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Availability of organic and inorganic forms of phosphorus to lupins (*Lupinus* spp.)

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Abstract

Inositol phosphate is at least equal to KH_2PO_4 as a source of P for the growth of lupins in sand but a much poorer source in soil. RNA and glycerophosphate were excellent sources of P for lupin growth in a P-fixing soil. Soil and root phosphatase activity were not altered by amendment of soils with either inorganic- or organic-P. The difference in availability of differing P-sources is related to their solubility in soils rather than susceptibility to phosphatases.

Introduction

Soil organic-P contributes a large proportion of total soil-P and is comprised largely of phosphomonoesters (especially penta- and hexa-inositol phosphates) and diesters (Dalal, 1977). Nutrient cycling is responsible for the re-distribution of primary mineral P into organic forms over time scales of $10^4 - 10^6$ years and in old and strongly weathered (e.g. Australian) soils organic-P may comprise more than half of total soil phosphorus. The contribution of organic-P to plant nutrition is difficult to assess but studies in soil (Stewart and Tiessen, 1987) and forest (Adams and Byrne, 1989) chronosequences suggest that this contribution increases with geological and stand age respectively. However, in contrast to detailed studies of inorganic-P availability, the formation and depletion of organic-P in soils have received scant attention, particularly since the precise nature of this fraction is as yet undefined.

Phosphatase enzymes in the soil, whether of microbial origin or associated with plant roots, are capable of catalyzing the mineralization of P from organic compounds similar to those present in soils (Bieleski, 1973; Speir and Ross, 1978; Theodorou, 1971). Enzyme activities are usually increased when the concentration of soluble inorganic-P is low, as in highly weathered soils, while rhizosphere activity in both agricultural (Helal and Sauerbeck, 1984) and forest (Häussling and Marschner, 1989) species has been shown to be many times greater than that in the bulk soil. This may be because the rhizosphere becomes depleted in organic-P (and enriched in inorganic-P) relative to the bulk soil (Tarafdar and Jungk, 1987).

Species from families such as the Proteaceae, Casuarinaceae, Fabaceae and Mimosaceae produce cluster roots and species of such propensity are generally known for their ability to grow on low-P soils (Bowen, 1981; Grierson and Attiwill, 1989; Groves and Keraitis, 1976; Lamont, 1984). Gardner et al. (1982; 1983) have proposed that the cluster roots of white lupin (*Lupinus albus* L.) possess an ability to modify the soil chemically in such a way as to increase the solubility of

inorganic-P forms, through production of acidifying and chelating agents. Theoretically, root excretions should also increase the solubility of organic-P compounds such as phytins and thereby the pool of P available for uptake.

In order to examine further some of the above problems this study examined the availability of organic forms of P to two species of lupins, one, *Lupinus albus*, normally developing prolific sets of cluster roots when growing in soil culture at relatively low levels of P, the other *L. angustifolius* not producing cluster roots under any circumstances.

Materials and methods

All experiments utilized plants of white lupin (*Lupinus albus* L.) and narrow leaf lupin (*L. angustifolius* L.) grown from seed in 16 cm diameter pots in a naturally-lit, temperature-controlled (max 25°C, min 10°C) glasshouse in Perth, Western Australia during the months May–November. Seeds were inoculated with *Rhizobium* at sowing and pots thinned to three plants/pot, once seedlings had emerged. Generally, each treatment comprised five replicates (total of 15 plants) and all results were expressed on a pot basis and data analyzed statistically by analysis of variance in which treatment means were compared at the 5% level by the Student Newman-Kuels (SNK) test.

Experiment 1

Plants were grown in washed, organic-matter-free quartz sand. The seedlings were fed regularly with a balanced, −N, culture solution incorporating different forms of P and pots were leached with distilled water twice each week to prevent the build-up of salts. Nutrient solution was applied initially at 1/10 strength increasing progressively to full strength during the first three weeks of plant growth. The specific treatments comprised the base culture solution with 0.5 m*M* of P in the following forms: KH_2PO_4/K_2HPO_4 (*ortho-P*, BDH), Inositol phosphate (*inositol-P*, Phytic acid, Sigma), Pyrophosphate (*pyro-P*, tetrasodium salt, BDH), Choline phosphate (*choline-P*, Sigma). Plants were harvested after eight weeks. Shoots, nodules and roots were separated, dried (70°C) and weighed. Plant parts were then ground and a subsample digested in H_2SO_4/H_2O_2 prior to automated analysis for N and P (Technicon, 1977).

