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Mineralisation of soil orthophosphate monoesters under pine seedlings and ryegrass

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Abstract. The effects of radiata pine (*Pinus radiata* D. Don.) seedlings and ryegrass (*Lolium perenne* L.) on the mineralisation of orthophosphate monoesters in 7 grassland soils were assessed in a 10-month pot trial using NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy. Extraction with NaOH–EDTA recovered 46–86% of the total soil P, and NaOH–EDTA-extractable organic P determined by molybdate colourimetry ranged between 194 and 715 mg/kg soil, representing 34–85% of the total soil organic P. Orthophosphate monoesters were the predominant species of the extracted organic P in all soils, with much smaller concentrations of orthophosphate diesters, and traces of phosphonates. Concentrations of orthophosphate monoesters were consistently lower in soils under pine (103–480 mg P/kg soil) compared with the initial soils (142–598 mg P/kg soil) and most soils under grass (122–679 mg/kg soil). Mineralisation of *myo*-inositol hexa*kis*phosphate accounted for 18–100% of the total mineralisation of orthophosphate monoesters in most soils under radiata pine. This suggests that supposedly recalcitrant inositol phosphates are available for uptake by radiata pine, although the extent of this varies among soils.

Additional keywords: Pinus radiata, *Lolium perenne*, solution ^{31}P NMR spectroscopy, *myo*-inositol hexa*kis*phosphate, spectral deconvolution.

Introduction

Continued advances in our understanding of the chemical nature and associated dynamics of soil phosphorus (P) are important to ensure that agricultural and forest ecosystems are managed in a sustainable manner. Soil organic P represents 15–80% of the total soil P, mostly derived from plant residues and microbially mediated processes (Stewart and Tiessen 1987).

Plant species vary in their ability to mobilise organic P in soils (Jungk 1996; Taranto *et al*. 2000; Chen *et al*. 2002) and thereby influence the nature and distribution of soil P (Nielsen and Dalsgaard 1987; Magid 1993; Chen *et al*. 2002; Solomon *et al*. 2002). Recently, a direct link between forest vegetation and soil organic matter composition has been reported (Quideau *et al*. 2001). In New Zealand the effects of recent widespread conversion from grassland to plantation forestry [predominately radiata pine (*Pinus radiata* D. Don.)] on soil fertility and nutrient dynamics have been extensively investigated. Results from field studies clearly demonstrated that afforestation of grassland resulted in significant mineralisation of soil organic P and concomitant increases in plant-available inorganic P concentrations (e.g. Condron *et al*. 1996; Chen *et al*. 2000). Furthermore, results from a short-term (10 month) glasshouse pot experiment on 15 New Zealand grassland soils confirmed that mineralisation of soil organic P was consistently and significantly greater under radiata pine seedlings compared with ryegrass (Chen *et al*. 2003). However, in such experiments specific information on the forms of organic P involved is scarce, mainly due to the analytical difficulties involved in soil P speciation. Indeed, more than 50% remains unidentified in many soils (Magid *et al*. 1996).

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Table 1. Chemical and physical properties determined for the selected New Zealand grassland soils before planting (Chen *et al.* 2003)
PSI, P sorption index

Soil Series	USDA Soil Classification	pH	Organic C (g/kg)	Total N (g/kg)	Total P (mg/kg)	Organic P (mg/kg)[A]	C/P
Mangamahu	Dystrochrept	5.3	39.3	3.8	402	315 (78)	98
Pukaki	Dystrochrept	5.2	49.1	3.1	663	534 (81)	74
Hurunui	Dystrochrept	5.6	78.7	6.7	904	670 (74)	87
Te Kauwhata	Humult	5.7	50.0	4.8	938	606 (66)	53
Temuka	Aquept	6.5	39.6	3.8	1056	509 (48)	38
Patoka	Udand	5.7	93.4	8.5	1585	1051 (66)	59
Stratford	Udand	5.4	67.8	7.0	2746	1166 (43)	25

