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Fate and effects of phosphorus additions in soils under N_2-fixing red alder

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Abstract. Soil phosphorus (P) dynamics are controlled by the interaction of geochemical, biochemical and biological processes. Changes in species composition or management could alter the relative importance of these processes. We examined soil P dynamics in two plantations of N_2-fixing red alder (*Alnus rubra*) by determining the fate and effects of added fertilizer P. History of the plantations varied such that sites were previously occupied by 60-yr-old stands of alder or non-fixing Douglas-fir (*Pseudotsuga menziesii*). Without fertilization, the soil with a longer period of alder influence had more organic P (P_o) and less sorbed inorganic P (Hydroxide- and Bicarb-extractable P_i). Fertilization increased soil total P, and 88% of the fertilizer was accounted for in the surface mineral soil (0–15 cm). Sorbed P_i was the major sink for fertilizer P (55–60%), independent of site history. Although P_o was 35–70% of soil P in unfertilized plots, added P did not accumulate as P_o. Neither site history nor P addition influenced phosphatase activity. Fertilization increased decomposition during incubation of the organic horizon, suggesting that late-stage decomposition is P-limited in these N-rich soils. On the time-scale of a few years, geochemical sorption and desorption of inorganic P were the most important processes controlling the distribution of added P. Organic P accumulation is expected to occur over a longer time frame, linked to the production and turnover of organic matter.

Introduction

Atmospheric inputs of phosphorus (P) are generally much smaller than inputs of carbon, nitrogen or sulfur, and therefore weathering, leaching and immobilization interact to control soil P availability over the long-term (Walker & Syers 1976; Crews et al. 1995). Soil organic matter is the long-term sink for nitrogen (N) within terrestrial ecosystems, with mineralization/immobilization dynamics strongly regulating N turnover and availability (Binkley & Hart 1989; Davidson et al. 1992; Stark & Hart

1997). In contrast, soil P dynamics are driven by competing mechanisms of adsorption/precipitation interactions, microbial assimilation and release, and extracellular enzyme activity (Cross & Schlesinger 1995; Sanyal & De Datta 1991; Stewart & Tiessen 1987; Walbridge et al. 1991). Interaction between these competing mechanisms has made it difficult to determine their relative importance in controlling P dynamics (Walbridge & Vitousek 1987; Zou et al. 1992). Much of our understanding of P cycling focuses on contrasts between soil orders (Tiessen et al. 1984; Cross & Schlesinger 1995), rather than finer-scale disturbance or species effects. Since many forest soils have high sorption capacity and high levels of organic matter, both geochemical and biological/biochemical processes may strongly influence P availability.

The soil P cycle involves strong chemical and biological sinks. Potential fates of P in soil solution include:

1) sorption as orthophosphate (P_i) to soil anion exchange sites,
2) sorption followed by occlusion within minerals as unavailable P_i,
3) uptake of P_i into plants or microbes and storage as P_i or polyphosphates,
4) conversion to easily decomposed organic P (P_o) compounds by the biota,
5) conversion to more complex and recalcitrant P_o compounds,
6) subsequent sorption as P_o on exchange sites, and
7) loss as P_i or P_o via leaching.

Both 1 and 2 result in P_i being held chemically within the soil, whereas 4, 5 and 6 result in P_i being converted to P_o by the biota. Unfortunately, the designation of organic vs. inorganic P does not reveal much about the potential availability of these components to the biota, since sorption reactions may be only somewhat reversible and the lability of P_o could vary. Mechanisms 1 and 6 represent P held on exchange sites, which could be released by exchange reactions occurring continually within the soil (Sanyal & De Datta 1991). Upon exchange, plants or microbes could assimilate P_i (3 and 4), while P_o could be attacked by phosphatases and taken up by microbes. Mechanisms 2 and 5 result in relatively inaccessible P that will not contribute to available soil P, until conditions are conducive for desorption or until the resistant P_o is enzymatically converted to P_i or simple P_o compounds. Soil P fractionation schemes (e.g., Chang & Jackson 1957; Hedley et al. 1982) allow for separation into different forms that correspond roughly to the pathways listed above.

Several studies have shown that increased atmospheric N inputs can decrease P availability, measured as plant uptake or soil extractable pools (Mohren et al. 1986; Clarholm & Rosengren-Brinck 1995; Emmett et al. 1995; Gundersen 1998). Changes in soil properties under N_2-fixers may alter P availability via geochemical mechanisms by decreasing soil pH (Binkley & Sollins 1990; Van Miegroet & Cole 1985), which in turn can

increase anion exchange capacity in acid soils by increasing the degree of protonation of Fe and Al hydroxides (McBride 1994). It also possible that biological/biochemical mechanisms associated with rapid organic matter accumulation under alder (Binkley et al. 1992; Bormann et al. 1994) reduce extractable P_i levels by promoting conversion to P_o (Compton & Cole 1998).

Previous research comparing the influence of N_2-fixing red alder and non-fixing Douglas-fir on soil P forms has shown that extractable P_o is consistently greater under alder as compared to pure Douglas-fir (Zou et al. 1995; Compton & Cole 1998), while extractable P_i is similar (Zou et al. 1995) or lower (Compton & Cole 1998). Fluxes of P in litterfall, uptake and resorption are also faster in pure and mixed alder stands than pure Douglas-fir (Binkley et al. 1992; Compton & Cole 1998). Mineralization of P_o to P_i through phosphatases and subsequent plant uptake of P_i may be enhanced under alder (Giardina et al. 1995; Zou et al. 1995), such that actual rates of P turnover are faster, even if available pool sizes of P_i are similar or smaller.