Experiment 2

A low-P subsoil (red earth, Uc 5.22 Northcote et al., 1975) was collected from a virgin jarrah (*Eucalyptus marginata*) forest at Jarrahdale, Western Australia. General properties of soil of this type have been described previously (Bolan et al., 1984). Three kg of air-dried and sieved (four mm) soil was placed in each plastic-bag-lined pot. Basal nutrients (−N, −P) were applied (at rates described by Bolan et al., 1983) in solution to the surface of each pot and phosphorus then applied at four rates (10, 25, 62.5 and 100 μg P g^{-1} dry weight soil). Phosphorus sources were: KH_2PO_4 (*ortho-P*, BDH), β-Glycerophosphate (*glycero-P*, Calbiochem, disodium salt), Ribonucleic acid (*RNA*, Sigma), Inositol hexaphosphate (*inositol-P*). The ortho-P and glycero-P were applied in solution to the surface of each pot, whereas the RNA and inositol-P were applied in powder form.

All added nutrients were distributed uniformly by shaking the dry pot-contents in a plastic bottle for one minute. The pots were watered gravimetrically to field capacity (12% w/w) each day and plants were harvested after nine weeks as described for Experiment 1.

Experiment 3

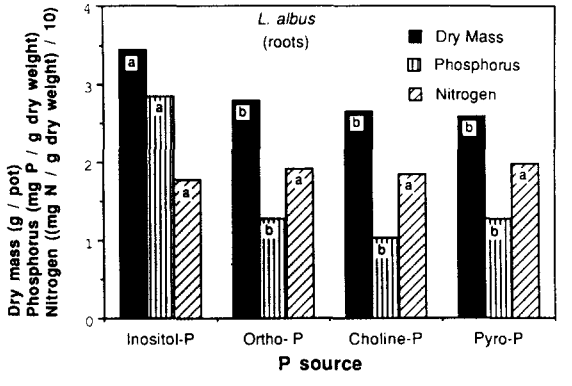
Plants were grown in the same soil as, and under similar conditions to, Experiment 2; except that P was supplied as three forms (ortho-P, RNA and glycero-P) at one rate only (40 μg P g^{-1} dry weight soil). After eight weeks, the soil was sampled by taking a 2 cm diameter core from the centre of each pot. After careful sieving to remove root fragments, soil phosphatase activity was assayed by the method of Tabatabai and Bremner (1969). At the same time the remainder of the root system was recovered by careful washing and root tip phosphatase activity as-

sayed using the method initially described by Woolhouse (1969).

Results

Experiment 1

The dry matter yield of whole plants of both lupin species in sand culture was greatest when P was supplied as inositol phosphate (see separate data for shoots in Fig. 1 and roots in Fig. 2). Root clusters were generally scarce on *L. albus* plants in all treatments, so no distinction is made between cluster roots and 'normal' roots in the data presented in the Figures. Root mass was not



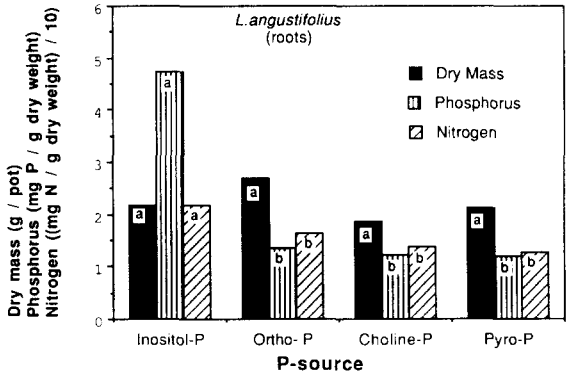
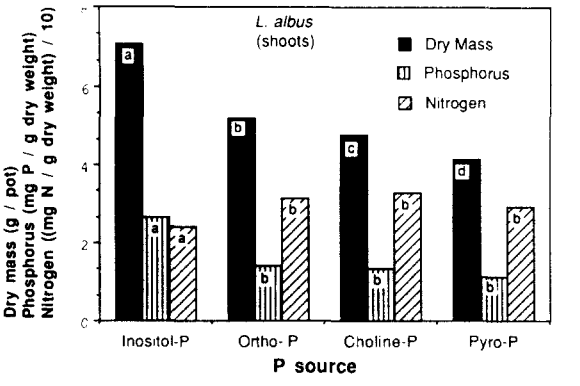


Fig. 1. Dry mass and N and P concentrations of roots (excluding nodules) of *L. albus* and *L. angustifolius* grown in sand culture with varying P-sources. Treatment means for mass or N or P concentrations marked with the same letter are not different ($p < 0.05$, SNK test).