		Dithionite (g/kg)		Oxalate (g/kg)		Clay (%)	Silt (%)	Sand (%)	PSI (mg 100/g)/(μmol/L)
		Fe	Al	Fe	Al				
Mangamahu	Dystrochrept	9.8	3.4	6.3	2.5	18	40	42	32.0
Pukaki	Dystrochrept	5.4	2.4	2.6	2.8	12	28	60	20.8
Hurunui	Dystrochrept	12.1	2.7	2.5	1.9	23	42	35	14.2
Te Kauwhata	Humult	19.3	4.1	5.9	2.9	26	38	36	18.8
Temuka	Aquept	4.1	2.0	2.8	1.2	21	40	39	3.7
Patoka	Udand	8.5	12.2	4.7	15.8	12	29	59	59.4
Stratford	Udand	9.0	7.9	7.4	14.0	11	27	62	48.2

[A]Data in parentheses are percentage of soil organic P over total P

Various chemical extraction techniques have been developed for characterising soil P in natural and managed ecosystems (Cross and Schlesinger 1995; Magid *et al.* 1996; Frossard *et al.* 2000), but these provide no structural information on soil organic P. In the past 2 decades, solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy has been used to characterise the effects of climate, land-use change, cultivation, and long-term fertilisation on soil P chemistry (e.g. Newman and Tate 1980; Condron *et al.* 1985, 1990; Cade-Menun and Preston 1996; Turrión *et al.* 2001; Cade-Menun *et al.* 2002; Solomon *et al.* 2002). The quantitative determination of functional P groups, including inorganic orthophosphate, orthophosphate monoesters, orthophosphate diesters, phosphonates, and condensed inorganic and organic phosphates, is well established (Cade-Menun and Preston 1996; Turner *et al.* 2003*b*).

The objective of this study was to examine the specific effect of radiata pine seedlings and ryegrass (*Lolium perenne* L.) on mineralisation of various forms of soil organic P, to improve our understanding of the relationship between chemical form and bioavailability. This involved using solution ^{31}P NMR spectroscopy to identify different forms of organic P in selected soils from the glasshouse pot trial described by Chen *et al.* (2003).

Materials and methods

Soil samples and glasshouse experiment

Details of soils used and the glasshouse experiment were described by Chen *et al.* (2003). In brief, 15 surface soil samples (0–7.5 cm) under grassland were collected from around New Zealand, air-dried (25°C), and sieved (4 mm) before the glasshouse experiment. Two plant species, radiata pine (Breed 'GF12') and perennial ryegrass (cultivar 'Grasslands Nui'), were used in this experiment. Seeds were directly sown into the pots and each species was thinned to 5 per pot after germination. The trial included 3 replicates of each plant species for each soil. All pots were placed on a capillary mat in the glasshouse in a completely randomised design, and soil moisture contents were maintained at *c.* 70% field capacity. Radiata pine seedlings were inoculated with mycorrhizae (*Rhizopogon rubescens* Tul.) at a rate of 1×10^7 spores per pot, applied in a water suspension, 2 weeks after sowing. No nutrients were added to the pots during the experiment. The average daily temperature during the experiment ranged from 12 to 25°C.

Seven soils with relatively high proportions of organic P were selected for inclusion in this investigation. These included soils from the North Island (Te Kauwhata, Stratford, Patoka, Mangamahu) and the South Island (Hurunui, Temuka, Pukaki) (Table 1, from Chen *et al.* 2003). Soil pH values were 5.2–6.5, and concentration of organic carbon (C), total nitrogen (N), and total P was 39.3–93.4, 3.1–8.5, and 0.40–2.75 g/kg, respectively. Organic P comprised 43–81% of the total P. Concentrations of dithionite-extractable iron (Fe) and aluminium (Al) were 5.4–19.3 and 2.0–12.2 g/kg soil, respectively. Oxalate-extractable Fe and Al concentrations were 0.5–7.4 and 1.0–15.8 g/kg soil, respectively. Clay content varied from 40 to 310 g/kg soil, and P sorption index (PSI) values ranged between 3.1 and 59.4 (mg 100/g)/(μmol/L).

