992; Clinton *et al.*, 1994; Richardson *et al.*, 1996; Mason and Milne, 1999; Watt *et al.*, 2003a and 2003b). In contrast, Richardson *et al.* (1996) reported that some species of grass, herbaceous broadleaves and buddleia have significantly increased P concentrations in needles of 3-year-old radiata pine trees (synergism), but broom, gorse, lotus and pampas had no significant effect on needle P concentrations when they were grown in a moderately fertile soil in the field (Richardson *et al.*, 1993). However, the mechanism by which the plant species influenced the P nutrition of the tree was not reported.

The dynamics of P transformations in soils are controlled by chemical and biological processes (Khanna and Ulrich, 1984). Plants may influence the chemical and biological properties within their rhizosphere, and in this way they may enhance the soil P availability and the P uptake of neighbouring plant species. The mechanisms involved in the nutrient uptake from the rhizosphere soil, especially P, are associated with: (i) rhizosphere acidification (Gijsman, 1990a and 1990b; Haynes, 1990); (ii) nutrient depletion by plants; (iii) increase of the activities of soil enzymes (Tarafdar and Jungk, 1987; Gahoonia and Nielsen, 1992); (iv) excretion of root exudates (Hedley *et al.*, 1982a; Gardner *et al.*, 1983a and 1983b; Jones, 1998); and (v) specialised root structure (Gardner *et al.*, 1982).

Gillespie and Pope (1989) have provided an evidence of the role of understorey vegetation in enhancing P uptake by trees. They reported that walnut tree seedlings had greater P uptake when they were grown with alfalfa compared with walnut seedlings grown alone. They considered that the diffusion of solubilised phosphate rock-P to the roots of walnut at the points of root intersection with alfalfa was the mechanism for the greater P uptake by walnut seedlings. This was caused by H⁺ ions diffusing from the roots of alfalfa decreasing pH, thereby, solubilising the rock phosphate. Increase of P availability following a decrease in soil pH (acidification) has been reported by Hedley *et al.* (1982a) in a study with rape fertilized with KH₂PO₄ (Hedley *et al.*, 1982b). They explained that the increased P availability with a decrease in rhizosphere pH was due to an enhancement of P dissolution from acid-soluble forms of soil P_i.

More recently, Sarno *et al.* (2004) reported that the presence of native understorey (*Chromolaena odorata*, *Clidemia hirta*, *Imperata cylindrica*, *Melastoma affine*, and *Mikania micrantha*) in a coffee plantation in a Vertic Dystrudept soil in Sumber Jaya, West Lampung, Indonesia increased available P compared with available P in the absence of these understorey species. In another study, Salam *et al.* (2001) reported that in a coffee plantation, soil phosphatase activities in bulk soils in the plots with native understorey (158 µg *p*-nitrophenol g⁻¹ h⁻¹) were higher than those in the plots without understorey (108 µg *p*-nitrophenol g⁻¹ h⁻¹). In the

rhizosphere soils the phosphatase activity difference between these plots might have been even higher (Trolove *et al.*, 2003) but this was not measured in this study.

In New Zealand *P. radiata* plantations where most soils are P deficient or marginally deficient, P is an important nutrient. Therefore, a better understanding of the soil P chemistry under *P. radiata* trees in association with other plants is required for the development of tree growth models that include a range of the understorey-associations and for the efficient P fertilizer management practices in forest plantations. As with most soils under pine plantations in New Zealand, most soils under pine trees in Indonesia are also acidic and P deficient. Accordingly, the information from the present study would also be useful for managing pine forest in the country. In the present study, we investigated the effect of broom (*Cytisum scoparius* L.) and ryegrass (*Lolium multiflorum*) grown with *P. radiata* seedlings in an Allophanic Soil treated with three rates of TSP on the pH and phosphatase activity in the rhizosphere soils under greenhouse condition.

