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Regulation of soil phosphatase and chitinase activity by N and P availability

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Abstract. Soil microorganisms and plants produce enzymes that mineralize organically bound nutrients. When nutrient availability is low, the biota may be able to increase production of these enzymes to enhance the supply of inorganic nitrogen (N) and phosphorus (P). Regulation of enzyme production may be a point where N and P cycles interact. We measured acid phosphatase and chitinase (N-acetyl ß-D-glucosaminide) activity in soil across a chronosequence in Hawaii where N and P availability varies substantially among sites and long term fertilizer plots had been maintained for over 4 years.

Phosphatase activity was high at all sites. Chitinase activity decreased significantly as age and N availability increased across the chronosequence. Phosphorus addition suppressed phosphatase activity at all sites, while N addition increased phosphatase activity at the young, N-limited site. In contrast, N addition repressed chitinase activity only at the N limited young site, and P additions had no effect on chitinase activity. These results suggest that the regulatory relationship between nutrient supply and nutrient mineralization are asymmetric for N and P, and that the differences could help to explain differences observed in patterns of N and P availability.

Introduction

McGill and Cole (1981) proposed a conceptual model that differentiates the factors regulating nitrogen (N) and phosphorus (P) mineralization in soils. They pointed out that carbon (C) and N are bonded together in soil organic matter and thus, N mineralization is coupled to respiration of C by soil organisms. In contrast, most organic phosphorus is bound in phosphate esters, and is mineralized independently of C through catalysis by phosphatase. This model implies that biotic demand for P drives phosphatase production and P mineralization, while demand for C or energy may be driving the mineralization of N. This would result in an inverse relationship between P availability

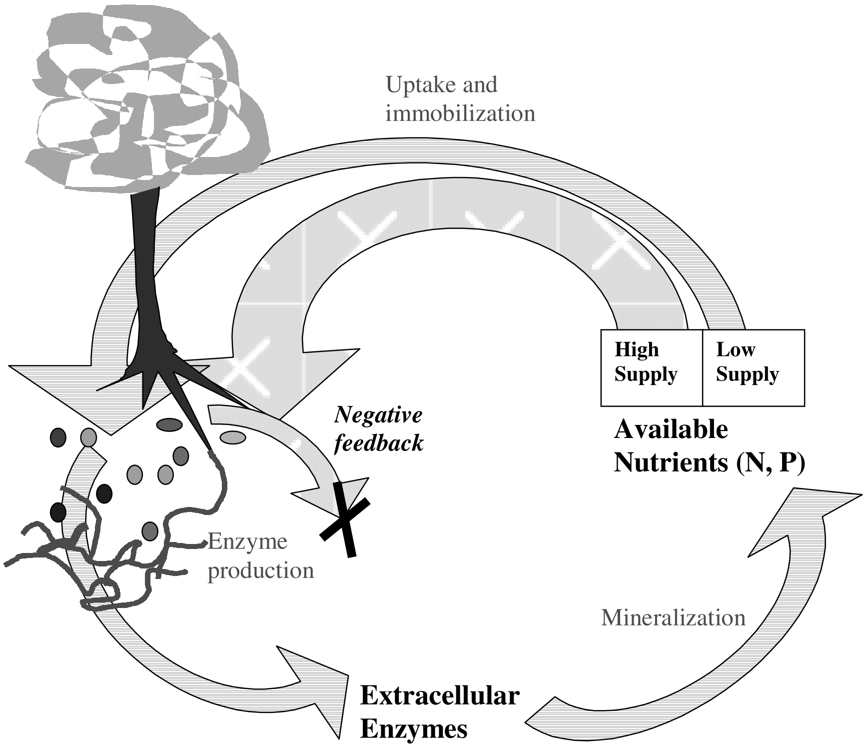

Figure 1. Relationship between nutrient supply and enzyme activity as regulated by a negative feedback mechanism. When nutrient supply is low, enzymes are induced and nutrients are mineralized, but when nutrient supply is high, enzymes are suppressed and mineralization ceases.

and P mineralization, but either a weak link or no link between N availability and N mineralization.