NaOH–EDTA extraction

Initial soils before planting and soils sampled after growth of ryegrass or radiata pine were air-dried (25°C) and sieved (≤2 mm) before NaOH–EDTA extraction. Solution ^{31}P NMR spectroscopy was performed on composite soils bulked from 3 replicates of the same treatments. The NaOH–EDTA extraction followed the method described by Cade-Menun and Preston (1996), whereby 5.0 g of air-dried soil was extracted with 100 mL of a solution containing 0.25 M NaOH and 0.05 M EDTA for 16 h at room temperature. Samples were filtered through Whatman 41 papers and subsamples taken for analysis

of inorganic P, organic P, and total P by molybdate colourimetry (Tiessen and Moir 1993). The remaining extracts were frozen and lyophilised for NMR analysis.

Solution ^{31}P NMR spectroscopy

Freeze-dried NaOH–EDTA extracts (approx. 200 mg) were re-dissolved in 0.9 mL of 1 M NaOH and 0.1 mL D_2O (for signal lock) and transferred to 5-mm diameter NMR tubes. The addition of NaOH ensures consistent chemical shifts and optimum spectral resolution at a solution pH >12. Solution ^{31}P NMR spectra were obtained using a Bruker AMX 600 spectrometer operating at 243 MHz. We used a 30° pulse width, a total acquisition time of 1.5 s (pulse delay 0.808 s, acquisition time 0.672 s), and broadband proton decoupling. Temperature was regulated at 24°C, although it is now recommended that temperature should be standardised at 20°C (Cade-Menun *et al*. 2002; Turner *et al*. 2003*b*). The number of scans collected (2000–10000) depended on the P concentration of the freeze-dried extracts. Compounds were identified by their chemical shift relative to 85% orthophosphoric acid after Lorentzian convolution with a width of 10 Hz. We used the deconvolution process of the Bruker WinNMR program to determine chemical shift and area of individual signals, which were identified from literature reports (Turner *et al*. 2003*b*), with general functional classes of P compounds as follows: phosphonates around 19 ppm, inorganic orthophosphate at approximately 6.1 ppm, orthophosphate monoesters at 3–6 ppm, orthophosphate diesters between –0.5 and 2.0 ppm, pyrophosphate at approximately –4 ppm, inorganic polyphosphates around –20 ppm. Within the orthophosphate diesters, signals between 0.6 and 2.0 ppm were assigned to phospholipids and alkali-labile nucleic acids, whereas the signal at approximately 0 ppm was assigned to DNA (Makarov *et al*. 2002; Turner *et al*. 2003*b*). Signals appearing between –0.5 and –2.0 ppm were defined as unknown P (Makarov *et al*. 1997). Phosphorus functional groups were expressed as a percentage of total signal area and concentrations in the NaOH–EDTA extracts were calculated on the basis of the total P in the extracts measured by molybdate colourimetry (see above).

Signals at 5.85, 4.92, 4.55, and 4.43 ppm in the ratio 1:2:2:1 were identified by spectral deconvolution and assigned to *myo*-inositol hexa*kis*phosphate (IP_6) (Turner *et al*. 2003*d*). The proportion of the spectral area under these 4 signals was then multiplied by the total P concentration in the NaOH–EDTA extract to give the concentration of IP_6 in the soil (mg P/kg dry soil).

Results

Phosphorus in the NaOH–EDTA extracts

Extraction with NaOH–EDTA recovered 46–86% of total soil P, of which organic P (determined by molybdate colourimetry) constituted 34–85% (Table 2). Extracts of pine soils contained more NaOH–EDTA-extractable inorganic P (88–1034 mg/kg), but less NaOH–EDTA-extractable organic P (218–681 mg/kg), than the grass soils (63–931 mg/kg inorganic P and 232–715 mg/kg organic P), despite more P being taken up by pine seedlings (Chen *et al*. 2003) (Table 2).