II. MATERIALS AND METHODS

A. Experimental Design and Treatments

The experiment was arranged in a split-plot design inside a greenhouse. The main-plot treatments were three rates of P fertilizer: 0, 50, and 100 mg P kg⁻¹ soil (equivalent to 0, 50 and 100 kg P ha⁻¹, bulk density = 1 g cm⁻³, depth = 10 cm) applied as TSP (granules ground to pass through 250 μ m; total P = 20.7%) to the soil. Each main-plot was split into four split-plots consisting of four plant combinations (Figure 1): (1) broom alone (compartment a), and (2) radiata with ryegrass (compartment b) (plants in both split-plot treatments grown within the same tray, but (1) and (2) separated by a nylon mesh (43 μ m opening) to stop plant roots from one compartment getting into the other); (3) ryegrass alone (compartment a), and (4) broom with radiata (compartment b) (grown within the same tray, but (3) and (4) separated by a nylon mesh as carried out for plant combinations (1) and (2)).

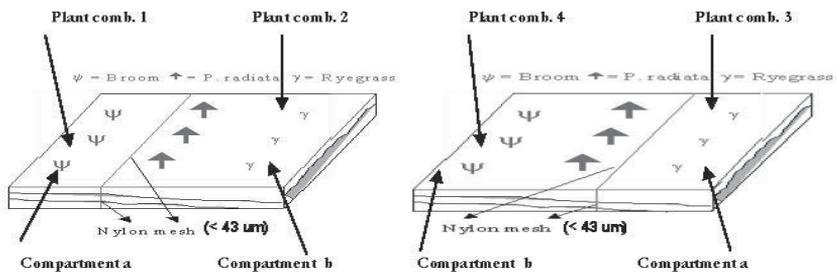


Figure 1. Plant combinations in trays (pots)

The treatments were replicated five times. This study employed the divided pots design using below-ground partitions to get the expected root interferences (Pannel, 1993), meanwhile the above-ground environment for all pots was homogeneous as the order of the plants in every pot was similar. The experiment was designed in such a way to compare the effects of below-ground interaction of radiata + ryegrass and radiata + broom on rhizosphere pH and phosphatase activity in the soil.

A bulk sample of soil collected from Kaweka forest, New Zealand (from a 0-10 cm depth) was used in this trial. This forest area had not received fertilizer for at least 30 years. The soil is classified as Orthic Allophanic Soil (Hewitt, 1998) or Hapludand (Soil Survey Staff, 1999).

The soil was air-dried and passed through a 5 mm sieve to remove debris. A subsample of soil was ground to pass through a 2 mm sieve and analyzed for chemical properties and soil plant-available P. The results are shown in Table 1.

Table 1. Properties of the Allophanic Soil prior to planting in the greenhouse trial

Parameter	Value
pH (H ₂ O)	5.70
Bray-2 P ($\mu\text{g P g}^{-1}$)	3.00
SO ₄ ($\mu\text{g P g}^{-1}$)	29.30
K (cmol _c kg ⁻¹)	0.29
Ca (cmol _c kg ⁻¹)	2.90
Mg (cmol _c kg ⁻¹)	0.58
Na (cmol _c kg ⁻¹)	0.12
CEC (cmol _c kg ⁻¹)	14.00
N (%)	0.27
C (%)	5.60
P ret. (%)	92.00

B. Planting and Maintenance of Trial

Rectangular plastic trays having internal dimensions of 245 mm wide, 307 mm long, and 130 mm deep were used to grow the plants. Each tray (pot) was partitioned into two compartments having 1/3 and 2/3 of tray volumes separated by a nylon mesh having 43 μm openings which was sealed with glue to the edges and bottom of the trays. The nylon mesh was expected to stop entry of roots and

most of the mycorrhizal hyphae from one compartment to the other. After 4.5 kg of air-dried soil (WC = 50%, equal to 3 kg oven-dried basis) was mixed homogeneously with the appropriate amounts of TSP, 1/3 and 2/3 of the soil weight was placed into compartments a and b, respectively in the trays (pots).