Negative correlations between enzyme activity and nutrient availability are consistent with a mechanism where the production or activity of the enzymes is regulated by the supply of the nutrient that the enzyme mineralizes (Spiers & McGill 1979; Dick et al. 1988; McCarty et al. 1992; Juma & Tabatabai 1978; Sinsabaugh 1994). This negative feedback mechanism could be the primary way in which plants and microbes regulate mineralization in response to nutrient supply (Figure 1). The McGill and Cole model suggests that enzymes involved in N mineralization should be less responsive to variations in N demand than P-mineralizing enzymes are to P demand. While inorganic P (PO_4) can be released directly by enzyme activity, N in organic matter is bound between C atoms in varied configurations, and inorganic N (NH_4) can only be released through multi-step pathways involving a suite of enzymes that selectively eliminate particular types of C-N bonds. In addition, the early products in the decomposition of organic N containing compounds often have fates other than complete mineralization.

The presence of a negative relationship between nutrient supply and enzyme activity has not been explored for N, but has been observed in many studies of P (Sinsabaugh et al. 1993; Tadano et al. 1993; Spiers & McGill 1979; Juma & Tabatabai 1978), supporting the idea that phosphatase production and activity are linked to biotic demand for P (Clarholm 1993). However, other studies have found weak or no evidence of this link (Adams 1992; Harrison 1983; Speir & Ross 1978; Speir & Cowling 1991). These contradictory results may be an incidental consequence of the study sites selected. Where no relationship is seen, P may not limit the study system, some other resource (N, C, organic P substrate . . .) may limit phosphatase production or activity, or enzymes may be stabilized by organic matter and clay minerals (thereby masking changes in synthesis and degradation). Alternatively, perhaps the conceptual model does not work as expected.

The supply of a nutrient could affect not only the enzymes mineralizing that same nutrient, but also those enzymes mineralizing other nutrients. For example, Zou et al. (1995) observed a positive effect of increased N on phosphatase activity. Soils underlying plantations of N-fixing trees increased in both phosphatase activity and P availability. Dick et al. (1988) found that N fertilization in agricultural systems increased acid phosphatase activity. In contrast, Clarholm (1993) found that N fertilization had no effect on phosphatase activity in young Spruce stands in Sweden. Where N supply is low, additions of N may stimulate production of phosphatases because N is essential for enzyme synthesis and because N addition may boost plant and microbial productivity and thus increase demand for P. The converse could also occur, P addition to low P systems could stimulate enzymes involved in N mineralization by boosting productivity and N demand. However, enzymes involved in N mineralization may be less responsive to the supply of P than P-mineralizing enzymes are to N, in that P is not an important component of these enzymes, and enzymes that mineralize N may not be tightly coupled to N demand.

In this study, we evaluated the activity of acid phosphatase (which mineralizes P) and a chitinolytic enzyme (N-acetyl-B-D glucosaminide) that is essential in the mineralization of N from chitin. Acid phosphatase enzymes are the dominant group of enzymes involved in mineralizing P in acidic soils. They cleave ester bonds in a variety of organic phosphorus compounds, and thereby release phosphate (Malcolm 1983; Duff et al. 1994). These phosphatases are produced by plants, fungi, and bacteria, for internal use and in forms that can be excreted. Chitinases are an essential class of enzyme in the main pathway of chitin degradation. They hydrolyze the glucosidic bonds of chitin releasing a smaller N containing organic compound. A common fate of this compound is mineralization to inorganic N (Davis & Eveleigh 1984; Gooday 1994). Chitin is one of the dominant forms of organic N entering soil; it is a major component of arthropod exoskeletons and of fungi (Paul & Clark 1996; Gooday 1994). Chitinases are produced by both fungi and bacteria with fungi being the dominant producers in soils (Gooday 1994). Both phosphatase and chitinase activity are suppressed by their products; high phosphate levels repress phosphatase activity (Rolstone et al. 1975; Nannipieri et al. 1978), while glucose and N acetyl-glucosamine can repress chitinase activity (Gooday 1994). There is no evidence for rapid chitinase repression by NH_4 or NO_3 additions.