Soil phosphorus composition determined by solution ^{31}P NMR spectroscopy of NaOH–EDTA extracts

Representative solution ^{31}P NMR spectra of NaOH–EDTA extracts of the initial soil, and soils under grass and pine (Pukaki, Mangamahu, and Stratford) are shown in Fig. 1.

Table 2. Soil total P contents (mg/kg) and NaOH–EDTA-extractable P (mg/kg) in the selected New Zealand grassland soils before (I) and after planting with ryegrass (G) and radiata pine seedlings (P)

Soil series	Sample	Total soil P	NaOH–EDTA extractable P		
			Total P[A]	Inorganic P[B]	Organic P[B]
Mangamahu	I	402	298 (74)	60 (20)	238 (80)
	G	377	295 (78)	63 (21)	232 (79)
	P	357	306 (86)	88 (29)	218 (71)
Pukaki	I	663	422 (64)	62 (15)	360 (85)
	G	652	441 (68)	67 (15)	375 (85)
	P	596	397 (67)	121 (30)	276 (70)
Hurunui	I	904	623 (69)	198 (32)	425 (68)
	G	814	498 (61)	136 (27)	362 (73)
	P	795	516 (65)	190 (37)	327 (63)
Te Kauwhata	I	938	633 (68)	275 (43)	359 (57)
	G	893	559 (63)	218 (39)	342 (61)
	P	853	522 (61)	272 (52)	250 (48)
Temuka	I	1056	626 (59)	306 (49)	320 (51)
	G	919	593 (65)	272 (46)	322 (54)
	P	958	565 (59)	371 (66)	194 (34)
Patoka	I	1585	749 (47)	409 (55)	340 (45)
	G	1482	677 (46)	328 (48)	348 (52)
	P	1422	687 (48)	384 (56)	303 (44)
Stratford	I	2746	1655 (60)	946 (57)	708 (43)
	G	2607	1645 (63)	931 (57)	715 (43)
	P	2616	1715 (66)	1034 (60)	681 (40)

[A]Data in parentheses are the proportion (%) of the total soil P.
[B]Data in parentheses are the proportion (%) of the NaOH–EDTA extractable P.

The relative proportions of inorganic P and organic P in the NaOH–EDTA extracts varied among soils (Tables 2 and 3). Orthophosphate was the dominant inorganic compound, with concentrations between 121 and 1098 mg/kg (27–74% total extractable P). There were smaller concentrations of pyrophosphate (<18 mg/kg), and polyphosphate (4 mg/kg) was detected only in Mangamahu soil after planting with radiata pine (Table 3). In general, concentrations of inorganic P determined by ^{31}P NMR were greater than by molybdate colourimetry.

Organic P compounds included orthophosphate monoesters, orthophosphate diesters, and phosphonates (Fig. 1, Table 3). Orthophosphate monoesters were the dominant P species in the NaOH–EDTA extracts, accounting for 24–64% of the total P (Table 3), and 80–97% of the total NaOH–EDTA-extracted organic P (data not shown). Of these, concentrations of IP_6 ranged between 17 and 142 mg/kg, representing 11–50% of the orthophosphate monoesters (Table 4). Concentrations of IP_6 increased as the orthophosphate monoesters increased ($r = 0.870$, $P < 0.01$). Concentrations of orthophosphate diesters ranged between 1 and 116 mg/kg (<19% total extracted organic P) (Table 3). Of these, the phospholipids constituted 28–100%, and DNA