Here we examine soil P retention pathways by comparing the fate and effects of fertilizer P in the soil of two alder plantations. Review of previous work led us to formulate two hypotheses: 1) conversion of added P_i to P_o is an important transformation in soils with long-term alder influence because these soils tend to have greater P_o; and 2) the phosphatase enzyme system operates as a negative feedback, such that increased P_i availability through fertilization results in decreased phosphatase activity. Soil P forms were determined sixteen months after P addition using the Hedley et al. (1982) sequential fractionation, and phosphatase activity was determined by incubation of soils with *p*-nitrophenyl phosphate (Tabatabai 1982). The Hedley procedure separates inorganic and organic P according to ease of chemical extraction, allowing comparison of organic to inorganic P, and easily extracted to more tightly bound P forms. Although this method has largely been used to provide insight into the nature of soil P across a wide range of soils (Lajtha & Schlesinger 1988; Walbridge et al. 1991; Crews et al. 1995; Cross & Schlesinger 1995), a growing number of studies have used this method to examine species or management impacts within forest soils (Giardina et al. 1995; Zou et al. 1995).

Methods

Site description

This work was conducted at the Thompson Research Center (200 m a.s.l.), located in the foothills of the western Cascade Mountains in the Cedar River Watershed, 60 km SE of Seattle, WA. The climate is typical of the mild, wet

winters and cool dry summers encountered in low elevations of the Cascades. Mean January air temperature is 2.8 °C; mean July air temperature is 16.8 °C. Mean annual precipitation is 1300 mm, the bulk of which falls as rain from October to April. Soil underlying the study site is of the Alderwood series, previously classified as a dystric entic Durochrept, recently reclassified as an mesic ortstein aquic Haplorthod (Soil Survey Staff 1986). The Alderwood series is a gravelly sandy loam, derived from ablation till overlying indurated basal till originating from the last glaciation.

The vegetation of the research site in the early 1900s was a mature Douglas-fir stand typical of the western hemlock zone (*Tsuga heterophylla*), and was logged by railroad between 1910 and 1920 (Turner et al. 1976). Most of the area was planted with Douglas-fir in 1931 at 2 × 2.5 m spacing; areas not planted or where Douglas-fir plantations were destroyed by fire became established with red alder over the next 10 years. These approximately 60-year-old alder and Douglas-fir stands have been the subject of intensive study focusing on the biogeochemical cycling of N, P, sulfur, cations, and organic matter for more than thirty years (Turner et al. 1976; Van Miegroet et al. 1990; Johnson & Lindberg 1992; Johnson et al. 1986; Homann et al. 1990; Homann et al. 1992; Compton & Cole 1998).

Experimental design

Stand conversion plots were created in September 1984 (Cole et al. 1995). Briefly, two 50 × 100 m (0.5 ha) plots were clearcut in both the 60-yr-old red alder and Douglas-fir stands mentioned above. In February 1985, those two plots were planted at 2 × 2.5 m spacing with 2-yr-old red alder, yielding one plot per treatment: first rotation (Douglas-fir cut and planted with alder: fir-to-alder [FA]) and second rotation (alder cut and planted with alder: alder-to-alder [AA]). Each plot was divided into eight 15 m × 15 m subplots.

A randomly chosen subplot within each plot (AA or FA) was fertilized with 400 kg triple superphosphate-P ha^{-1} on 27 April 1991. Triple superphosphate is ground rock phosphate dissolved with phosphoric acid, having the general formula $Ca(H_2PO_4)_2$. An adjacent subplot was used as a control. In August 1992, six soil samples (0–15 cm depth) were collected from the side of small pits in both the control and 400 kg P ha^{-1} subplots in the FA and AA plots. Average soil moisture ranged from 9–38% of fresh weight. Bray-2 extractable P concentrations in 1984, prior to the establishment of the clearcut plots, were similar among subplots within a given plot: 105 vs. 115 mg P kg^{-1} in FA control vs. fertilized subplots (s.d. = 32 for all eight subplots); 9 vs. 34 mg P kg^{-1} in the AA control vs. fertilized subplots (s.d. = 17). Because of the similarity between subplots prior to replanting and fertilization, the changes in P fractions after fertilization are expected to be controlled by the added

P. Eight O horizon samples were collected in the control and P fertilized subplots in October 1993 using an 80.12 cm^2 soil corer.

Soil P fractionation and interpretation of P fractions

Sequential fractionation procedures remove progressively more chemically resistant fractions, separating them into inorganic (P_i) and organic (P_o) forms. We used a modification of the Hedley et al. (1982) soil P fractionation procedure, replacing the resin extraction with a water extraction step, and removing the sonication step, since these young soils have little clay and weak aggregation. First, 0.5 g dry-weight equivalent of fresh sieved soil was shaken for sixteen hrs with 25 mL distilled, deionized water, then centrifuged for fifteen min at 12,500 rpm. The supernatant was decanted and analyzed for P_i by the phosphomolybdate-ascorbic acid procedure (Olsen & Sommers 1982) using a Perkin-Elmer 55E spectrophotometer (Perkin-Elmer, Norwalk, Connecticut, USA). This water-extractable fraction is termed Water-P_i. The soil residue was then shaken for 16 hrs with 25 mL 0.5 *M* $NaHCO_3$ (Bicarb-P_i and P_o), 25 mL 0.1 *M* NaOH (Hydroxide-P_i and P_o), and 25 mL 1 *M* HCl (Acid-P_i), with centrifugation and decanting of the supernatant before each extractant was added. Each supernatant was analyzed for P_i as described above and total P by inductively coupled plasma emission spectroscopy (Thermo Jarrell Ash, Franklin, Massachusetts, USA). Organic P (P_o) in each fraction was calculated as total P minus P_i. Only P_i was analyzed in the HCl extract, since initial tests found very little P_o in this extract. Total P (P_T) on a duplicate soil sample was determined by H_2O_2-$LiSO_4$-H_2SO_4 digestion (Parkinson & Allen 1975), and residual P (Resid-P) was calculated as the difference between total P and the sum of the measured fractions.