Pinus radiata seeds obtained from Forest Research Ltd., Rotorua were germinated according to the following procedure: seeds were soaked overnight on December 10, 2001 in running tap water, planted in moist perlite in a box with a lid (box of 10 cm depth), and kept in a dark place at 22-24°C. All the seeds germinated in 7 days.

A week after germination of the seeds, three radiata seedlings were transplanted into compartment 2 in each tray on 26 December 2001. At the same time, 10 broom seeds (obtained from Forest Research Ltd.) were sown directly (after soaking 5 minutes in hot water at approximately 95°C) into compartment a or compartment b depending on the treatment and a week later the seedlings were thinned to four plants per tray. Four months later (9 April 2002), ryegrass (variety Moata) seeds were sown and seven days after they germinated the seedlings were thinned to 10 plants per tray.

Five months after the planting of the *P. radiata* seedlings, a complete but -P nutrient solution (Middleton and Toxopeus, 1973) was added to all trays. The nutrient solution was applied at a rate of 450 ml per tray four times during a two-week period, except the nitrogen stock solution which was applied only two times. Applications of nutrient solutions were made at three-four day interval. In total, each tray received 54.2 mg N/kg soil and 35 mg K/kg soil (equivalent to 54 kg N/ha and 35 kg K/ha respectively).

The greenhouse was maintained at 28°C maximum and 13°C minimum temperatures. Soil water content was maintained at 80% field capacity by bringing the weight of tray and soil to the required weight by adding distilled water (field capacity of Kaweka forest soil was 87% gravimetric moisture content). The weight of soil in each tray at 80% field capacity was 5.1 kg. Radiata trees, broom and ryegrass were harvested on 24 February 2003 (56 weeks after planting).

C. Soil Sampling

Soil samples were taken after plants had been harvested (56 weeks after planting). The root-soil mass was shaken gently and the fallen soil mass was collected. This represented the bulk soil (bk). The soil adhering to the roots after the bulk soil had fallen away was collected by aggressively shaking the roots (Adamo *et al.*, 1995; Wang and Zabowski, 1998). This soil represented the rhizosphere (rh) soil. Each main-plot treatment (P fertilizer rates) had four bulk soils and six rhizosphere soils, which are referred to as:

1. bulk soil from broom alone (compartment a (B₁-bk))
2. rhizosphere soil from broom alone (compartment a (B₁-rh))

3. bulk soil from radiata grown with grass (compartment b ($R_2 + G_2$ -bk))
4. rhizosphere soil from radiata grown with grass (compartment b (GR_2 -rh))
5. rhizosphere soil from grass grown with radiata (compartment b (RG_2 -rh))
6. bulk soil from broom grown with radiata (compartment b ($B_2 + R_2$ -bk))
7. rhizosphere soil from broom grown with radiata (compartment b (RB_2 -rh))
8. rhizosphere soil from radiata grown with broom (compartment b (BR_2 -rh))
9. bulk soil from grass alone (compartment a (G_1 -bk))
10. rhizosphere soil from grass alone (compartment a (G_1 - rh)).

All soil samples were passed through a 2 mm sieve to remove debris and stored at 4°C for measuring Bray-2 P.