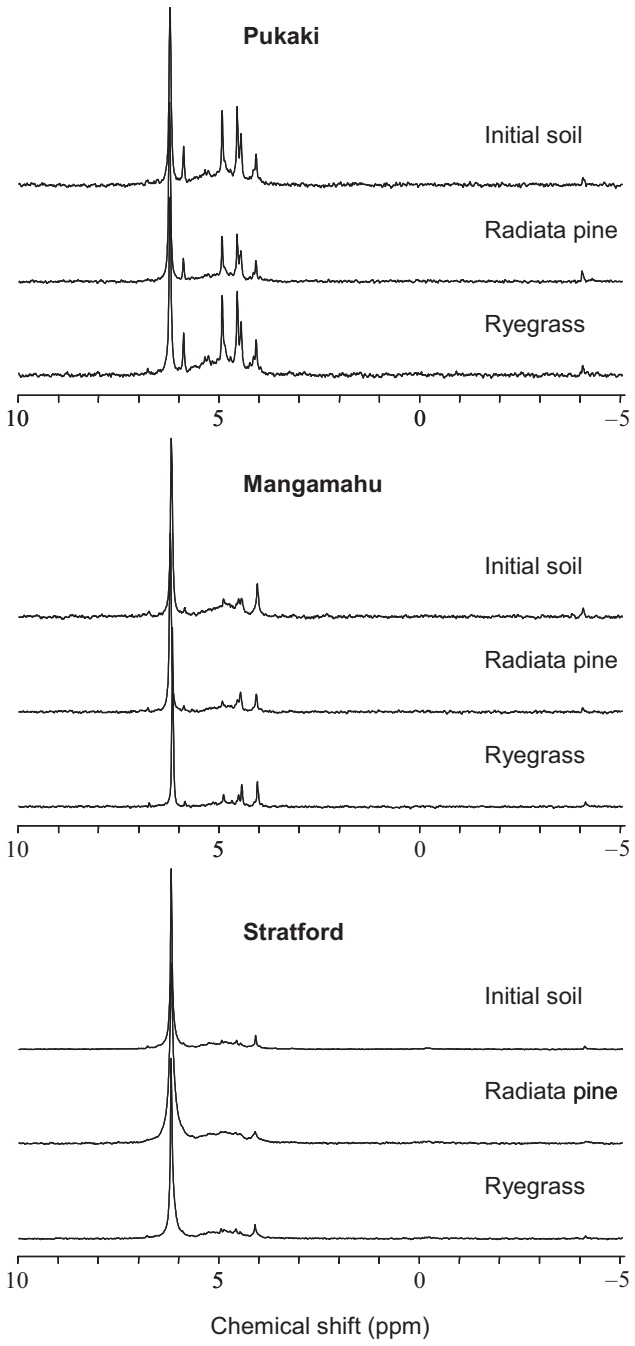

Fig. 1. ^{31}P NMR spectra of representative soils (Pukaki, Mangamahu, and Stratford) before and after 10 months growth of ryegrass and radiata pine.

constituted up to 72%. Small concentrations of phosphonates (<13 mg/kg) were detected in some soils, and a trace (1 mg/kg) of an unknown P compound was detected in the initial Manganmahu soil (Table 3).

Influence of plant species on soil phosphorus composition

Concentrations of orthophosphate monoesters consistently decreased following growth of pine (103–480 mg/kg soil) compared with the initial soil (142–598 mg/kg) and soils under grass (122–679 mg/kg) (except the Hurunui soil) (Table 3). In addition, the relative proportion of orthophosphate monoesters was generally smaller in soil under pine than in initial and grass soils (except the Hurunui soil) (Table 3). In soils under ryegrass, concentrations of orthophosphate monoesters decreased in Mangamahu, Hurunui, Te Kauwhata and Patoka soils, but increased in Pukaki, Temuka, and Stratford soils, compared with the initial soils. Clearly, mineralisation of orthophosphate monoesters induced by ryegrass varied among soils.

Concentrations of IP_6 were 7–48 mg P/kg lower in soils under pine than in most initial soils (except the Patoka soil) and those under grass (except Patoka and Mangamahu soils). These decreases accounted for 18–100% of mineralised orthophosphate monoesters (Table 5). Ryegrass induced limited mineralisation of IP_6 (3–20 mg P/kg) in 4 soils (Mangamahu, Hurunui, Temuka, Stratford soils) (Tables 4 and 5).

Concentrations of orthophosphate diesters showed no clear trend among plant species within a soil (Tables 3). For example, phospholipid concentrations decreased in the Mangamahu, Pukaki, and Temuka soils after growth of radiata pine compared with the initial soils, but accumulated in the Hurunui, Te Kauwhata, Patoka, and Stratford soils (Table 3).