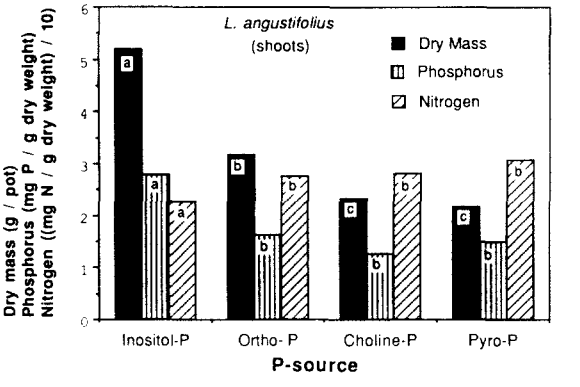


Fig. 2. Dry mass and N and P concentrations of shoots of *L. albus* and *L. angustifolius* grown in sand culture with varying P-sources. As for Fig. 1.

generally affected by P-source (Fig. 2) to as great an extent as were both shoot (Fig. 1) and nodule mass (results not presented) both of which were greater in plants supplied with inositol-P than with other sources. Differences in the shoot mass among P-sources decreased in the order inositol-P > ortho-P > choline-P > pyro-P.

Phosphorus concentration of both roots and shoots of both species were greater in plants grown with inositol-P than with other P-sources (Figs. 1, 2). N concentrations were less in roots and shoots of *L. albus* and shoots of *L. angustifolius* and greater in roots of *L. albus*, when grown with inositol-P than with other sources (Figs. 1, 2). Phosphorus contents of inositol-P grown *L. angustifolius* (24.8 mg P/pot) and *L.*

albus (28.6 mg P/pot) were between 2 and 3 times those of plants grown with other P-sources (5.3–8.8 and 7.9–10.8 mg P/pot respectively). In contrast, the nitrogen content (and hence nitrogen fixation) were more similar among treatments (*L. angustifolius* 91–166 mg N/pot; *L. albus* 173–232 mg N/pot); the highest values being those for inositol-P treatments.

Experiment 2

L. albus produced cluster roots in all treatments of this soil-based study; fewer in number and mass in pots supplied with ortho-P at 100 μg P g^{-1} dry weight soil, than in other pots (results not shown). Increasing addition of P in any form at rates up to 62.5 μg P g^{-1} soil increased the growth (dry matter accumulation) of both lupin species (Fig. 3), suggesting P limitation below this level of availability. In complete contrast to the sand culture results of Experiment 1, inositol-P was found to be by far the poorest form of P supplementation to the soil for both species, although the effect was somewhat more pronounced with *L. angustifolius* than *L. albus*. Glycero-P was a slightly better P-source than ortho-P or RNA at lower rates of addition. At 100 μg P g^{-1} soil, RNA appeared toxic to both species and glycero-P became a less effective source of P than ortho-P. In general, the concentration of P in plant tissues of both species

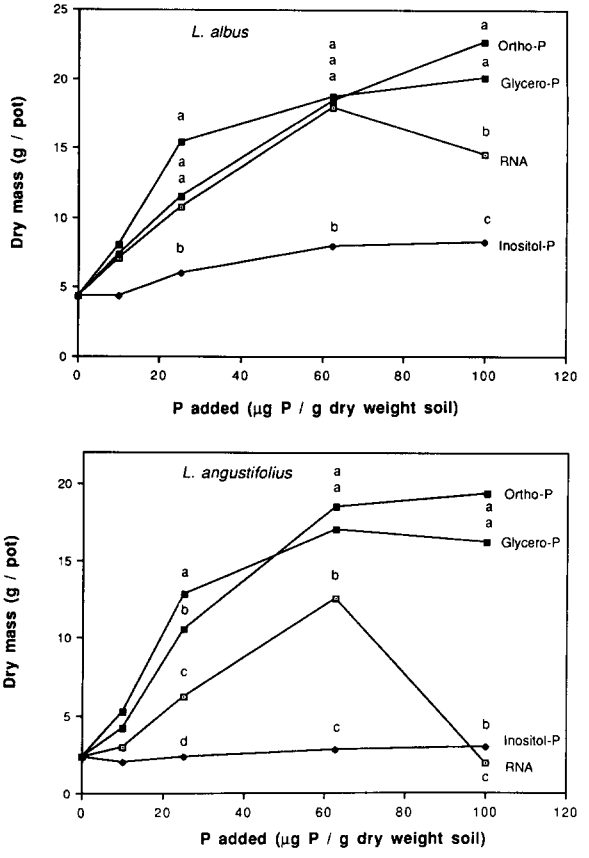


Fig. 3. Dry mass of *L. albus* and *L. angustifolius* grown in a P-deficient subsoil with varying sources and rates of applied P. Treatment values marked by the same letter at each of the three highest rates of P application are not different ($p < 0.05$, SNK test).