The objectives of this study were to determine: (1) how N and P supply may differentially regulate chitinase and phosphatase activity, and (2) to determine whether the supply of one nutrient can play a role in regulating the mineralization of the other through enzyme production.

Methods

Study sites

We used a soil chronosequence in the Hawaiian archipelago along which parent material, elevation, precipitation, slope position, disturbance history, and dominant vegetative cover are relatively consistent (Crews et al. 1995). The sites are between 1,100 and 1,200 m above sea level, in aseasonal montane rainforest dominated by the tree *Metrosideros polymorpha*, with mean annual rainfall near 2,500 mm. We used three sites that vary in nutrient availability, as measured by in situ resin bags (see text below – Crews et al. 1995), and in nutrient limitation to tree growth, as measured by the response of above ground plant productivity to long-term fertilization (Vitousek et al. 1993; Vitousek et al. 1997; Vitousek & Farrington 1997). The 300 year old site has relatively low N and P availability (n = 5: resin P(mean = 0.20 μg/bag/day), resin N-NH_4(3.09), and resin N-NO_3(0.22)), and N limits plant growth. The soil is classified as a *Lithic Hapludand*, dominated by olivine and glass (Vitousek et al. 1997). The 20,000 year old site is high in both N and P (n = 8: P(1.21), NH_4(8.12), and NO_3(4.25)), and neither N nor P alone limit plant growth. The soil is classified as a *Hydric Hapludand*, dominated by noncrystalline allophane and imogolite minerals. The 4.1 million year old site is low in P and high in N (n = 6: P(0.41), NH_4(4.12), and NO_3(10.29)), and P supply limits plant growth. The soil is classified as a *Plinthic Acrudox*, dominated by secondary kaolin and crystalline sesquioxide minerals. (For additional soil characteristics see Table 1).

All three sites contain long term fertilization experiments which have been fertilized with N (50 kg urea-N/ha/yr and 50 kg ammonium nitrate-N/ha/yr), P (100 kg triple super-phosphate P /ha/yr), and N+P (100 kg N and P /ha/yr). At the time of sampling, the plots at the youngest site had been fertilized for 11 yrs, at the intermediate aged site for 4 yrs, and at the oldest site for 6 yrs. The long-term fertilizations are fully factorial randomized complete blocks replicated 4 times at each site. A short-term fertilization was also conducted for 1 month at the youngest and oldest sites, with the same rates of fertilization. The short-term fertilization was fully factorial and randomized plots were replicated 4 times at each site.

Our study system provides an ideal setting to compare how differences in nutrient availability and limitation influence enzyme activity. Well established differences in nutrient availability and limitation between sites allow us to better account for confounding factors such as alternative nutrient limitation. In addition, the long term experimental fertilization plots, provide single nutrient manipulations and have been in place long enough to reduce the possibility that changes are masked by stabilization of enzymes in the soils.

For soil analyses we sampled the complete depth of the O horizon (10–12 cm) and the underlying mineral horizon, that most represented the A horizon, to 10 cm depth. Three samples of each horizon were collected in each plot and composited by horizon. Samples for each study (long or short-term fertilization) at a site were taken in the same day. All three sites were sampled within a one month period.

Laboratory analyses

Soils from O and A soil horizons from all sites and all fertilization treatments were refrigerated at 4 °C for less than 48 hours before enzyme analysis. Soils were hand sorted to remove large roots and rocks. The pool size of inorganic nitrogen was estimated using KCl extraction, in which 10 g soil subsamples were shaken in 50 ml of 2N KCl for 1 minute and then allowed to equilibrate for 24 hours before filtration (Whatman #1, pre-leached with 90 ml of 2N KCl) (Bremner & Keeney 1966). The extractable P pool was estimated using an NH_4F extract (0.03 N NH_4F and 0.025 N HCl), using a ratio of 1:5 soil to solution shaken for one minute and then allowed to equilibrate for 2 hours before filtration (Whatman #1, pre-leached with 90 ml of distilled water) (Bray & Kurtz 1945). Nitrate-N, NH_4-N, and PO_4-P concentrations in extracts were measured using an Alpkem autoanalyzer (Alpkem Corporation, Wilsonville, Oregon, U.S.A.).