D. Chemical Analysis

Soil pH was determined using a soil:water w/w ratio of 1:2.5. Soil suspensions were stirred and kept overnight at $20 \pm 2^\circ\text{C}$ after which pH was determined using a pH meter equipped with a glass electrode (Blakemore *et al.*, 1987). The organic matter content of the soils (expressed as percentage carbon) was determined by heating the samples in a stream of high purity oxygen in a Leco furnace to produce CO_2 . The CO_2 was measured with an infrared detector (Leco Co., 1996) and the quantity of that gas used to determine the total organic carbon. Cation exchange capacity (CEC) and exchangeable cations were determined by ammonium acetate leaching at pH 7 (Blakemore *et al.*, 1987). The concentrations of K, Ca, Mg, and Na in the leachates were determined by atomic absorption spectrometry (AAS), and the ammonium concentration was determined using an Autoanalyser (Blakemore *et al.*, 1987). Phosphorus retention (an index of P fixation) was determined by measuring the P concentration in soil solution after 5 g soil was shaken with 25 ml solution containing $1000 \mu\text{g P ml}^{-1}$ for 16 h. Bray-2 P was determined by shaking 2.5 g of air dry soil for one minute in 25 ml of a solution containing 0.3 M NH_4F and 0.1 M HCl and measuring the P concentration in the solution by the colorimetric technique of Murphy and Riley (1962 *in* Blakemore *et al.*, 1987).

E. Acid Phosphatase Enzyme Activity

Only acid phosphatase enzyme activity was measured in this study because the soil used in the trial was acidic (pH = 5.7), and acid phosphatase is expected to be the dominant phosphatase enzyme (Eivazi and Tabatabai, 1977). Acid phosphatase activity was measured according to the method described by Tabatabai (1994). Moist soils (1 g oven-dried weight equivalent, < 2 mm) were incubated with a 0.20 ml toluene and a 1 ml *p*-nitrophenyl phosphate solution (*p*-nitrophenyl phosphate, disodium hexahydrate) in a 4 ml modified universal buffer (MUB) solution of pH 6.5 at 37°C for 1 h. The MUB was made by mixing 12.1 g of tris (hydroxymethyl) aminomethane (THAM), 11.6 g of maleic acid, 14.0 g of citric acid, and 6.3 g of boric acid (H_3BO_3) in 488 ml of 1 M sodium hydroxide (NaOH) and diluting the

solution to 1 L with water. The amount of *p*-nitrophenol released after one hour was measured on a UV spectrophotometer at 412 nm and the enzyme activity was expressed as μg *p*-nitrophenol released per g of dry-soil per hour.

F. Statistical Analysis

An analysis of variance (ANOVA) for a split-plot design was performed using SAS (SAS Institute, 2001). The least significant difference (LSD) test at $P < 0.05$, unless otherwise stated, was used to separate the means when the ANOVA results indicated that there were significant treatment effects (Steel *et al.*, 1997).

III. RESULTS AND DISCUSSION

A. Soil pH

The rates of P fertilizer application had no effect on soil pH, but significant differences ($p < 0.0001$) in soil pH were observed between plant combinations. The interactive effect of the P rate and plant combinations on soil pH was also significant ($p < 0.0001$).

Table 2. Effect of P fertilizer rate and plant combinations on pH in bulk and rhizosphere soils

Sampling position	P rate ($\mu\text{g P.g}^{-1}$ soil)		
	0	50	100
B ₁ -bk	5.76 a*	5.77 ab	5.67 bc
B ₁ -rh	5.74 a	5.70 bc	5.57 c
R ₂ +G ₂ -bk	5.59 bc	5.79 ab	5.77 a
GR ₂ -rh	5.61 bc	5.17 e	5.30 d
RG ₂ -rh	5.68 ab	5.57 d	5.61 bc
R ₂ +B ₂ -bk	5.73 ab	5.88 a	5.80 a
RB ₂ -rh	5.72 ab	5.59 cd	5.60 c
BR ₂ -rh	5.50 c	5.17 e	5.29 d
G ₁ -bk	5.69 ab	5.77 ab	5.80 a
G ₁ -rh	5.66 ab	5.75 b	5.73 ab

Note: *Numbers under each P rate followed by the same letters are not different at $P < 0.05$