Table 1. P and N content (mg/pot) of lupins grown with different sources of P added to a red earth (Uc 5.22), P-deficient subsoil. At each rate of application, means followed by the same letter are not different

Source	Rate of application (μg P g^{-1} soil)									
	L. albus					*L. angustifolius*				
	0	10	25	62.5	100	0	10	25	62.5	100
	Phosphorus									
Ortho-P	2.6	3.7^{b}	9.2^{b}	21.7^{a}	30.4^{a}	1.4	3.5^{a}	9.9^{b}	20.5^{a}	23.9^{a}
Glycero-P		4.3^{b}	10.9^{ab}	22.3^{a}	34.1^{a}		3.3^{a}	11.7^{a}	19.7^{a}	26.3^{a}
Inositol-P		2.6^{c}	3.8^{d}	6.6^{b}	15.3^{c}		1.2^{c}	1.5^{d}	2.1^{c}	2.5^{b}
RNA		4.4^{b}	7.1^{c}	17.9^{a}	23.3^{b}		2.1^{b}	4.7^{c}	12.0^{b}	2.5^{b}
Seed[z]	2.8					1.0				
	Nitrogen									
Ortho-P	100	121^{b}	263^{b}	482^{a}	595^{a}	39	71^{a}	188^{c}	368^{a}	607^{a}
Glycero-P		139^{ab}	281^{b}	453^{a}	628^{a}		71^{a}	220^{b}	341^{a}	386^{b}
Inositol-P		78^{c}	118^{d}	150^{c}	229^{c}		32^{c}	39^{e}	46^{c}	53^{c}
RNA		146^{a}	199^{c}	385^{b}	368^{b}		48^{b}	105^{d}	287^{b}	50^{c}
Seed	46					31				

[z] For three seeds.

increased with increasing rates of P addition, so plant total P-content generally reflected trends in plant mass (cf. Figs. 1 and 2, Table 1). Corresponding values for plant total N-content per pot were more variable (Table 1) and the data suggests that N-fixation by *L. angustifolius* was greatly reduced when glycero-P was applied at 100 μg P g^{-1} soil whereas N-fixation by *L. albus* remained proportional to plant growth at this level of P-amendment.

Experiment 3

Phosphatase activity was greater per unit fresh weight of cluster root tips of *L. albus* than in comparable mass of non-cluster root tips (Table 2). Addition of ortho-P to the soil reduced root tip phosphatase in both species whereas addition of RNA and inositol-P increased activity in cluster roots of *L. albus* and the roots of *L. angustifolius*. Soil phosphatase activity was not affected by the addition of inorganic-P or organic-P but was much greater in soil sampled from pots containing plants than in soil from pots without plants, especially in the case of *L. albus* (Table 3).

Table 2. Root tip phosphatase activity (mg nitrophenol produced h^{-1} g^{-1} fresh weight)

Root tips		40 μg P g^{-1} soil added as		
	No added P	Ortho-P	Inositol-P	RNA
L. angustifolius	11.0	7.2	12.8	23.1
	(1.0)	(1.0)	(1.1)	(3.3)
L. albus	11.9	5.9	8.7	9.6
	(2.0)	(0.5)	(0.7)	(0.9)
L albus	15.7	10.7	34.7	16.6
(cluster roots)	(2.7)	(0.5)	(6.1)	(2.6)

Table 3. Soil phosphatase activity (μg nitrophenol produced h^{-1} g^{-1} dry weight soil)

Assay		40 μg P g^{-1} soil added as		
	No added P	Ortho-P	Inositol-P	RNA
L. angustifolius	288	361	351	343
	(39)	(30)	(51)	(45)
L. albus	507	471	477	496
	(31)	(52)	(51)	(34)
No plants	256	236	277	236
	(25)	(30)	(32)	(19)