Table 1. Soil characteristics for control plots on long age chronosequence in the Hawaiian Islands (Chadwick, pers. com.).

Site	Horizon	Depth (cm)	pH H_2O (1:01)	Bulk Density	Organic C %	Total N %	Total P %	CEC pH 7.0	Exch. Ca	Exch. Mg	Exch. K
								Cmol(+)/kg			
300	O	0–10	4.7	0.32	19.7	0.90	0.070	44.7	5.2	3.82	0.98
years	A	10–18	4.9	1.00	4.0	0.28	0.055	11.1	1.7	0.5	0.6
20,000	O	0–12	3.7	0.29	42.1	1.92	0.070	87.12	2.3	3.2	0.9
years	A	12–20	4.0	0.34	13.9	0.95	0.098	67.0	1.0	0.7	0.3
4,100,000	O	0–7	4.1	0.25	50.7	2.08	0.028	133.3	4.5	9.3	1.6
years	A	7–11	3.8	0.60	27.8	1.59	0.031	79.2	2.2	4.4	0.7

Potential chitinase and phosphatase activity were measured using a method in which enzyme activity releases p-nitrophenol (pNP) from added substrates and levels of pNP are determined colorimetrically (Kuperman & Carreiro 1997). Approximately 2–3 g of soil (DW equivalent) were mixed with 150 ml acetate buffer at pH 5. pNP-phosphatase (Sigma 104 phosphatase substrate) or p-NP-N-acetyl- ß-D-glucosaminide (Sigma N9376) were added to this slurry and incubated at 25 °C for 2 or 6 hours, respectively. These were then centrifuged and the supernatant was mixed with NaOH to stop enzyme activity and bring out the yellow color. Levels of pNP were measured at 410 nm absorbance on a Hitachi U-2000 spectrophotometer. It is not possible to distinguish among abiontic, extracellular, or released intracellular enzymes using this method. However, for enzyme activity to be accounted for in this assay, the enzymes must cleave substrates that are too large to pass through cell membranes; thus, all activity measured occurred outside of microbial cells and plant roots.

Differences between sites were tested with a one-way analysis of variance and means were separated using a least squares mean test. Differences among factorial fertilization treatments were tested using a two-way analysis of variance. Unless otherwise stated, results are reported as significant when $p < 0.05$. Correlations between soil nutrients and enzyme activity were determined using linear regression. (Systat 7.0-Wilkinson 1990).

Results

Potential phosphatase activity in the O and A horizons ranged from 7.8 to 22.7 μmol/g/hr, with significantly higher activity in the O horizon at the fertile intermediate aged site (Table 2, Figure 2). The pattern across the sites was the same for the O and A horizons, with activity in the O horizon higher than that found in the A horizon (Figure 2). Potential chitinase activity in the O and A horizons decreased across the sites, ranging from 5.0 to 0.4 μmol/g/hr (Table 2, Figure 2). Activity at the N limited, young site was more than double that at the two N rich older sites (Figure 2). The pattern in activity was the same in both O and A horizons, but activity levels in the O horizon were double that found in the A horizon at all sites.

Long-term fertilization with P significantly increased levels of extractable P in soils at all sites at the time these samples were collected (data not shown). Long-term fertilization with N significantly increased levels of exchangeable N (NO_3 and/or NH_4) in soils at the youngest and oldest sites, but not in the fertile intermediate aged site (data not shown).

P fertilization significantly decreased phosphatase activity at all sites for both O and A horizons (Table 2). Phosphatase activity in the O horizon at the

Table 2. Potential soil enzyme activity across a soil age and nutrient gradient in the Hawaiian Islands with a factorial fertilization experiment; N, P, and N+P at each site.