A duplicate sample of the water-extracted soil was treated with 1 mL chloroform for sixteen hours, then extracted with 0.5 *M* $NaHCO_3$. The increase in P extracted by chloroform-bicarbonate when compared with bicarbonate alone originates from lysed microbial cells, and is referred to as Microbe-P. No correction was made for fumigation efficiency or adsorption of P released upon fumigation, although others have found that 40% of microbial biomass P was released as P_i by chloroform fumigation (Brookes et al. 1982; Walbridge et al. 1991).

The water-extractable P_i (Water-P_i) is directly available to the biota, and represents the most available fraction. Bicarb-P_i represents an easily desorbed form, and is used as an index of available P, since plants can release bicarbonate. Bicarb-P_o is the most easily exchanged and presumably labile organic fraction. Hydroxide-P_i originates from P_i sorbed to Al and Fe hydroxides and from Fe-phosphates such as strengite (Williams et al. 1971). Hydroxide-P_o includes sorbed P_o, as well as P_o associated with humic substances since

NaOH also solubilizes humic and fulvic acids (Schnitzer 1982). Although chemically recalcitrant, the NaOH fractions are somewhat accessible to plants (Gahoonia & Nielsen 1992). Acid-P_i represents apatite or Ca-bound P (Williams & Walker 1969), and may be a small portion of total P in acid soils. Residual P is not released in the sequential extraction procedure; it is considered to be extremely recalcitrant organic P (Williams & Walker 1969; Compton & Cole 1998), but could be dominated by strongly occluded Fe- and Al-bound P_i in some soils (Williams et al. 1980; Tiessen & Moir 1993).

Soil organic P

Soil organic P was determined by a modification of the ignition-extraction method (Saunders & Williams 1955; Olsen & Sommers 1982), using two temperatures to separate labile aliphatic-associated P from recalcitrant aromatic-associated P. As a measure of organic P, the ignition-extraction method was recommended over pure extraction methods when comparing treatments within similar soils, and represents the upper limit of P_o values (Bowman 1989). The thermal combustion of organic matter occurs in two main sequences (Kristensen 1990): 1) the low temperature region (100–350 °C) where evaporation and oxidative degradation of weak aliphatic bonds occurs, and 2) the high temperature region (350–600 °C) where oxidation of aromatic groups such as in polyphenols and humic substances occurs. Subsamples (4 g) were heated to 300 or 550 °C. Unheated and heated soils (0.5 g each) were extracted with 25 mL of 0.5 M H_2SO_4, shaken on a reciprocating shaker for sixteen hours, then centrifuged at 12,500 rpm for fifteen minutes. The filtered extracts were then analyzed for P_i as described above. All heated samples were corrected for P_i released by H_2SO_4 from unheated soils, and the following fractions were obtained: Total P_o (P_i extracted from sample ignited at 550 °C minus P_i extracted from unignited sample); low temperature or Labile P_o (P_i extracted from sample heated to 300 °C minus P_i extracted from unignited sample): and high temperature or Recalcitrant P_o (total P_o – low temperature P_o).

Phosphatase activity

Potential acid phosphomonoesterase (EC 3.1.3) activity was determined using the procedure of Tabatabai (1982), where the release of phosphate from an added *para*-nitrophenyl phosphate substrate produces para-nitrophenol. Soil pH may influence enzyme activity; since red alder can lower soil pH by one unit (Van Miegroet & Cole 1984), we measured phosphatase activity at pH 4.0 and 5.0. The substrate was added to field moist soils, and incubated at 37 °C for 1 hour. The reaction was halted by the addition of 0.5 *M* NaOH,

and $CaCl_2$ was added to prevent dispersion of clay and extraction of organic matter, both of which interfere with the measurement of *p*-nitrophenol. The sample was filtered (Whatman #1) and analyzed for absorbance at 400–420 nm with a Perkin-Elmer 55E spectrophotometer. This method is simple and quick, but it is necessary to point out that it measures phosphatase activity at relatively high temperatures against a synthetic phenolic ester, which may not be a direct measure of native phosphatase activity for alcoholic esters, such as inositol phosphates or nucleotides, commonly identified in soils (Cosgrove 1977). Differential adsorption of *p*-nitrophenol between samples is another potential concern (Vuorinen 1993; Amador et al. 1997), however we assume that any sorption of negatively charged *p*-nitrophenol by variable charge sites is reversible after the addition of a strong base.

Organic horizon mass and decomposition

Organic horizon samples were weighed, air-dried and ground to <2 mm in a small Wiley mill. Moisture content was determined by drying at 105 °C. Total P was determined as described above using H_2O_2-$LiSO_4$-H_2SO_4, followed by colorimetric analysis for P_i. Water-extractable P_i in the O horizon was determined by placing 0.5 g of ground material in a centrifuge tube with 25 mL distilled, deionized water. The tubes were shaken for 16 hours on a reciprocating shaker, followed by centrifugation for 15 min at 12,500 rpm. Supernatants were filtered with Whatman #1 filter paper and analyzed the same day for P_i.

To examine the effect of field P addition on the decomposition of the O horizon, a 5 g subsample of the field-moist O horizon was placed in a 50 mL beaker and placed in an incubator at 25 °C for 4 months (25 October 1993 through 5 March 1994). The samples were kept field moist by adding distilled, deionized water weekly to maintain constant weight. Upon completion, the beakers were dried at 75 °C, weighed and analyzed as described above. Subsamples of pre- and post-incubation material were dried to 105 °C to determine oven-dried material lost and heated to 550 °C for 4 hours to determine organic matter content. Percent mass loss was determined by the following equation:

$$\%\, mass\ loss = (X_o - X_T)/X_o \times 100.$$

Decomposition rate (k) was determined by the following equation for decay with no additions:

$$k = -ln(X_T/X_o)/t$$

where X_o is the initial dry mass (105 °C), X_T is the final mass, and t is 0.333 yr (4 months).

