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Responses of extracellular enzymes to simple and complex nutrient inputs

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Abstract

Soil microbes produce extracellular enzymes that mineralize organic matter and release carbon and nutrients in forms that can be assimilated. Economic theories of microbial metabolism predict that enzyme production should increase when simple nutrients are scarce and complex nutrients are abundant; however, resource limitation could also constrain enzyme production. We tested these hypotheses by monitoring enzyme activities and nutrient pools in soil incubations with added simple and complex nutrient compounds. Over 28 days of incubation, we found that an enzyme's activity increased when its target nutrient was present in complex but not simple form, and carbon and nitrogen were available. β-Glucosidase and acid phosphatase activities also increased in treatments where only carbon and nitrogen were added. Glycine aminopeptidase and acid phosphatase activities declined in response to ammonium and phosphate additions, respectively. In some cases, mineralization responses paralleled changes in enzyme activity—for example, β-glucosidase activity increased and respiration was 5-fold greater in soil incubations with added cellulose, ammonium, and phosphate. However, a doubling of acid phosphatase activity in response to collagen addition was not associated with any changes in phosphorus mineralization. Our results indicate that microbes produce enzymes according to 'economic rules', but a substantial pool of mineral stabilized or constitutive enzymes mediates this response. Enzyme allocation patterns reflect microbial nutrient demands and may allow microbes to acquire limiting nutrients from complex substrates available in the soil.

Keywords: Decomposition; Extracellular enzymes; Microbes; Carbon; Nitrogen; Phosphorus

1. Introduction

Extracellular enzymes are the primary means by which soil microbes degrade complex organic compounds into small molecules that can be assimilated. In addition to allowing microbes to access energy and nutrients present in complex substrates, extracellular enzymes catalyze the initial, rate-limiting step of decomposition and nutrient mineralization (Asmar et al., 1994; Sinsabaugh, 1994). This dual role of enzymes in soils means that changes in enzyme production can affect ecosystem processes directly.

Because enzyme production is nitrogen (N) and energy intensive, microbes should only produce enzymes at the expense of growth and metabolism if available nutrients are scarce (Koch, 1985). Fertilization with phosphorus (P) consistently suppresses phosphatase activity (Clarholm, 1993), and concentrations of available nutrients often negatively correlate with the activities of nutrient-releasing enzymes (Pelletier and Sygush, 1990; Chróst, 1991; Sinsabaugh and Moorhead, 1994). Conversely, when available nutrients are scarce, microbes can produce enzymes to mobilize resources from complex sources (Harder and Dijkhuizen, 1983). For example, additions of organic P and cellobiose (an intermediate in cellulose degradation) stimulated phosphatase and β-glucosidase production, respectively, in lake water samples (Chróst, 1991). Similarly, cellulose additions stimulated cellulase activities in wetland soils (Shackle et al., 2000). These findings demonstrate that enzyme production can be an inducible response to the presence of complex substrates.

Many enzymes are also produced constitutively by microbes, which may allow them to detect complex resources in the environment (Chróst, 1991; Koroljova-Skorobogatko et al., 1998; Klonowska et al., 2002). These constitutive enzymes generate low concentrations of

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microbially-available products that induce additional enzyme synthesis when complex substrates are abundant. Once concentrations of products increase enough, enzyme synthesis becomes suppressed and production returns to constitutive levels (Chróst, 1991). In soils, both constitutive and inducible enzymes may associate with mineral and organic compounds, thereby establishing a slow-turnover pool of abiotic enzymes that could aid microbes in the detection and degradation of complex substrates (Burns, 1982).

Although reasonable theories exist to explain enzyme production, the links between enzyme production and nutrient acquisition have not been resolved. Based on microbial economic theory (Koch, 1985), enzyme production should be induced only when it will lead to greater resource acquisition. However, this induction feedback may be weak if substrates are not available, microbes do not strongly regulate enzyme production, or enzyme costs are low enough to allow continuous production. Although one experiment with birch sticks showed that decomposition rates are strongly related to the activities of cellulose- and lignin-degrading enzymes (Sinsabaugh et al., 1993; Sinsabaugh and Moorhead, 1994), the relationship between enzyme concentrations and substrate degradation remains poorly understood for most carbon compounds.