Age	Layer	Treatment	n	Phosphatase		Chitinase	
				μmol/g/hr			
				X	SE	X	SE
300 years	Organic	Control	4	10.30	1.86	5.00	1.16
		N	4	16.70*	1.26	2.96**	0.4
		P	4	3.82*	0.44	3.69	0.75
		NP	4	7.90	2.22	2.62	0.45
	A	Control	4	7.86	0.19	2.76	0.46
		N	4	8.39	0.49	2.17	0.42
		P	4	3.48*	0.74	2.19	0.77
		NP	4	3.87	0.10	1.63	0.47
20,000 years	Organic	Control	4	22.71	3.76	1.99	0.19
		N	4	21.53	2.08	1.90	0.41
		P	4	11.38*	1.66	2.27	0.11
		NP	4	12.71	1.09	2.04	0.27
	A	Control	4	9.86	1.11	0.53	0.06
		N	4	11.73	3.31	0.50	0.12
		P	4	6.44*	0.72	0.62	0.03
		NP	4	5.87	0.23	0.44	0.10
4,100,000 years	Organic	Control	4	10.31	0.79	0.83	0.17
		N	4	13.57**	2.02	0.82	0.06
		P	4	7.87*	1.78	0.38**	0.07
		NP	4	4.69	0.59	1.41**	0.23
	A	Control	4	7.82	1.24	0.38	0.11
		N	4	7.57	0.42	0.41	0.05
		P	4	2.73*	0.40	0.51	0.11
		NP	4	3.65	1.11	0.48	0.08

*indicates a fertilizer treatment significantly different from the control ($p < 0.05$).
**indicates a potentially significant treatment ($p < 0.1$).

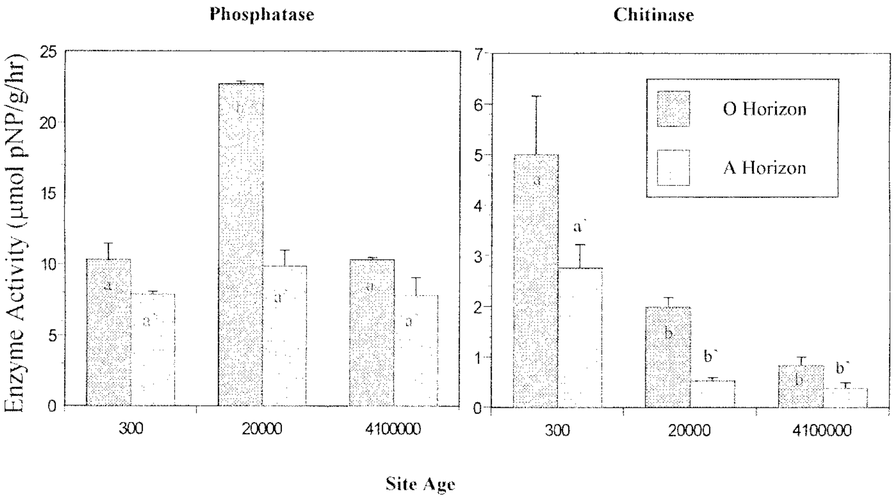

Figure 2. Potential enzyme activity in soil O and A horizons across the Hawaiian Islands. Letters indicate significant differences between means ($p < 0.05$).

N limited young site was significantly stimulated by N fertilizer (Table 2). The stimulatory effect of N combined with the inhibitory effect of P in the O horizon of the youngest plot explains why phosphatase activity in the N+P fertilized plot was higher than that found in the P addition only plots. Nitrogen fertilization had no effect on phosphatase activity in the N-rich intermediate and old-aged sites (Table 2). No significant effects of fertilization were observed in the short-term fertilization study.

Chitinase activity in the O horizon of N fertilized plots at the N poor young site, decreased to about half that found in the control plots (p = 0.06, Table 2). Again, no significant effects of fertilization were seen in the short-term fertilization study.

Discussion

The levels of phosphatase and chitinase activity observed in this study were unusually high (Table 3). The range of phosphatase activities in the unfertilized plots, 7.8 to 22.7 μmol pNP/g soil/hr, is higher than the range found in most other studies across a variety of ecosystems, 0.2 to 12.0 μmol pNP/g soil/hr (Adams 1992; Clarholm 1993; Nannipieri et al. 1978; Harrison 1983; Hausling & Marschner 1989; Amador et al. 1997; Bonmati et al. 1991; Dick et al. 1994, 1988; Tarafdar & Marschner 1994; Rojo et al. 1990; Juma & Tabatabai 1978; Speir & McGill 1979). Only two studies found levels of phosphatase activity within the same range as reported here; 2.0–21.0 μmol

Table 3. The range of acid phosphatase or chitinase activity found in the top soil horizons (O and A) in a variety of ecosystems.