Statistical analyses

Concentrations of P within each fraction were compared by two-way ANOVA for site history (length of alder occupation or plot: FA vs. AA), fertilizer treatment and interaction between site history and fertilization. Differences in the means ($p < 0.05$) between subplots were determined by Tukey's honestly significant difference using SYSTAT (Wilkinson 1992). If the assumption of homogeneity of variances (Bartlett's test) was not met, data was natural log transformed and statistical analyses were performed using the transformed data (Wilkinson 1992). Pearson correlation coefficients and probabilities were determined between phosphatase activity at both pH levels and all P fractions. Mass loss and decomposition rates for each O horizon substrate were compared by two-way ANOVA using site history and fertilizer addition as independent variables.

Results

Phosphorus fractions as influenced by site history

Only a small proportion of soil P was found in the available fractions (Water-P_i plus Bicarb-P_i). Approximately 2% of total P was found in these fractions in the control AA soil and 7% in the control FA soil. In the unfertilized soils, over 80% of total soil P was found in the most chemically resistant forms (Figure 1; Hydroxide-P, Acid-P_i and Resid-P). Acid-P_i was approximately 20% of total P in both stands. Organic P varied from 35% of total P in the FA soil to 70% in the AA soil.

The AA stand, with 67 years of alder influence, had more organic P (Bicarb-P_o, Hydroxide-P_o and Resid-P; Figure 1, Table 1), while the FA soil with only 7 years of alder influence contained more sorbed P_i (Bicarb-P_i and Hydroxide-P_i). Total P did not differ by site history. Both Labile and Recalcitrant P_o as determined by ignition-extraction were significantly greater in the AA control soil than the FA control (Table 2). The AA soil also had greater carbon and nitrogen concentrations and N:P ratios (Table 2).

Fertilizer P

The fate of added P was examined using a mass balance approach. We determined the fate of added P in a given form as a proportion of the recovery of fertilizer P in the fertilized soils (Table 3). Recovery was calculated as the difference in total P between the control and fertilized soils (755 mg P kg^{-1} in the FA and 588 mg P kg^{-1} in AA plots). Converting these differences to

Table 1. Soil P fractions in the control and fertilized (+P) plots. Effects represent the probability that site or fertilizer has no effect on P form (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns = not significant). Differences ($p < 0.05$) among means within a column are shown by letters.

Plot		Total P (P_T)	Water P_i	Microbe P	Bicarbonate P_i	Bicarbonate P_o	Hydroxide P_i	Hydroxide P_o	HCl P_i	Resid. P
FA	mg kg^{-1}	1166^{bc}	2^{bc}	36^{b}	79^{b}	40^{b}	477^{ab}	49^{b}	217^{ab}	285^{ab}
	s.d.	385	2	30	58	20	332	22	98	121
	% of P_T	100	0	3	7	3	41	4	19	24
FA + P	mg kg^{-1}	1921^{a}	33^{a}	242^{a}	309^{a}	50^{ab}	724^{a}	86^{ab}	373^{a}	161^{b}
	s.d.	622	33	160	201	25.2	232	54	148	153
	% of P_T	100	2	13	16	3	38	5	19	8
AA	mg kg^{-1}	943^{c}	1^{c}	64^{ab}	16^{b}	88^{a}	113^{b}	147^{a}	151^{b}	362^{ab}
	s.d.	315	1	23	23.7	18.3	103	58	58	110
	% of P_T	100	0	7	2	10	12	16	16	38
AA + P	mg kg^{-1}	1531^{abc}	7^{b}	114^{ab}	105^{b}	93^{a}	382^{ab}	145^{a}	306^{ab}	441^{a}
	s.d.	465	7	93	71	45.2	227	53	156	129
	% of P_T	100	0	7	7	6	25	10	20	29
Effects										
Site		ns	ns	ns	**	***	**	**	ns	**
Fert.		**	*	*	**	ns	*	ns	**	ns
SxF		ns	ns	ns	ns	ns	ns	ns	ns	ns

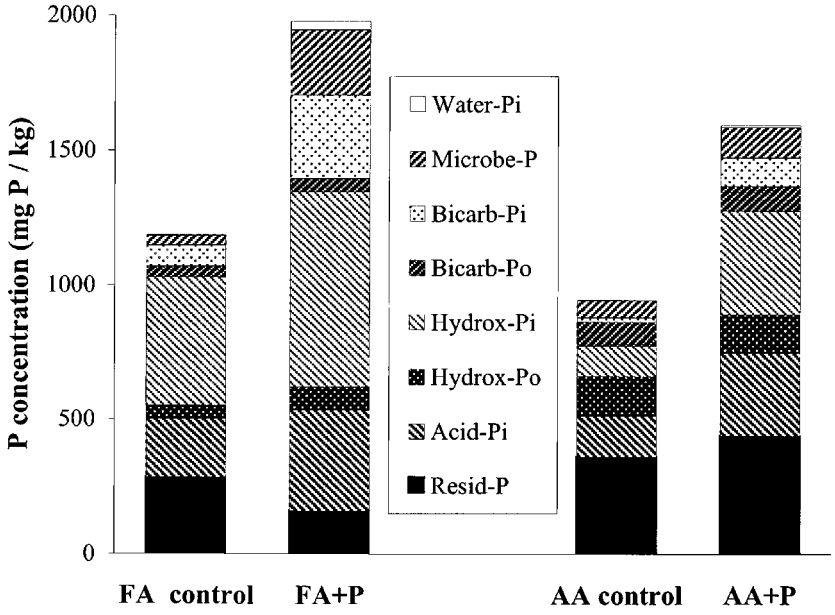

Figure 1. Soil phosphorus fractions in the conversion plot control and fertilized stands.

an areal basis using the <2 mm soil mass for the 0–15 cm soil depth of 522 Mg ha^{-1} (Homann et al. 1992) yields an increase of 394 kg P ha^{-1} in FA + P soil and 306 kg P ha^{-1} in AA + P, accounting for 98% and 77% of the added P, respectively.