The goal of this paper is to evaluate the relationships between resource availability, extracellular enzyme production, and nutrient dynamics in an infertile tropical soil. Given that microbes should limit enzyme costs while improving resource acquisition (Koch, 1985), we tested the hypothesis that microbial enzyme production increases in the presence of complex resources, but decreases when assimilable carbon (C) and nutrients are available. We also tested whether resource limitation stimulates enzyme production to acquire the limiting resource (sensu Harder and Dijkhuizen, 1983). Finally, we determined if enzyme responses were associated with increases in resource availability, or constrained by resource limitation.

2. Materials and methods

2.1. Soil collection

We collected rainforest soil derived from a 300-year-old volcanic tephra substrate at 1200 m elevation on the Island of Hawaii (Crews et al., 1995). We chose this site because the soils are relatively low in available N and P (Vitousek and Farrington, 1997) and would allow us to apply treatments to a low nutrient background. Four cores separated by at least 1 m were taken to 10 cm depth with a bulb corer every 10 m along a 50 m transect. After shipment to Stanford University, we passed the 20 cores through a 4 mm sieve and mixed the soil by hand to form a homogeneous substrate for laboratory incubations. Subsamples were taken to determine water content, and all subsequent results are reported per gram dry soil.

2.2. Substrate additions

For each of the nutrients C, N, and P, we set up a sub-experiment that included different combinations of complex and simple nutrient additions. We used cellulose, collagen, and cellulose phosphate as complex sources of C, N, and P, respectively, because they are all insoluble and require specific extracellular enzymes for their degradation. Our simple C, N, and P additions were sodium acetate (417 mM), ammonium chloride (286 mM), and sodium phosphate (26 mM), with all solutions buffered to pH 5.0 to mimic the native soil pH (Olander and Vitousek, 2004). Because it is impossible to add N and P in complex form without adding C as well, we minimized the effect of added C by choosing compounds with narrow C:nutrient ratios. Nutrient concentrations in the complex substrates were 44% C in cellulose (assuming a molecular formula of $C_6H_{10}O_5$), and 11% N in collagen and 2.1% P in cellulose phosphate based on Kjeldahl digestion followed by colorimetric analysis on an Alpkem autoanalyzer (Alpkem Analytical, OI, Inc.).

For each sub-experiment (Table 1), we measured enzyme responses to the following treatments: (1) control, (2) complex nutrient added alone, (3) other simple nutrients added alone, (4) other simple nutrients + complex nutrient, and (5) simple nutrient added alone. In each sub-experiment, we expected that complex nutrient addition (treatment 2) would stimulate activity of the focal enzyme, while simple nutrient addition (treatment 5) would suppress it. We expected that other simple nutrients (treatments 3 and 4) would increase demand for the focal nutrient and stimulate focal enzyme activity. For example, adding ammonium + phosphate would increase C demand and stimulate microbes to produce more β-glucosidase activity to obtain C from soil organic matter (treatment 3) or added cellulose (treatment 4).

We added nutrient substrates at rates of 8 mg C (cellulose and sodium acetate), 1.6 mg N (collagen and ammonium chloride), or 320 μg P (cellulose phosphate and sodium phosphate) g^{-1} soil. The quantity of C was chosen to exceed the amount we expected microbes to respire during the incubation, and the quantities of N and P were meant to approximate the stoichiometry of microbial biomass (C:N:P ratio of 25:5:1). On day zero, we added each complex substrate to a batch of soil and partitioned the batch into aliquots for assessing enzyme activities and nutrient pools. Dissolved nutrients were added to the partitioned aliquots in eight increments over time, beginning at day zero and then every 4 days until day 28. The same amount of water was added to all treatments, and soils were kept moist throughout the experiment. We used this addition design because we did not want to further disrupt the soil structure by mixing in complex nutrients every 4 days, and we

Table 1
Experimental design

	C sub-experiment	N sub-experiment	P sub-experiment
Focal nutrient	C	N	P
Focal enzyme	β-glucosidase	Glycine aminopeptidase	Acid phosphatase
Treatment			
(1)	Control	Control	Control
(2) Complex nutrient	Cellulose	Collagen	CelluloseP
(3) Other simple nutrients	Ammonium + phosphate	Acetate + phosphate	Acetate + ammonium
(4) Other simple nutrients + complex	Ammonium + phosphate + cellulose	Acetate + phosphate + collagen	Acetate + ammonium + celluloseP
(5) Simple nutrient	Acetate	Ammonium	Phosphate