Discussion

The two major determinants of the availability of organic-P to plants are generally suggested to be (a) solubility of the particular source and (b) its susceptibility to plant and soil phosphatases (e.g. Sanyal and De Datta, 1991). In this study, it was shown that addition of either inorganic- or organic-P to a strongly-fixing red earth soil, did not alter phosphatase activity of the soil but respectively inhibited or promoted plant-root phosphatase. Soil phosphatase may be of either plant or microbial origin and the very low concentration of organic matter in the poor subsoil used in this experiment was likely to have specifically restricted microbial growth and phosphatase activity. In sand culture, in which microbial activity would be expected to be much reduced, both lupin species proved capable of utilizing pyro-P, choline-P and inositol-P, presumably by enzymic hydrolysis of substrates (Furlani et al., 1987; Tarafdar and Claasen, 1988).

There have been a number of published studies of the availability of different P sources in soil. For example, Tarafdar and Claasen (1988) demonstrated that glycerophosphate, lecithin and phytin (inositol) added to soil were at least equal to inorganic P sources in their availability to clover, barley, oats and wheat whereas Furlani et al. (1987) noted that ethyl ammonium, glyceryl and phenyl phosphates were preferable to inorganic-P sources for the growth of sorghum genotypes. In both of these studies, as well as the present, glycero-P was generally a preferable source of P to ortho-P when plants were grown in soil. DNA was a better source of P for the growth of barley in a near neutral soil than was KH_2PO_4 (McKercher and Tollefson, 1978) i.e. similar to the results shown here for RNA. Tarafdar and Claasen (1988) concluded that when organic sources of P were added to soil, the limiting factor on plant utilization was the availability of phosphatase-hydrolyzable P sources. Overall, experimental results support the general contention that the soil solubility of each class of compound determines its availability. For example, in alkaline or neutral soils, glycero-P was sorbed at a much slower rate than KH_2PO_4 or inositol hexaphosphate (McKercher and Anderson, 1989). Simi-

larly, some phosphomonoesters are more mobile in soils than orthophosphate ions (Rolston et al., 1975) and inositol phosphates may accumulate in the B-horizon (relative to the A-horizon) of some soils (Martin, 1970).

Inositol phosphates are strongly competitive with orthophosphate for sorption sites in neutral and slightly alkaline soils (McKercher and Anderson, 1989) and the assumption of similar behaviour in acidic soils may explain the present results (Expt. 2) of poor availability of inositol-P to soil grown lupins, especially *L. angustifolius*, a species lacking cluster roots. In contrast, P-sorption in sand culture is likely to be negligible, a supposition consistent with the finding (Expt. 1) that inositol-P was the *best* P-source for lupins.

There was little difference between the two lupin species in respect of either phosphatase activity in their root tips or that exhibited in soil surrounding the root system yet soil activities were appreciably higher when plants were present than in similarly treated unplanted soil, a result expected from general observations in the literature (Sanyal and De Datta, 1991). Phytase enzymes have been widely examined (e.g. Theodoru, 1971; Bartlett and Lewis, 1973; MacDonald and Lewis, 1978) as phytates make up a large proportion of soil organic-P. Both lupin species grew well with a variety of P-sources in sand and soil culture and substrate solubility appeared to be dominant in determining P-availability to the plants. There was some evidence (viz. the better growth of *L. albus* than *L. angustifolius* with inositol-P in soil culture) that the known capability of cluster roots for solubilization of inorganic P-sources (through the production of chelating agents for Fe and Al), also applies to organic P-sources. This aspect would be especially interesting to follow up in future comparisons of the species or between other closely related taxa (e.g. *Acacia* spp., *Daviesia* spp., *Kennedia* spp.) in which cluster roots are present in certain species but not in others.

Acknowledgements

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References

Bartlett E M and Lewis D H 1973 Surface phosphatase activity of mycorrhizal roots of beech. Soil Biol. Biochem. 5, 249–257.

Bieleski R L 1973 Phosphate pools, phosphate transport and phosphate availability. Annu. Rev. Plant Physiol. 24, 225–278.

Blanchar R W and Hossner L R 1969 Hydrolysis and sorption reactions of orthophosphate, pyrophosphate, tripolyphosphate and trimetaphosphate anions added to an Elliot soil. Soil Sci. Soc. Am. Proc. 33, 141–144.

Bolan N S, Robson A D and Barrow N J 1983 Plant and soil factors including mycorrhizal infection causing sigmoidal response of plants to applied phosphorus. Plant and Soil 73, 187–201.