Nearly all of the fertilizer P went into inorganic forms. Adsorbed P_i (Hydroxide-P_i plus Bicarb-P_i) was the largest sink, accounting for 55–60% of added P (Table 3). The fertilized soils contained significantly more Bicarb-P_i, Microbe-P, Bicarb-P_i, Hydroxide-P_i and Acid-P_i. Fertilization did not alter Bicarb-P_o, Hydroxide-P_o or Resid-P. Acid-P_i, bound to calcium as in primary mineral apatite, increased by 20–24% in the fertilized soils.

Site history had a minor influence on fertilizer P fate (Table 3). The only interactions between site history and fertilization were for Bicarb-P_i and Microbe-P ($p < 0.10$), where these forms increased only in the FA soil after fertilization. The Microbe-P fraction was a large sink in the FA soil (26% of P added), but was a much smaller sink in the AA soil (8%).

Fertilization did not significantly affect soil C or N concentrations, but decreased C:P and N:P ratios (Table 2). There was a significant interaction between site history and fertilization in C, N, C:P and N:P.

Table 2. Soil C, N and P_o concentrations and element ratios from 0–15 cm depth. Significant effects of site history and fertilizer were analyzed by a two-way ANOVA (†$p < 0.06$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), and mean comparisons were conducted using Tukey's HSD. Differences ($p < 0.05$) among means within a column are shown by letters.

Plot	Total C	Total N	Labile P_o (300°C)	Rec. P_o (550°C)	C:N	C:P	N:P
	g kg^{-1}		mg P kg^{-1}		g g^{-1}		
FA	47.7^{a}	2.32^{a}	315^{a}	87	22^{ab}	43^{a}	2^{a}
s.d.	23.0	0.67	94	28	5	19	1
FA + P	74.8^{a}	2.99^{a}	499^{a}	149	24^{b}	39^{a}	2^{a}
s.d.	30.0	0.69	215	95	6	19	0
AA	121.7^{b}	6.75^{b}	723^{b}	207	18^{ab}	136^{b}	8^{c}
s.d.	36.8	1.250	251	153	3	62	2
AA + P	91.9^{ab}	5.44^{b}	665^{b}	179	17^{a}	62^{a}	4^{b}
s.d.	26.1	1.28	195	178	2	13	1
Effects							
Site	***	***	**	†	**	***	***
Fert.	ns	ns	ns	ns	ns	**	**
SxF	*	*	ns	ns	ns	*	*

Phosphatase activity

While site history and fertilization strongly influenced the concentrations of labile P_i (Table 1), neither of these factors influenced phosphatase activity (Figure 2). The only factors affecting phosphatase activity were incubation pH ($p < 0.10$) and the interaction between pH and site history ($p < 0.05$). Phosphatase activity ranged from 0.95–3.60 μmol *p*-nitrophenol g^{-1} hr^{-1} and was lowest in the AA control soil at pH 4.0. The highest activity was in the same soil at pH 5.0. Incubation pH did not affect phosphatase activity in the FA soil, but the higher pH slightly increased phosphatase activity in the AA soil. At pH 5.0, phosphatase activity was positively correlated with several P_o forms (Bicarb-P_o, $r^2 = 0.542$; Labile ignition-extraction P_o, $r^2 = 0.514$; and total ignition-extraction P_o, $r^2 = 0.539$; all $p < 0.01$).

Table 3. Differences in soil P fraction concentrations between fertilized and control soils. Each fraction is also presented as a percentage of the fertilizer P recovery. * Significantly greater than control soil, results of t-test ($p < 0.05$).

	Difference mg P kg^{-1}	Recovery (% of diff.)	Difference mg P kg^{-1}	Recovery (% of diff.)
	FA		AA	
Water-P_i	30*	4%	6*	1%
Bicarb-P_i	230*	29%	89	14%
Bicarb-P_o	10	1%	9	1%
Microbe-P	205*	26%	50	8%
Hydroxide-P_i	247	31%	269	41%
Hydroxide-P_o	37	5%	–2	0%
Acid P_i	156	20%	155	24%
Residual P	–124	–16%	79	12%
Total	792	100%	655	100%
Labile P_o	184	23%	–58	–9%
Recalc. P_o	66	8%	–14	–2%

Organic horizon mass, decomposition and P release

The AA stand, with a longer period of alder influence, had a larger organic (O) horizon mass (Table 4). Fertilization increased the concentrations of total P and P_i in the O horizon of both stands. Concentrations of P_i averaged approximately 10% of O horizon P in the control plots, and 20% in the fertilized plots. The O horizon mass and P content were not significantly affected by P fertilization, although the FA + P and AA + P O horizons contained 34 and 10 kg P ha^{-1} more P than the respective control plot O horizons. The O horizon was a very small sink for the added P, accounting for an average of 5% of the P added.

During the 4-month incubation, there was a net accumulation of P_i, ranging from 8 to 19% of O horizon P (Figure 3). Final P_i concentrations were 25–100% higher than initial P_i concentrations. Magnitude and percentage of P_i release was highest in the FA + P O horizon, and lowest in the AA control. Average P_i release ranged from 83 to 442 mg P kg^{-1}. Initial O horizon P_i concentration was correlated with release of P_i ($r^2 = 0.681$), but was not correlated with the decomposition rate.

