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Changes in phosphorus fractions caused by increased microbial activity in forest soil in a short-term incubation study

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ABSTRACT

The effects of adding larch (*Larix kaempferi*) leaf litter and nitrogen (N) on microbial activity and phosphorus (P) fractions in forest soil were examined in a short-term (28-d) laboratory incubation study. The soil was analyzed using a modified Hedley sequential extraction procedure and an acid phosphatase assay. The addition of larch litter and N increased the acid phosphatase activity and decreased the labile P (H_2O-P + $NaHCO_3$-P) concentration. Compared with addition of larch litter only, addition of both inputs decreased the proportion of inorganic P (Pi) and increased that of organic P (Po) in the NaOH fraction, bound to aluminum and iron oxides. The results of nutrient (carbon, N, or P) addition indicated that acid phosphatase was synthesized to acquire P. This study suggests that, in this forest soil, P in the H_2O-P + $NaHCO_3$-P and in the NaOH-Pi fractions was available for soil microorganisms to decompose leaf litter and that increase in microbial activity eventually translated in an increase in the proportion of Po found in the NaOH fraction in this forest soil.

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KEYWORDS
Phosphorus; acid phosphatase; nitrogen; forest soil; larch leaf litter

Introduction

In forest ecosystem, soil microorganisms play a critical role in the transformation and cycling of P. Organic P in soil organic matter (including plant litter and dead microorganisms) is mineralized to Pi through the action of phosphatases primarily produced by microorganisms, and then Pi is taken up by plants and microorganisms [1]. Microorganisms also secrete organic acids to solubilize P bound to oxides via ligand exchange as well as by ligand-enhanced dissolution of the oxides [2]. The microorganisms can then assimilate the released Pi and decompose the released Po via phosphatase activity [3,4]. Although large amounts of organic matter are added to forest soils by litterfall [5], little is known about the effects of newly added plant litter on microbial activity and the distribution of P in soils. In the present study, we evaluated the effects of larch leaf litter and N inputs on P transformation and acid phosphatase activity in forest soil in a short-term laboratory incubation study.

Materials and methods

Soil and litter samples

The soil sample (Aluandic Andosol) was taken from the A horizon, and larch needle-leaf samples were taken from the Oi horizon in a larch plantation at 1270 m elevation at the Nishikoma Station, Education and Research Center of Alpine Field Science, Shinshu University (35.83°N, 137.87°E). The soil was sieved through 2-mm mesh and homogenized thoroughly. A portion of the soil was air dried for chemical analysis, while the rest was maintained in a field-moist state at 4 °C. The soil properties were as follows (on a dry weight basis): pH (H_2O), 4.5; organic carbon (C), 62.6 mg g^{-1}; total N, 4.8 mg g^{-1}; sand, 370 mg g^{-1}; silt, 350 mg g^{-1}; clay, 280 mg g^{-1} (mineral fraction only). Larch needle-leaves were air dried and then ground with a vibrating sample mill TI-100 (Heiko Seisakusho, Tokyo, Japan) prior to chemical analysis and use in incubation. The larch needle-leaf sample indicated total C, 542 (mg g^{-1} dry weight); total N, 10.6; total P, 0.67; lipids, 87; water-soluble polysaccharides, 76; hemicellulose, 116; cellulose, 109; and lignin, 405 [6].

Incubation experiment

We conducted a laboratory incubation study to evaluate the effects of adding larch litter and N to stimulate microbial activity on P transformation and acid phosphatase activity in the soil. We added N to stimulate soil microbial activity in this study, because previous study showed that N addition remarkably increased microbial activity in larch litter-amended soil [6]. The forest soils were pre-incubated at 60% of water holding capacity for

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1 week at 22 °C and then the following treatments were applied: (1) soil + larch litter, and (2) soil + larch litter + N. After adding ground larch leaf (157 mg g^{-1} soil) and N (1.5 mg N g^{-1} soil as a solution of ammonium chloride), the treatments done in triplicate were mixed well and then incubated at 22 °C for 3, 7, 14, and 28 d. We kept 17 g of soil (on a dry weight) in a loosely capped 50-mL bottle, and distilled water was added occasionally to maintain soil moisture at a constant level.