Bolan N S, Robson A D, Barrow N J and Aylmore L A G 1984 Specific activity of phosphorus in mycorrhizal and non-mycorrhizal plants in relation to the availability of phosphorus to plants. Soil Biol. Biochem. 16, 299–304.

Bowen G D 1981 Coping with low nutrients. *In* The Biology of Australian Plants. Eds. J S Pate and A J McComb. pp 33–64. University of Western Australia Press, Nedlands.

Dalal R C 1977 Soil organic phosphorus. Adv. Agron. 29, 85–117.

Furlani A M C, Clark R B, Maranville J W and Ross W M 1987 Organic and inorganic sources of phosphorus on growth and phosphorus uptake in sorghum genotypes. J. Plant Nutr. 10, 163–186.

Gardner W K, Barber D A and Parberry D G 1983 The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil-root interface is enhanced. Plant and Soil 70, 107–124.

Gardner W K, Barber D A and Parberry D G 1982 The acquisition of phosphorus by *Lupinus albus* L. I. Some characteristics of the soil-root interface. Plant and Soil 68, 19–32.

Grierson P F and Attiwill P M 1989 Chemical characteristics of the proteoid root mat of *Banksia integrifolia* L. Aust. J. Bot. 37, 137–143.

Groves R H and Keraitis K 1976 Survival and growth of seedlings of three sclerophyll species at high levels of phosphorus and nitrogen. Aust. J. Bot. 24, 681–690.

Häussling M and Marschner H 1989 Organic and inorganic soil phosphates and acid phosphatase activity in the rhizosphere of 80-year-old Norway spruce. Biol. Fertil. Soils. 8, 128–133.

Heinrich P A, Mulligan D R and Patrick J W 1988 The effect of ectomycorrhizas on the phosphorus and dry weight acquisition of Eucalyptus seedlings. Plant and Soil 109, 147–149.

MacDonald R M and Lewis M 1978 The occurrence of some acid phosphatases and dehydrogenases in the vesicular arbuscular mycorrhizal fungus *Glomus mosseae*. New Phytol. 80, 135–141.

Martin J K 1970 Distribution of organic P in a sequence of soils under tussock grassland. N.Z.J. Agric. Sci. 13, 522–533.

McKercher R B and Anderson G 1989 Organic phosphate sorption by neutral and basic soils. Comm. Soil Sci. Pl. Anal. 20, 723–732.

McKercher R B and Tollefson T S 1978 Barley response to phosphorus from phospholipids and nucleic acids. Can. J. Soil Sci. 58, 103–105.

McLachlan K D 1980 Acid phosphatase activity of intact roots and phosphorus nutrition in plants. I. Assay conditions and phosphatase activity. Aust. J. Agric. Res. 31, 429–440.

Northcote K H, Hubble G D, Isbell R F, Thomson C H and Bettenay E 1975 A description of Australian soils. CSIRO, Australia. 20 p.

Rolston D E, Rauschkolb R S and Hoffman D L 1975 Infiltration of organic phosphorus compounds in soil. Soil Sci. Soc. Am. Proc. 39, 1089–1094.

Sanyal S K and De Datta S K 1991 Chemistry of phosphorus transformations in soil. Adv. Soil Sci. 16, 1–120.

Speir T W and Ross D J 1978 Soil phosphatase and sulphatase. *In* Soil Enzymes Ed. R G Burns. pp 197–250. Academic Press, London.

Stewart J W B and Tiessen H 1987 Dynamics of soil organic phosphorus. Biogeochemistry 4, 41–60.

Tabatabai M A and Bremner J M 1969 Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307.

Tarafdar J C and Jungk A 1987 Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biol. Fertil. Soils. 3, 199–204.

Tarafdar J C and Claasen N 1988 Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. Biol. Fertil. Soils. 5, 308–312.

Technicon Instruments. 1977(b). Individual/simultaneous determination of nitrogen and/or phosphorus in BD acid digests. Industrial method No. 329–74W/B. Tarrytown, New York, USA.

Thoedorou C 1971 The phytase activity of the mycorrhizal fungus *Rhizopogon luteolus*. Soil Biol. Biochem. 5, 89–90.

Woolhouse H W 1969 Differences in the properties of the acid phosphatases of plant roots and their significance in the evolution of edaphic ecotypes. *In* Ecological Aspects of the Mineral Nutrition of Plants. Ed. I H Rorison. pp 357–380. Blackwell Scientific Publications, Oxford.