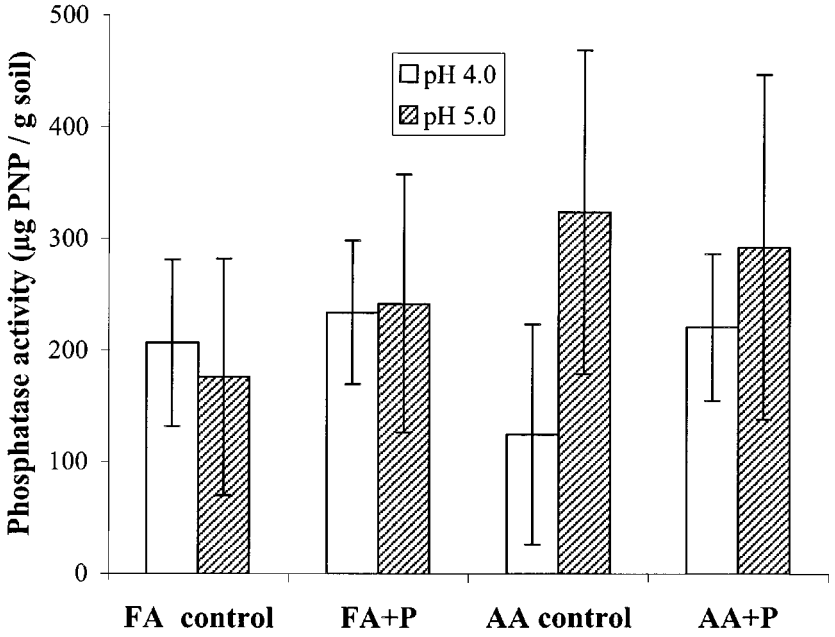

Figure 2. Mean acid phosphatase activity in the conversion plot control and fertilized soils at two pH levels. Phosphatase activity is expressed as μmol *p*-nitrophenol released g soil^{-1} hour^{-1}. No significant differences were observed between mean values ($p < 0.05$). Error bars are $\pm$1 SD.

Mass loss of the O horizon over the 4-month laboratory incubation ranged from 7–15% (Figure 4). Both the decomposition constant (k) and percent mass loss of the O horizon were greater in the fertilized plots ($p < 0.05$ and $p < 0.06$, respectively). Decomposition rate was negatively correlated with initial C/P ratio ($r^2 = 0.472$) and positively correlated with O horizon C content ($r^2 = 0.640$), yet only weakly correlated with C:N ratio ($r^2 < 0.120$).

Discussion

Influence of site history on soil P forms

The soil with a 60-yr longer period of alder influence (AA) had more P_o and less sorbed P_i than the FA soil. This is consistent with previous findings where soils under red alder had more P_o and less sorbed P_i than soil from an adjacent non-fixing Douglas-fir stand (Compton & Cole 1998; Zou et al. 1995). Long-term presence of alder may result in greater accumulation of P_o than found under associated conifers.

Table 4. Organic horizon mass, P concentration and content, and P_i concentration as influenced by site history and fertilization (n = 8 samples per site). Effects are the result of a two-way ANOVA (*p < 0.05, ***p < 0.001).

Plot	Mass Mg ha^{-1}	Total P mg P kg^{-1}	Water P_i mg P kg^{-1}	P Mass kg P ha^{-1}
FA	36.4	1290	141	45.5
s.d.	8.0	58	20	8.6
FA + P	35.3	2370	523	79.7
s.d.	5.6	379	130	14.9
AA	78.2	994	103	78.0
s.d.	12.4	54	20	13.4
AA + P	45.3	2100	413	87.6
s.d.	12.4	256	80	22.1
Effects Site	*	ns	ns	ns
Fert.	ns	***	***	ns
SxF	ns	***	ns	ns

Possible pathways for increasing soil P_o include conversion of P_i into P_o by biotic uptake and turnover, or more rapid formation of recalcitrant P_o forms. Because alder rapidly cycles P through uptake and litterfall (Binkley et al. 1992; Compton & Cole 1998), sorbed P_i may be incorporated into plant and microbial biomass, then converted to P_o. The two-step P_o determination yielded some insight into the nature of this P_o accumulation. Based on the work of Kristensen (1990), low temperature combustion is expected to release P_i from nucleic acids, triglycerides and inositol phosphates, while high temperature combustion is expected to release P_i contained in humic substances. More Labile- and Recalcitrant-P_o was found in the soil with longer alder influence, which suggests that organic matter produced under alder may contain more of this recalcitrant, humic-bound P than that produced by Douglas-fir. Soil solutions under alder had greater concentrations of phenolics (Balci 1964). The high concentrations of phenolics could facilitate the formation and stabilization of humic-bound P under alder.

The patterns and values for soil P_o obtained by the sum Hedley approach were similar to ignition-extraction estimates, although both yielded high coef-