Phosphorus fractionation

Soil P was fractionated according to Hedley et al. [7] with some modifications [8,9]. Soil samples were dried at 65 °C for 16 h immediately after collection. Then, each sample was sequentially extracted with distilled water, 0.5 M $NaHCO_3$, 0.1 M NaOH, 1 M HCl, and concentrated HCl. After the conc. HCl extraction, residual P was determined in the remaining soil material by digestion with conc. H_2SO_4 + H_2O_2 at 250 °C on a hot plate; H_2O_2 addition was repeated until liquid was clear. The Pi concentrations in all fractions were determined by the Murphy–Riley method [10]. The H_2O, $NaHCO_3$, NaOH, and conc. HCl extracts were digested with persulfate in an autoclave at 120 °C (60 min for H_2O, $NaHCO_3$, and conc. HCl extracts, and 90 min for NaOH extract) [8] for determination of total P. There is rarely any Po in 1 M HCl extracts [8], so only Pi was measured in this fraction. The Po concentration was calculated as the difference between total P and Pi. The entire sequential extraction procedure was conducted only for samples just after preincubation (day 0) and at the end of incubation (day 28). Only H_2O and $NaHCO_3$ extractions were conducted on days 3, 7, and 14 because the floating leaf debris in the extracts made it impossible to accurately fractionate P in these samples.

Because the H_2O-Pi and H_2O-Po concentrations were very low, total P in the H_2O fraction was expressed as H_2O-P. The sum of total P concentrations from H_2O and $NaHCO_3$ fractions was considered to represent labile P. The NaOH extract contained Pi associated with Al- and Fe-oxides, Po originating from humic substances, and Po adsorbed onto Al- and Fe-oxides. The 1 M HCl-Pi fraction represented P derived from primary apatite in carbonate-free soils [11]. The conc. HCl-Pi and conc. HCl-Po fractions represented more stable pools of Pi and Po, respectively, but the conc. HCl-Po fraction may also contain particulate organic matter Po, which may be readily available [8,12]. Residual P was likely to consist of Pi thoroughly occluded by sesquioxides, constituent Pi in resistant primary minerals, and Po in more recalcitrant organic forms [13]. In this study, the sum of P concentrations in each fraction (1070 µg P g^{-1} for original soil) is regarded as the total P concentration in soil.

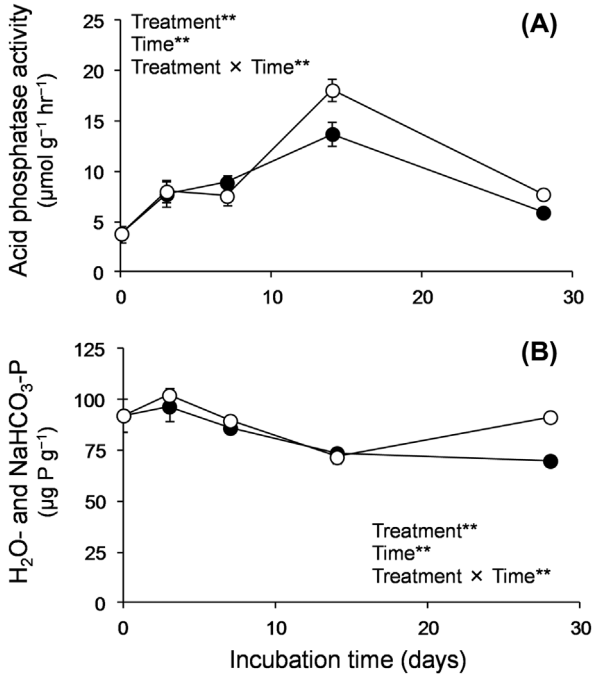

Figure 1. Changes in acid phosphatase activity (A) and H_2O-P + $NaHCO_3$-P concentration (B) in larch leaf litter-amended soil in the presence (○) or absence (●) of N during incubation. Error bars show standard deviation (n = 3); some error bars are smaller than the symbols. **denotes significance at $p < 0.01$ (two-way ANOVA).

Acid phosphatase activity measurement

Acid phosphatase activity was determined for moist soil samples on days 0 (after the 1-wk pre-incubation), 3, 7, 14, and 28. Acid phosphatase activity was determined with *p*-nitrophenyl phosphate as the substrate in a modified universal buffer with pH 6.5 [14]. In brief, the buffer and the substrate were added to 1 g of soil, and then it was allowed to stand at 37 °C for 1 h [14]. After filtration, the color intensity of the filtrate was measured with a spectrophotometer at 400 nm.

Effects of C, N, or P addition on acid phosphatase activity

Four treatments were prepared to examine the effects of C, N, or P addition on the acid phosphatase activity in the soil amended with larch litter: (1) soil + larch litter, (2) soil + larch litter + C, (3) soil + larch litter + N, and (4) soil + larch litter + P. After adding the ground larch leaf (157 mg g^{-1} soil) and C (or N or P) to the preincubated soil, the treatments replicated five times were incubated at 22 °C for 1 week and then acid phosphatase activity was determined. The amounts of added C, N, and P were intended to approximate the stoichiometry of microbial biomass accounting for about half of added C to be released as CO_2. C, N, and P were added, respectively, in the form of glucose (15 mg C g^{-1} soil), ammonium chloride (1.5 mg N g^{-1} soil), and disodium hydrogen phosphate (0.3 mg P g^{-1} soil).