The effect of interaction between the P rate and plant combinations on soil pH (Table 2) is explained below. The pHs of *P. radiata* rhizosphere soils either grown with broom or grass were significantly ($p < 0.05$) lower than those in the bulk soils and the bulk and rhizosphere soils of grass and broom whether they were grown alone or grown with *P. radiata* for the additions of 50 and 100 $\mu\text{g P g}^{-1}$ soil. However, such differences did not occur for the control treatment (0 $\mu\text{g P g}^{-1}$ soil). The lower pH under *P. radiata* is due to predominantly NH_4^+ rather than NO_3^- uptake by *P. radiata* plants (Olykan and Adams, 1995), which results in excess cation over anion uptake causing H^+ release by roots to maintain electroneutrality within plant cells (Gijman, 1990a and 1990b; Haynes, 1990; Hinsinger and Gilkes, 1995). The acidification in the *P. radiata* rhizosphere could also be due to oxalate anion released by *P. radiata* roots (Malajczuk and Cromack, 1982) with an associated H^+ release. The reason for *P. radiata* rhizosphere soil in treatments receiving 0 $\mu\text{g P g}^{-1}$ soil not having any significant reduction in pH is that the root growth (Rivaie, 2005) and mycorrhizal hyphae development at this P rate were too low to release sufficient H^+ by root and hyphae to cause significant pH reduction in the rhizosphere.

No significant pH difference was observed between grass or broom rhizosphere soils and the associated bulk soils in the absence of *P. radiata* (G_1 -rh vs G_1 -bk and B_1 -rh vs B_1 -bk), suggesting that these two plants do not have the same mechanism of ion uptake or organic anion excretion as *P. radiata*. In contrast to *P. radiata*, grass has been shown to take up predominantly NO_3^- rather than NH_4^+ (Gahoonia *et al.*, 1992). Broom, being a legume, was expected to export H^+ into the rhizosphere when actively fixing N_2 , thus causing the acidification of rhizosphere soils (Nyatsanga and Pierre, 1973; Bolan *et al.*, 1991). But such rhizosphere acidification under broom was not observed in this study.

Davis (1995) reported that the soil pH increased slightly under grass and decreased slightly under pines one year after growth of these plants in a glasshouse trial. Scott (2002) also reported that in a soil which was low in total P and carbon content, soil pH decreased significantly under pines compared with soil pH under grass and the legume lucerne in a greenhouse trial. The pH under lucerne was lower than that under grass.

At the highest P rate (100 $\mu\text{g P g}^{-1}$ soil) the pH in rhizosphere and bulk soils of broom grown alone (B_1 -rh, B_1 -bk) were significantly lower than that in the rhizosphere and bulk soils of grass grown alone (G_1 -rh, G_1 -bk). This may be because at high P rates the NO_3^- uptake by grass would have been higher resulting in higher alkalinity production in the soil. At this P rate, broom would have fixed higher amounts of atmosphere N, producing higher acidity in the soil.

For the 50 and 100 $\mu\text{g P g}^{-1}$ soil treatments, the soil pH was significantly lower in the grass rhizosphere soil compared to the bulk soil when grass was in association with *P. radiata* (RG_2 -rh vs $R_2 + G_2$ -bk) and the broom rhizosphere soil compared to the bulk soil when broom was in association with *P. radiata* (RB_2 -rh vs $R_2 + B_2$ -bk). This acidification is probably due to the effect of *P. radiata*, because in the absence

of *P. radiata* (compartment a) there was no significant difference in pH between rhizosphere and bulk soils for both grass and broom. When broom and grass were with *P. radiata* (compartment b) there was an intermingling of the roots of these plants with those of *P. radiata* and, hence, the rhizosphere of *P. radiata* influenced those of the two associated plants. Scott (2002) also observed that the soil pH under ryegrass or broom in association with *P. radiata* was significantly lower than that when they were grown alone in a greenhouse trial.