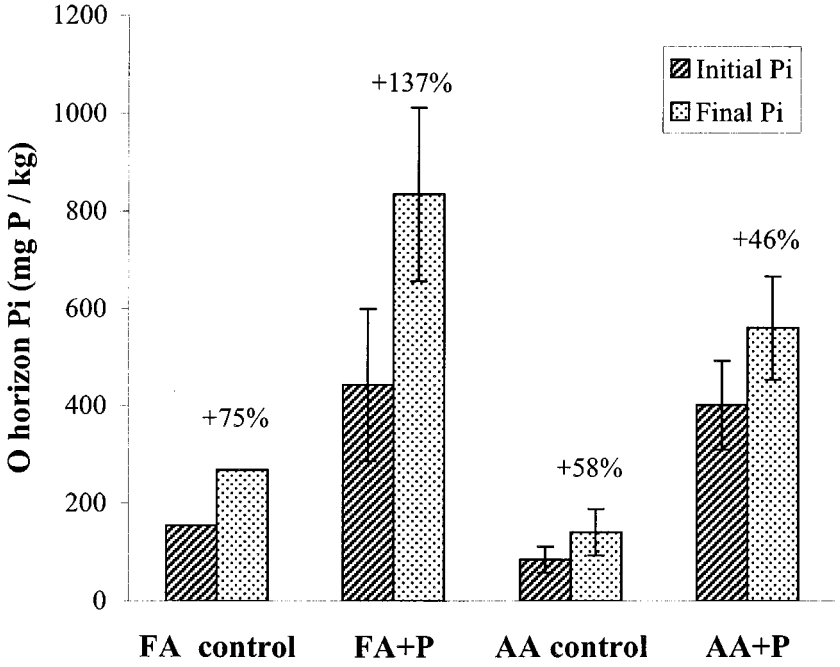

Figure 3. Concentrations of water-extractable P_i in the O horizon before and after the four-month incubation period. Error bars are one standard error above the mean. Relative increase in P_i – P concentration as a percentage of initial concentration is shown in text above bars.

ficients of variation. The ignition-extraction method (Labile + Recalcitrant P_o) and the sum of P_o fractions in the Hedley method (Microbe-P + Bicarb-P_o + Hydroxide-P_o + Resid-P) yielded similar estimates of total P_o for all soils (FA control: 402 vs. 410 mg P kg^{-1}; 648 vs. 539, fertilized; AA control: 930 vs. 663 mg P kg^{-1}; AA + P: 682 vs. 793). The values for the two methods were quite variable, but were in agreement to within one standard deviation of the ignition method ($\pm$249 mg P kg^{-1}). Lajtha et al. (1999) recently indicated that ignition-extraction provides unreliable estimates for certain soils and can yield negative values in tropical soils. The two methods of estimating organic P gave similar estimates in our high P soils, if the Resid-P fraction is considered to be an organic form.

The residual fraction comprised a variable and often large proportion (8–38%) of total soil phosphorus. Few studies examine the nature of this fraction, but the similarity between our sum Hedley and the ignition-extraction estimates suggests that residual P is largely organic in nature in our soils. Tiessen and Moir (1983) include a new step in their fractionation procedure; extracting the final residue with concentrated HCl allows the determination of

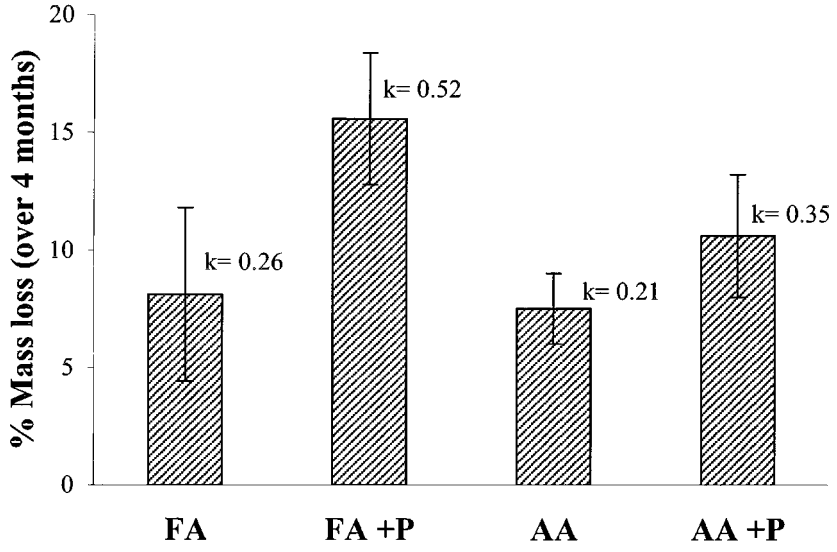

Figure 4. Percent mass loss (bars) and decomposition rate (k, yr^{-1}) of the O horizon as influenced by site history and fertilization. Significant effects as determined by two-way ANOVA on % mass loss: Fertilization ($p < 0.05$ for % mass loss) and ($p < 0.06$ for k). Error bars are ± 1 SD.

extractable P_i and P_o in the residue. In Oxisols, this Conc. HCl-P was largely inorganic in nature, while 71% of residue P was found as P_o in the Mollisols tested (Tiessen & Moir 1983). Residual P may be largely organic in relatively young temperate soils. Research exploring the nature and accessibility of this and other recalcitrant P fractions is needed for a more complete understanding of soil P cycling and availability.

Phosphorus turnover and decomposition rates

Our hypothesis that soil P_i availability is inversely related to phosphatase activity was not supported. There was no relationship between phosphatase activity and site history or P additions; instead we found a positive correlation between phosphatase activity and Labile- and Bicarb-P_o. This correlation with soil P_o is expected, if the presence of hydrolyzable P_o stimulates release of phosphatases by plants or microbes. Phosphatase activity was slightly higher at pH 5 than pH 4 ($p < 0.10$), suggesting that acidic inhibition of the enzymes or sorption of phosphates decreases phosphatase activity at lower pH. Choosing the appropriate incubation pH is clearly important; although all soils had similar activity at pH 5, the AA soil will have lower phosphatase activity in an unbuffered setting. Both the production and mineralization of

P_o may be more rapid in pure and mixed stands of N_2-fixers, since they have greater phosphatase activity than associated non-fixers while at the same time having more P_o (Giardina et al. 1995; Zou et al. 1995). Because P supply often limits the growth of N_2-fixers (Griffith 1978; Sprent & Sprent 1990; Smith 1992; Crews 1993), increasing the production and turnover of P_o may allow this limiting nutrient to be rapidly recycled rather than sorbed to mineral surfaces.