Table 1. Acid phosphatase activity and P concentration in soil ($n = 3$).

		Day 0	Day 3	Day 7	Day 14	Day 28
Acid phosphatase	Litter alone	3.8 ± 0.8	7.7 ± 1.3	8.9 ± 0.7	13.7 ± 1.2	5.9 ± 0.1
(μmol g^{-1} hr^{-1})	Litter + N		8.0 ± 1.1	7.5 ± 0.9	18.1 ± 1.1	7.7 ± 0.4
P concentration (μg P g^{-1})						
H_2O-Pt	Litter alone	9 ± 1	5 ± 1	7 ± 1	8 ± 1	5 ± 1
	Litter + N		7 ± 1	8 ± 1	6 ± 0	6 ± 0
$NaHCO_3$-Pi	Litter alone	29 ± 10	32 ± 2	24 ± 1	21 ± 1	18 ± 1
	Litter + N		28 ± 5	27 ± 0	26 ± 0	38 ± 4
$NaHCO_3$-Po	Litter alone	60 ± 1	60 ± 5	55 ± 1	44 ± 2	47 ± 0
	Litter + N		67 ± 4	54 ± 0	39 ± 3	47 ± 4
NaOH-Pi	Litter alone	299 ± 11				220 ± 20
	Litter + N					136 ± 3
NaOH-Po	Litter alone	341 ± 55				327 ± 18
	Litter + N					611 ± 46
1 M HCl-Pi	Litter alone	48 ± 5				40 ± 4
	Litter + N					43 ± 6
Conc.HCl-Pi	Litter alone	103 ± 7				158 ± 7
	Litter + N					185 ± 11
Conc.HCl-Po	Litter alone	27 ± 1				20 ± 6
	Litter + N					17 ± 1
Residual P	Litter alone	154 ± 45				166 ± 20
	Litter + N					192 ± 44
Sum	Litter alone	1070 ± 107				1002 ± 33
	Litter + N					1275 ± 71

Data at day 0 were for soil without amendment.

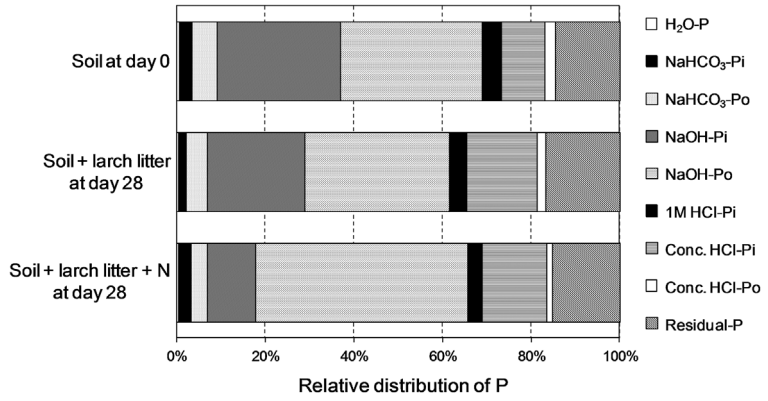

Figure 2. Distribution of P in the different fractions according to the modified Hedley sequential extraction procedure.

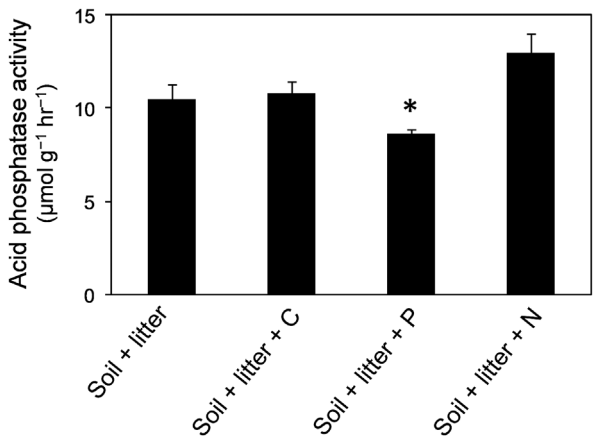

Figure 3. Effects of C, N, or P addition to larch litter amended soil on acid phosphatase activity compared with soil amended with litter alone. Error bars show standard error ($n = 5$); *denotes significant difference ($p < 0.05$) with soil amended with litter only (Dunnett's multiple comparisons test).