The ratio of orthophosphate monoesters to orthophosphate diesters has been used as an index of mineralisation capability (Condron *et al*. 1990; Turrión *et al*. 2001), but varied widely among soils in the current study (4–286) and showed no clear trend in response to plant growth (Table 3).

Discussion

The predominance of orthophosphate monoesters in these soils is consistent with reports of the organic P composition of world soils (e.g. Newman and Tate 1980; Condron *et al*. 1985; Turrión *et al*. 2001; Turner *et al*. 2003*c*). Orthophosphate monoesters include inositol phosphates, sugar phosphates, and mononucleotides (Condron *et al*. 1990). Of these, the inositol phosphates are most stable, being protected from enzymatic attack by strong complexation with soil minerals, clays, and humic compounds (Turner *et al*. 2002). This suggests that soil IP_6 is relatively unavailable for uptake by plants, and although some studies have reported that plants could use IP_6 amended in solution or soil culture, there are few reports of uptake of IP_6 from soil (e.g. Martin and Cartwright 1971; Shibata and Yano 2003). However, we detected consistent decreases in orthophosphate monoesters and IP_6 in soil under pine seedlings, which clearly indicated the utilisation of such compounds by these plants. Mineralisation of orthophosphate monoesters and IP_6 by ryegrass was

Table 3. Concentrations of P compounds (mg/kg) in the NaOH–EDTA-extracts as determined by ^{31}P NMR spectroscopy

Data in parentheses are the proportion (%) of the total extracted P calculated from total signal area. Trace amounts (1 mg/kg) of unknown P compounds were found in initial Manganmahu soil only; polyphosphate (4 mg/kg) was found only in Mangamahu soil after planting with radiata pine

Soil series	Sample[A]	Inorganic P		Organic P				Monoester-P/diester-P ratio[B]
		Orthophosphate	Pyrophosphate	Orthophosphate monoester	Phospholipids	DNA	Phosphonate	
Mangamahu	I	133 (45)	6 (2)	142 (48)	9 (3)	3 (1)	4 (1)	12
	G	146 (50)	9 (3)	122 (41)	4 (1)	3 (1)	12 (4)	19
	P	187 (61)	2 (1)	103 (34)	4 (1)	3 (1)	3 (1)	15
Pukaki	I	131 (31)	7 (2)	270 (64)	9 (2)	3 (1)	3 (1)	24
	G	121 (27)	10 (2)	283 (64)	19 (4)	8 (2)	n.d.	11
	P	174 (44)	9 (2)	204 (51)	2 (0.5)	2 (<1)	6 (2)	51
Hurunui	I	285 (46)	14 (2)	297 (48)	3 (1)	15 (2)	7 (1)	16
	G	222 (45)	13 (3)	241 (48)	6 (1)	8 (2)	9 (2)	18
	P	201 (39)	11 (2)	251 (49)	30 (6)	17 (3)	6 (1)	5
Te Kauwhata	I	402 (64)	13 (<1)	209 (33)	2 (<1)	4 (<1)	3 (1)	35
	G	329 (59)	14 (3)	199 (36)	6 (1)	8 (1)	4 (1)	15
	P	347 (66)	9 (2)	153 (29)	5 (1)	3 (1)	4 (1)	18
Temuka	I	448 (72)	n.d.	169 (27)	7 (<1)	2 (0.4)	n.d.	19
	G	362 (61)	12 (2)	200 (34)	13 (2)	6 (1)	n.d.	11
	P	416 (74)	8 (1)	136 (24)	1 (<1)	n.d.	5 (<1)	286
Patoka	I	340 (45)	9 (1)	358 (48)	13 (2)	23 (3)	6 (1)	10
	G	301 (45)	11 (2)	327 (48)	9 (1)	23 (3)	6 (1)	10
	P	319 (47)	10 (2)	318 (46)	16 (2)	20 (3)	4 (1)	9
Strafford	I	970 (59)	16 (1)	598 (36)	31 (2)	28 (2)	13 (1)	10
	G	880 (54)	18 (1)	679 (41)	40 (2)	28 (2)	n.d.	10
	P	1098 (64)	18 (1)	480 (28)	68 (4)	48 (2.8)	3 (0.2)	4

n.d., Not detected.
[A] I, initial soil before planting; G, ryegrass; P, radiata pine.
[B] The ratio of orthophosphate monoesters to the sum of phospholipids and DNA.