Decomposition of the O horizon was stimulated by P addition in our laboratory incubation, suggesting that P limits late-stage decomposition of this N-rich material. The decomposition rate (k) was not correlated with O horizon N concentration. The decomposition rate was negatively correlated with C/P ratios, which ranged from 100–700, often above the 'critical ratio' of 200–300, below which P_i is released (McGill & Cole 1981; Stevenson 1982).

Inhibition of decomposition by high N levels or limitation by other nutrients might result in slow decomposition of alder litter. There is some agreement that nitrogen addition inhibits late stage decomposition where lignin content is high (Berg 1986; Fog 1988), through formation of recalcitrant products resulting from abiotic reactions between ammonium or amino acids and phenolic groups in lignin byproducts (Stevenson 1982). Red alder leaves have high N levels and average lignin content (Edmonds 1980), which may explain their slow long-term decomposition rate relative to associated species (Cole et al. 1995) or slower short-term rates than predicted from their lignin:N ratios (Harmon et al. 1990). Hobbie and Vitousek (2000) found that both N and P additions stimulated leaf litter decomposition in a low P Hawaiian soil where net primary production was also P limited. In a stream mesocosm, Melillo et al. (1984) found that alder wood chips amended with P decomposed more rapidly than unamended chips, suggesting that available P limited heterotrophic activity. Because P fertilization increased decomposition and P_i release from alder O horizon, the present study indicates that late-stage decomposition of organic matter produced by alder is P limited.

Fate of fertilizer P additions

Retention of P within the mineral soil was much more important than incorporation into the O horizon over the sixteen-month period. An average of 88% of the fertilizer added was accounted for in the surface mineral soil (0–15 cm) after sixteen months, while only 5% was found in the O horizon. The behavior of added P is in strong contrast to the widely-recognized importance of immobilization and mineralization dynamics in soil N cycling. Tracer studies using ^{15}N reveal strong incorporation of N into the O horizon, accounting

for 34–44% of added fertilizer N and 42–62% of simulated atmospheric N inputs were found in the O horizon (Nadelhoffer et al. 1999; Buchmann et al. 1996). Geochemical processes appear to be more important than biotic immobilization in controlling fertilizer P distribution over the time-span of a few years.

The large proportion of P sorbed is not unexpected, given the high sorption capacity of these soils (5000 mg P kg^{-1} under 60-year-old red alder; Johnson et al. 1986). The addition of inorganic fertilizers in cultivated systems consistently increased soil total P and labile P_i in Ultisols (Beck & Sanchez 1996), Mollisols (McKenzie et al. 1992a; O'Halloran et al. 1987), Alfisols (Nziguheba et al. 1998) and Luvisols (McKenzie et al. 1992b). There appears to be a strong sorption sink for added P as P_i in a wide range of soils.

Conversion of fertilizer P to P_o was not an important process in either soil. While P_o accounted for approximately 70% of soil P in the AA control soil, retention as P_o did not occur in the AA + P soil. Thus our first hypothesis was not supported for the sixteen-month study period: site history had no influence on P_o accumulation. Fertilization with inorganic P has been shown to decrease Hydroxide-P_o (Nziguheba et al. 1998; Tran & N'Dayegamiye 1995) or have no effect on soil P_o (O'Halloran et al. 1987; McKenzie et al. 1992a, 1992b; Beck & Sanchez 1994). In P-deficient ecosystems, P_o increased after fertilization, perhaps because of enhanced plant growth and incorporation of plant residues into the mineral soil (Wagar et al. 1986; Richards et al. 1995). Soil P_o accumulation appears to be a longer-term process, involving decades of plant uptake, litter turnover and accumulation of organic matter.

The increase in Acid-P_i after fertilization most likely indicates that not all the fertilizer P has dissociated, even after sixteen months in a moist acidic soil, rather than formation of Ca-phosphates. Other studies have shown an increase in Acid-P_i within a few years of fertilization (McKenzie et al. 1992a; Beck & Sanchez 1994; Richards et al. 1995). Several interpretations of the P fractionation scheme consider Acid-P_i very stable over time; however the fertilizer may act as a substitute for weatherable minerals in these acid soils, and continue to supply P_i for several years.

Site history influenced the retention of added P in the surface soils, with slightly lower retention in the soil with longer alder influence. Approximately 98% of the added 400 kg P ha^{-1} was found in the FA 0–15 cm soil depth, while only 77% was found in the AA soil. The difference in total retention may result from slightly greater P movement downward through the profile in the soil with longer-term alder influence. A 60-year-old red alder stand had slightly greater movement of P below 10 cm soil depth than did an adjacent

Douglas-fir stand, however the net leaching losses below 40 cm were very low and similar between species (0.03 vs. <0.01 kg P ha^{-1} in alder vs. Douglas-fir; Compton & Cole 1998). These small differences in loss rates could be important, however, if P limits decomposition.

Summary

The long-term presence of N_2-fixing red alder increases the accumulation and cycling of P_o through biological/biochemical processes. Over the short-term, however, added fertilizer P did not accumulate as soil P_o, suggesting that P_o accumulation through turnover and decomposition of plant tissues requires more than a few years. Sorption of P in the mineral soil as P_i was the most important sink for fertilizer P. Some portion of this added P was exchanging with the most available pools (Water-P_i, Microbe-P and Bicarb-P_i) sixteen months after P addition in the soil with shorter-term alder influence. Decomposition of organic matter under alder releases relatively large quantities of P_i, which may support the growth and relatively high P demand of this N_2-fixer. In addition, P fertilization increased decomposition of the O horizon, suggesting that late stage decomposition may be P-limited in these N-rich soils.

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