B. Phosphatase Activity

The main effects of P fertilizer rates ($p=0.0012$) and plant combinations ($p<0.0001$) on acid phosphatase activity in soils were significant. But the P fertilizer rate x plant combination interaction was not significant. The acid phosphatase activity decreased with an increased rate of P application (Figure 2). This is probably because the acid phosphatase enzyme is an adaptive enzyme whose activity increases when there is a need for it to function, that is, when the soils are P deficient (Haußling and Marschner, 1989). These results are consistent with those of Pank and Kolenko (1986) who found that addition of 20 μmol orthophosphate to 1 g soil sampled from a 34-yr-old Douglas-fir forest reduced phosphatase activity in the soil. Fox and Comerford (1992) also reported that the application of KH_2PO_4 , at the rate of 6.7 and 13.3 mg P kg^{-1} soil as KH_2PO_4 , decreased phosphatase activity in rhizosphere soils collected from slash pine plantations.

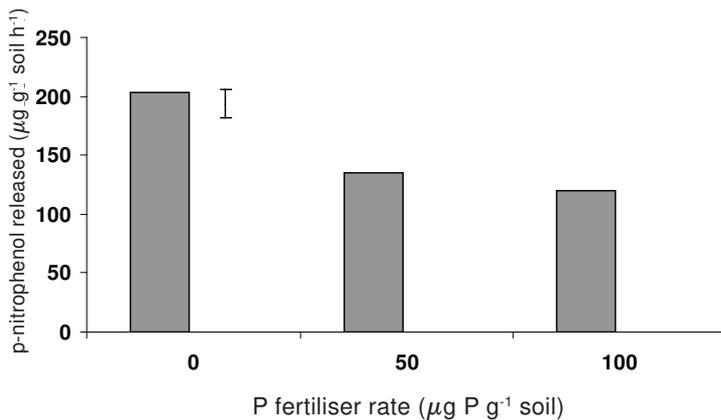


Figure 2. Effect of P fertilizer rates on acid phosphatase activity in soil (mean of plant combinations, and rhizosphere and bulk soils)

Table 3. Effect of plant combinations on acid phosphatase enzyme activity in rhizosphere and bulk soils under different plant combinations

Plant combination	<i>p</i> -nitrophenol released ($\mu\text{g g}^{-1}$ soil h^{-1})
B ₁ -bk	183.0 a*
B ₁ -rh	194.4 a
R ₂ +G ₂ -bk	121.2 b
GR ₂ -rh	139.5 b
RG ₂ -rh	143.7 b
R ₂ +B ₂ -bk	138.2 b
RB ₂ -rh	173.5 a
BR ₂ -rh	178.9 a
G ₁ -bk	129.5 b
G ₁ -rh	135.6 b

Note: *Numbers followed by the same letters are not different at $P < 0.05$

In general, the acid phosphatase activity in all rhizosphere soils was consistently higher than that in the bulk soils, but the differences were significant ($p < 0.05$) only between the bulk soils and rhizosphere soils of *P. radiata* and broom when they were in association with each other (BR₂-rh and RB₂-rh vs R₂+B₂-bk) (Table 3). The higher activity of this enzyme in the rhizosphere is due to the influence of plant roots and higher soil microbial activity in the rhizosphere than in the bulk soil (Dinkelaker and Marschner, 1992). Others have also reported that the soil in the rhizosphere of plants had higher phosphatase activity than that in the bulk soils (Hedley *et al.*, 1982a; Tarafdar and Junk, 1987; Haußling and Marschner, 1989; Jungk *et al.*, 1993; Asmar *et al.*, 1995; Chen *et al.*, 2002; Scott, 2002). The differences in phosphatase activity between rhizosphere soils and bulk soils were lower for broom and grass when they were grown without *P. radiata* (compartment a) than when they were grown with *P. radiata* (compartment b). The higher differences in phosphatase activity between broom and grass rhizosphere and the corresponding bulk soils in the presence of *P. radiata* are probably due to the higher phosphatase enzyme production by the ectomycorrhizal *P. radiata* roots (Haußling and Marschner, 1989; Antibus *et al.*, 1992; Scott, 2002), which were intermingled with broom or grass roots in compartment b. Due to this intermingling of roots the enzyme activity did not differ between the rhizosphere soils of *P. radiata* and the associated plants (GR₂-rh vs RG₂-rh and BR₂-rh vs RB₂-rh).