Results and discussion

Changes in pH, acid phosphatase activity, and labile P concentration in soil

On day 0, the pH (H_2O) in the soil amended with larch litter was 5.1. It gradually increased to pH 5.5 at day 28, while it dropped to pH 4.2 at day 3 and then slightly increased to pH 4.5 at day 28 when the larch litter was supplemented with N.

Acid phosphatase activity increased after adding the larch litter (Figure 1(A); Table 1), suggesting that this addition stimulated microbial activity. The activity was higher in the larch litter + N-amended treatment than in the treatment amended with larch litter only, especially at days 14 and 28 ($p < 0.01$; Figure 1(A)). The concentrations of labile P (H_2O-P + $NaHCO_3$-P) increased slightly at day 3, and then gradually decreased from day 3 to day 14 in both treatments (Figure 1(B)), even though

the concentration of mineralized P (i.e. available P) was expected to increase during this period because of the increase in acid phosphatase activity (Figure 1(A)). Hence, the microbial uptake rate of P might have been greater than the Po mineralization rate during this period. In the larch litter + N-amended soil, the labile P concentration increased from day 14 to day 28, while it remained unchanged in the soil with litter only. The increase in the labile P in the larch litter + N-amended soil may have been due to the increase in acid phosphatase activity, and/or the release of P from dead microorganisms [7].

Changes in soil P fractions

After the incubation of 28 days, the proportion of NaOH-Pi was lower in the larch litter-amended soil than at day 0, but the opposite was observed for the conc. HCl-Pi fraction (Figure 2; Table 1). In the larch litter + N-amended soil, the proportion of NaOH-Pi was further reduced, with 51% smaller value, and that of NaOH-Po was increased by 47% compared to the soil amended only with litter. These changes could reflect microbially mediated transformation of P in the soil. The decrease in NaOH-Pi, which represents Pi bound to Fe- and Al-oxides, might be driven by microbial assimilation of Pi desorbed by organic acids secreted by microorganisms [2]. Although NaOH-Pi is more stable than H_2O-P + $NaHCO_3$-P [15], this fraction is thought to be available for plants in Luvisols [16], Gleysols [17], and Andosols [18]. The increase in the proportion of NaOH-Po in the larch litter + N-amended soil may be due to the adsorption of Po released from dead microorganisms by Fe- and Al-oxides. Hedley et al. [7] and Hartono et al. [19] suggested that most of the Po released from dead microorganisms are adsorbed to Fe- and Al-oxides in the NaOH-Po fraction. Our results suggested that stimulation of microbial activity by adding plant litter and N increased the desorption and microbial uptake of Pi bound to Fe- and Al-oxides and the release of Po from dead microorganisms back into the soil. The latter event could explain the displacement of a part of NaOH-Pi to NaOH-Po during the incubation of forest soil since the increase observed in microbial activity may have depleted the readily mineralizable organic matter. Moreover, due to the acidic nature of this forest soil, the most important fractions to be affected by decomposition of larch litter would be those associated with Fe and Al, which is reflected in this study.

We noted that the microbially mediated transformation of P in the larch litter into various P fractions was obscure in this study. This is because the analytical error (variation between replicates) of total P measurements (coefficient of variation was ca. 6%) could not accurately determine the fate of P in larch litter (106 μg P g^{-1}), which was much lower than total soil P content (1070 μg P g^{-1}).

Nutrients controlling acid phosphatase activity

To explore the factors affecting acid phosphatase activity in the soil, we assessed the effects of C, N, or P addition. Acid phosphatase activity was higher (but not significant) in the larch litter + N-amended soil than in the soil amended only with litter (Figure 3), consistent with the results described above. This result might reflect its greater microbial P demand (i.e. relatively low P availability) due to the N amendment, which would lead to increased acid phosphatase synthesis to acquire more P. Phosphatase is also suggested to be synthesized to acquire C in soils [e.g. 20]. In the present study, acid phosphatase activity was not affected by C addition, but was significantly suppressed by P addition. These findings are consistent with those of previous studies showing that microbial phosphatase synthesis is primarily controlled by the need for P [12,21–23].

Conclusion

The results of this study suggest that plant litter and N inputs increased microbial activity and P demand, which decreased the labile P concentration and increased the desorption and microbial uptake of Pi bound to Al- and Fe-oxides. This eventually increased the proportion of Po bound to oxides after readily mineralizable organic matter was depleted in the forest soil.

Disclosure statement

No potential conflict of interest was reported by the authors.

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