Ecosystem	Phosphatase μmol pNP/g soil/hr	Chitinase μmol pNP/g soil/hr	Reference
Tropical Montane Forest (no fertilizer)	7.8–22.7	0.38–5.00	This study
Temperate Conifer Forest	2.2–20.8		Pang & Kolenko 1986
Temperate Conifer Forest	0.2–0.3		Carlholm 1993
Norway Spruce Forest	2.0–9.0		Haussling & Marschner 1989
Temperate woodland	2.3–7.9		Harrison 1983
Dry Forest (Australia)	3.6–9.3		Adams 1992
Riparian forest	0.2–2.5		Amador et al. 1997
Temperate seasonal pasture	8.6–25.6		Speir & Cowling 1991
Grassland (legumes)	0.9–2.6		Bonmati et al. 1991
Grassland	2.0–5.0		Nannipieri et al. 1978

Table 3. Continued.

Ecosystem	Phosphatase μmol pNP/g soil/hr	Chitinase μmol pNP/g soil/hr	Reference
Temperate pasture			
– Calciferous alkaline soil	4.4		Rojo et al. 1990
– Acidic soil	11.6		Rojo et al. 1990
Temperate agricultural	1.2–9.1		Speir & McGill 1979
– Variety of crops	0.5–1.5		Juma & Tabatabai 1978
– Wheat	1.1		Dick et al. 1988
– Wheat	0.3–0.7		Tarafdar & Marschner 1994
Peru abandoned terraces	1.8–2.5		Dick et al. 1994
Acidic forest soil		0.092–0.129	Spalding & Duxbury 1977
Corn field (fertilized)		0.002–0.012	Rodriguez-Kabana et al. 1983

pNP/g soil/hr in a conifer forest (Pang & Kolenko 1986) and 8.6–25.6 μmol pNP/g soil/hr in a temperate seasonal grassland (Speir & Cowling 1991). In the few published studies where chitinase activity in soils has been measured, it ranges from 0.002 to 0.129 μmol pNP/g soil/hr (Rodriguez-Kabana et al. 1983; Spalding & Duxbury 1977), an order of magnitude less than the range of activity observed in this study.

One possible explanation for these extremely high levels of enzyme activity may be the ability of enzymes, particularly phosphatases, to persist in soils for long periods of time by binding to soil humics and clays (Burns 1982; Sinsabaugh 1994; Rojo et al. 1990), which are abundant and active in soils of volcanic origin like ours (Table 1) (Torn et al. 1997). Bound enzymes may be released during the potential activity assays, stimulating their activity and increasing the levels of activity measured. However, while the high organic matter and clay content in our soils may explain the particularly high activities observed, the pattern in activity observed across the sites is not explained by the variability in organic matter and clay contents found across the sites (Table 1). An alternative explanation is that the aseasonal, warm, moist climate studied here could enhance enzyme persistence and activity.

Although the inhibitory effects of N and P fertilization on enzyme activity were strong in the long-term plots with at least 4 years of fertilization, no response was observed in the short, 1 month fertilizations. This suggests that the presence of high levels of the inorganic products (NO_3, NH_4, and PO_4) does not directly inhibit enzyme activity, but instead represses production (Spiers & McGill 1979; McCarty et al. 1992). It takes time before a negative feedback results in lower levels of activity.

Phosphatase activity changed little across the chronosequence despite significant variation in P supply, while chitinase activity decreased across the chronosequence, reflecting increasing N supply. This difference in the response of phosphatase and chitinase may suggest that N supply in the older soil reaches levels high enough to trigger a negative feedback and inhibit enzyme production, while the range of P supply is never high enough to trigger inhibition.