The rhizosphere and bulk soils under broom in compartment a (B_1 -bk and B_1 -rh) and the rhizosphere soil under *P. radiata* in association with broom in compartment b (BR_2 -rh and RB_2 -rh) had a significantly higher phosphatase enzyme activity than the soils under *P. radiata* in association with grass in compartment b (GR_2 -rh and RG_2 -rh) and under grass grown alone in compartment a (G_1 -bk and G_1 -rh). In addition, the phosphatase activity in the rhizosphere soil under broom grown alone in compartment a (B_1 -rh) was slightly higher than that in the rhizosphere soil under *P. radiata* in association with broom in compartment b (BR_2 -rh and RB_2 -rh). These suggest that probably the presence of broom under *P. radiata* was also the cause for the increase in phosphatase activity in the rhizosphere soil of *P. radiata*. This is probably because higher N availability under the leguminous broom plant as a result of N_2 fixation by this legume may have enhanced soil phosphatase activity (Giardina *et al.*, 1995; Zou *et al.*, 1995). Also, N addition through N_2 fixation by the broom may have increased the plants demand for P. Hence phosphatase activity increased to supply more P to the plants. Olander and Vitoisek (2000) reported that in a N limited site in a chronosequence of soil in Hawaii, the addition of N stimulated phosphatase activity. They suggested that the stimulation of phosphatase activity by N addition could be through the direct use of N as building material for the production of N rich enzymes and indirectly through the increased productivity of the roots.

The high phosphatase activity in the rhizosphere soil when *P. radiata* grown with broom could also be due to the interaction between *P. radiata* and broom rather than the effect of broom might have produced a high phosphatase enzyme by the ectomycorrhizal *P. radiata* roots (Haußling and Marschner, 1989; Antibus *et al.*, 1992; Scott, 2002) along with the higher N availability with the presence of broom which may also have enhanced soil phosphatase activity (Giardina *et al.*, 1995; Zou *et al.*, 1995). Soil phosphatase activity under red alder + mixed conifer stand was higher than that in the pure mixed conifer stand and red alder (Zou *et al.*, 1995). However, in the present study it is difficult to assess whether the cause is interaction or not because there was no combination of *P. radiata* grown alone.

IV. CONCLUSION

The pHs of *P. radiata* rhizosphere soils either grown with broom or grass were lower than those in the bulk soils and the bulk and rhizosphere soils of grass and broom whether they were grown alone or grown with *P. radiata* at the fertilizer applications of 50 and 100 $\mu\text{g P g}^{-1}$ soil. This acidification could be due to excess cation over anion uptake (NH_4^+ rather than NO_3^-) causing H^+ release by roots to maintain electroneutrality within plant and oxalate anion released by *P. radiata* roots with an associated H^+ release in the rhizosphere. Phosphate fertilizer application enhanced root induced acidification in a P deficient Orthic Allophanic Soil under *P. radiata*. The soils in the rhizosphere of grass and broom, grown in association with *P. radiata*, were also acidified by the effect of *P. radiata* roots.

Acid phosphatase activity in soils under *P. radiata*, grass and broom decreased with an increased rate of P application. At all P rates, acid phosphatase activity was higher in the rhizosphere of *P. radiata* grown with broom than in the bulk soils. The phosphatase activity in the rhizosphere soil of *P. radiata* grown with broom was also higher than that of *P. radiata* grown with grass, but it was slightly lower than that in the rhizosphere of broom grown alone. These results suggest that broom may have also contributed to the higher phosphatase activity in the rhizosphere soils than in the bulk soils of broom and *P. radiata* when they were grown together.

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