Table 4. Concentrations (mg P/kg soil) of *myo*-inositol hexa*kis*phosphate determined by spectral deconvolution and solution ^{31}P NMR spectroscopy

Values in parentheses are the proportion (%) of the total extracted P

Soil series	Initial soil	Ryegrass	Pine seedlings
Mangamahu	38 (13)	20 (7)	31 (10)
Pukaki	131 (31)	142 (32)	83 (21)
Hurunui	107 (17)	87 (18)	60 (12)
Te Kauwhata	27 (4)	39 (7)	17 (3)
Temuka	53 (8)	50 (9)	37 (7)
Patoka	72 (10)	78 (11)	88 (13)
Stratford	124 (8)	106 (7)	77 (5)

Table 5. Mineralisation (–) or accumulation (+) of *myo*-inositol hexa*kis*phosphate (IP_6) in soil over the 10-month growth of ryegrass or radiata pine

Soil series	Sample[A]	IP_6 mineralised (–) or accumulated (+) (mg P/kg)	RP[B] %
Mangamahu	G	–19	93
	P	–7	19
Pukaki	G	11	n.a.
	P	–48	73
Hurunui	G	–20	36
	P	–47	104
Te Kauwhata	G	12	n.a.
	P	–10	18
Temuka	G	–3	n.a.
	P	–16	47
Patoka	G	6	n.a.
	P	16	n.a.
Stratford	G	–18	n.a.
	P	–47	40

n.a., Not applicable.
[A] G, soils after planting ryegrass; P, radiata pine.
[B] Relative proportion of the amount of IP_6 mineralised over orthophosphate monoester mineralised in the 10 months of growth.

observed in some soils (e.g. Mangamahu and Hurunui soils), but was more limited than under pine (Tables 4 and 5).

Variability in the utilisation of IP_6 by pine seedlings and ryegrass among soils, is almost certainly explained by differences in soil properties, which are known to exert marked controls on the biological availability of inositol phosphates. For example, Martin and Cartwright (1971) demonstrated that IP_6 was more available to plants grown in

a weak P-fixing soil compared with a high P-fixing soil. Organic P derived from microbial and plant sources is chemically and biochemically modified and stabilised differently in different soil matrices. For example, IP_6 may have been less biologically available in the more acidic soils due to the formation of insoluble Fe or Al complexes (Jackman and Black 1951). Indeed decreases in IP_6 concentrations following growth of radiata pine were significantly correlated with the original level of the dithionite and oxalate-extractable Al in soil ($r = –0.709$ and $–0.660$, $P < 0.05$), and no mineralisation of IP_6 occurred in the Patoka soil (for both pine seedlings and ryegrass) that contained a high concentration of oxalate and dithionite-extractable Al. It is unlikely that ryegrass synthesises significant quantities of IP_6, so accumulation of orthophosphate monoesters (e.g. Temuka and Stratford soils under ryegrass) and IP_6 under ryegrass (e.g. in the Pukaki and Te Kauwhata soils) probably reflects either differences in extraction efficiency, or possibly the synthesis of IP_6 by microbes (Caldwell and Black 1958).