Results of the fertilization study further support this possibility. Without fertilization, inorganic P pool size and phosphatase were positively correlated. However, fertilization with P significantly decreased phosphatase activity at all sites, showing that the negative feedback mechanism can strongly inhibit phosphatase activity when high enough levels of P are reached. In contrast, chitinase activity and N supply were negatively correlated across the unfertilized chronosequence plots, with apparent inhibition occurring in the two N-rich older sites. Fertilization with N did inhibit chitinase at the

N-limited young site, but had no additional inhibitory effect in the older sites, suggesting that levels of N at these sites were high enough to saturate the negative feedback mechanism.

These results suggest that the background levels of nutrient supply can affect whether a relationship is observed between enzyme activity and the supply of the nutrient mineralized by that enzyme. If the levels of a nutrient are below some threshold level, no negative feedback will be observed without fertilization, as observed for P here. However, if background levels of a nutrient are high, enzyme activity will already be repressed and further additions of the nutrient will not result in additional suppression of enzyme activity as observed here for N.

Differences between the levels of N and P found in our sites and the levels necessary for inhibition of chitinase and phosphatase production can be partially explained by the McGill and Cole (1981) model. P availability and P mineralization are tightly coupled. When high P availability inhibits phosphatase production there is no other pathway of P mineralization. Therefore mineralization of P in excess of demand would rapidly result in a negative feedback repressing phosphatase production and maintaining levels of P below or near the threshold level for inhibition to occur. In contrast, when high availability of N inhibits chitinase production, there are other pathways for N mineralization. In particular, enzymes that mineralize C can continue to release N. Therefore, if C rather than N limits microbial activity, mineralization of N in excess of N demand could occur in response to demand for C. This might sustain levels of N substantially above the threshold level needed to inhibit activity of chitinase (or other enzymes that mineralize N) in unfertilized systems. Alternatively, abiotic sorption may be more important than negative feedback controls on mineralization in maintaining low levels of P in our soils (Frossard et al. 1995).

Overall, this study provides evidence that the role of N and P supply in regulating activity of enzymes that mineralize N and P, and the complementary role of these enzymes in regulating N and P supply, differ for N and P. Phosphatase activity is strongly regulated by P supply and it is possible that production of phosphatase may also play an important role in the regulation of P supply. Similarly, chitinase activity is tightly controlled by N supply. However chitinase production by the biota probably does not regulate N supply. Asymmetry between N and P cycles can be seen not only in self-regulation of enzyme production, but also in the interaction between nutrient cycles. N additions stimulated phosphatase activity in the N limited site, but P additions did not increase chitinase activity at any site.

The stimulation of phosphatase activity by N addition could be through direct use of N as building material for production of N rich enzymes, or more indirectly through increased productivity and thus P demand in response to the alleviation of N deficiency (or both). Where N fertilization increases site productivity and demand for P, the tight coupling of P demand to phosphatase production may result in increased phosphatase activity to meet the increased P demand. However, as McGill and Cole (1981) suggest, demand for N and chitinase activity may not be so tightly linked. P availability did not play any role in the regulation of N mineralization through the pathway of chitin degradation, even in the P limited sites. Even if P fertilization increases site productivity and demand for N, demand for C rather than N may be driving N mineralization. As a result, N may accumulate, increasing N supply above the threshold levels required to suppress chitinase activity. Explanations outside the McGill and Cole model are also plausible. First, a substrate other than P (such as C or a co-factor) may be limiting enzyme production. Second, enzymes involved in other pathways of N mineralization, even those not linked to C mineralization, may be more responsive than chitinase to the increased N demand, elevating N levels above the threshold for chitinase.

Additions of N, whether through fertilizer, N-fixation, or atmospheric deposition, can enhance P mineralization and availability, possibly increasing short-term productivity, in N limited systems. However, an increase in P mineralization may deplete the soil organic P pool over time, accelerating decreases in productivity in the long term.

The strong regulatory cycle between P demand and P mineralization may result in tight regulation of P supply, maintaining levels that meet demand where possible, but never greatly exceed it. In contrast, the incidental mineralization of N that occurs in the process of decomposition (C mineralization) may weaken the regulatory cycle between N demand and N mineralization, potentially resulting in accumulation of N supply substantially above biotic demand.

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