The mechanisms involved in mineralisation of soil orthophosphate and IP_6 by pine seedlings remain unknown. Differences in utilisation of organic P by ryegrass and pine seedlings may be linked to different types of mycorrhizal associations, because ryegrass is associated with vesicular arbuscular mycorrhizae (VAM) (Powell 1977), whereas roots of radiata pine seedlings are associated with ectomycorrhizae (ECM) (Chu-Chou and Grace 1979). In addition to more efficient P uptake by ECM-associated roots compared with VAM-associated roots (Marschner and Dell 1994), ECM hyphae have been found to produce phosphatase and phytase enzymes and low molecular weight organic acids, which may enhance the solubility and enzymatic hydrolysis of recalcitrant organic P in soil (e.g. Jones *et al*. 1998; Yadav and Tarafdar 2003). Chen *et al*. (2003) showed that phosphatase activity of ectomycorrhizal roots of radiata pine seedlings were consistently and significantly higher than that of ryegrass roots, and concentrations of soluble organic C in pine soils was also significantly greater than in grass soil. We hypothesise that these account for the greater utilisation of orthophosphate monoesters and IP_6 by pine seedlings compared with ryegrass.

Although the results from this study provide direct evidence that radiata pine seedlings are better able to utilise orthophosphate monoesters (including IP_6) than ryegrass, it is acknowledged that the results from this 10-month glasshouse experiment can only reflect the difference in utilisation of orthophosphate monoesters and IP_6 among plant species at the early stages of growth and development, particularly for radiata pine. Data from field studies have also demonstrated that concentrations of orthophosphate monoesters were lower in soil under pine plantations (up to 30 years old) compared with adjacent grassland (Condron *et al*. 1996), but it is difficult to extrapolate the results from this study to long-term effects of radiata pine trees on soil in the field. In fact, it is nearly impossible to investigate the effects of pure plant species on soil in the field due to the different management and existence or invasion of other plant species (e.g. understorey).

Orthophosphate diesters originate from plant and microbial sources (Makarov *et al*. 2002) and are considered relatively labile in temperate soils. The low charge density and shielded phosphate groups mean that they sorb only weakly in the soil and are, therefore, accessible to microbial and enzymatic attack (Magid *et al*. 1996). Solomon *et al*. (2002) found that concentrations of orthophosphate diesters were greater in soils under natural forest (24–27% of the total extracted P) compared with adjacent plantation forest (13–15%) and a cultivated field (9–10%). Orthophosphate diesters can accumulate in the clay fraction of soil (Amelung *et al*. 2001) and under waterlogged conditions (Mahieu *et al*. 2000). Makarov *et al*. (2002) reported that DNA accumulated in acid soils under cool conditions, whereas phospholipids and teichoic acids accumulated in soils with higher microbial activity. Concentrations of DNA are also strongly correlated with soil microbial biomass in temperate pasture soils (Turner *et al*. 2003*c*). The inconsistent effects of plant species on the levels of orthophosphate diesters among soils in the present study probably reflect the interaction of plant species and soil type, and might be related to a combination of factors including various soil mineral matrix and the diverse microbial activities induced by plant species.

This study showed a much wider range of orthophosphate monoester to diester ratios than those typically reported in the literature. Turrión *et al*. (2001) suggested that soil organic matter quality and rainfall affect microbial activity and the orthophosphate monoester to diester ratio, and Turner *et al*. (2003*a*) used temporal changes in this ratio to detect seasonal mineralisation of orthophosphate diesters in upland soils of northern England. Solomon *et al*. (2002) indicated an increase in the orthophosphate monoester to diester ratio after land-use change from the natural forest to plantation forest and crop land, showing that more orthophosphate diesters had been depleted in response to land-use change. However, we detected no significant relationships between the orthophosphate monoester to diester ratio and soil pH, soil C:N ratio, and microbial activity (as CO_2 respiration) across soils (data not shown), suggesting that the ratio may not be a good indicator of changes in quality of soil organic P in the short term.

Conclusions

Our results confirm that radiata pine enhances the mineralisation of soil organic P compared with ryegrass (Chen *et al*. 2003). In particular, orthophosphate monoesters, including IP_6, were mineralised by radiata pine, whereas the capability for ryegrass to utilise them was limited. Mineralisation varied among soils, almost certainly due to

differences in soil properties that influence the degree of stabilisation of inositol phosphates. Clearly, inositol phosphates cannot be regarded as 'unavailable' or 'recalcitrant' organic P in all soils, although further studies are required to elucidate the precise mechanisms involved in the stabilisation and turnover of inositol phosphates in soil.

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