Within a sub-experiment, the complex nutrient alone (Treatment 2) provides an incentive for microbes to produce the focal enzyme, while the simple nutrient alone (Treatment 5) should suppress the focal enzyme. Other simple nutrients should stimulate microbes to produce the focal enzyme to obtain the focal nutrient from soil organic matter (Treatment 3) or the added complex nutrient (Treatment 4). CelluloseP = cellulose phosphate.

anticipated that mineralization of the insoluble substrates would be relatively slow. Adding the dissolved nutrients incrementally was intended to mimic this slow release, thereby making microbial responses to complex and simple nutrient additions more comparable.

2.3. Enzyme activities

On days 0, 3, 7, 14, and 28 of the incubation, we measured the activities of extracellular β-glucosidase, glycine aminopeptidase, and acid phosphatase with assay techniques modified from Sinsabaugh et al. (1993). For each treatment, duplicate soil aliquots (0.5 g) were combined with 60 ml 50 mM sodium acetate buffer, pH 5, and homogenized in a blender for 1 min. In a 2 ml centrifuge tube, 0.75 ml homogenate was combined with 0.75 ml pNP (*p*-nitrophenol) substrate in 50 mM acetate buffer and shaken vigorously at 21 °C for 3–4 h (β-gluco-glucosidase), 2–4 h (glycine aminopeptidase), or 1–2 h (acid phosphatase). Substrates were 5 mM pNP-β-D-gluco-pyranoside for β-glucosidase, 2 mM glycine *p*-nitroanilide pre-dissolved in a small volume of acetone for glycine aminopeptidase, and 5 mM pNP-phosphate for acid phosphatase. We then centrifuged the tubes and measured the absorbance of pNP in the supernatant at 410 nm on a Hitachi U-2000 spectrophotometer (Hitachi Instruments, Inc.). Enzyme activities were expressed in units of μmol substrate consumed g^{-1} dry weight h^{-1} using a standard curve of absorbance versus pNP (Sigma 104-1) concentration.

2.4. Nutrient pools

We measured CO_2–C respiration from duplicate 5 g soil aliquots in sealed 1 l mason jars using a base trap followed by titration (Horwath and Paul, 1994). Traps were changed and titrated on days 4, 8, 16, 24, and 31, and the values for each time interval were summed to calculate cumulative respiration. We included soil-free controls to account for background CO_2 levels, which were negligible compared to soil respiration.

After 31 days of incubation, we extracted soluble C and N pools from duplicate 5 g soil aliquots in 50 ml 0.5 M K_2SO_4. C concentrations in extracts were determined using a Shimadzu TOC-5000A analyzer (Shimadzu Corp.), and N concentrations were measured colorimetrically on the Alpkem autoanalyzer after modified high-temperature persulfate digestion (D'Elia et al., 1977; Hedin et al., 1995) and addition of ascorbic acid to remove manganese complexes (Williams et al., 1995). We determined soluble P concentrations by extracting duplicate 2.5 g soil aliquots with 25 ml acid fluoride solution (Olsen and Sommers, 1982) followed by Alpkem colorimetric analysis. These results do not include organic forms of P.

On a separate set of duplicate soil aliquots, we determined microbial biomass C, N, and P using the chloroform fumigation-direct extraction technique (Brookes et al., 1982, 1985). After fumigation, aliquots were extracted and analyzed according to the procedures used for soluble C, N, and P. We used extraction efficiencies of 0.45, 0.54, and 0.50 for microbial biomass C, N, and P, respectively.

2.5. Statistical analyses

For each sub-experiment, we tested for significant treatment and time effects on enzyme activities using two-way analysis of variance (ANOVA) within SAS PROC GLM (SAS Institute, 2001). Treatment effects on nutrient pools were tested with one-way ANOVAs. Post-hoc mean separations were done using Tukey's HSD test with the threshold for significance adjusted to $P = 0.0125$ to account for multiple comparisons between each of four treatments and the control within each sub-experiment.

3. Results

When cellulose was added in combination with ammonium and phosphate, β-glucosidase activity increased sharply by day 7 and reached levels 36% greater than controls by day 28 (Fig. 1(A)). By contrast, cellulose added

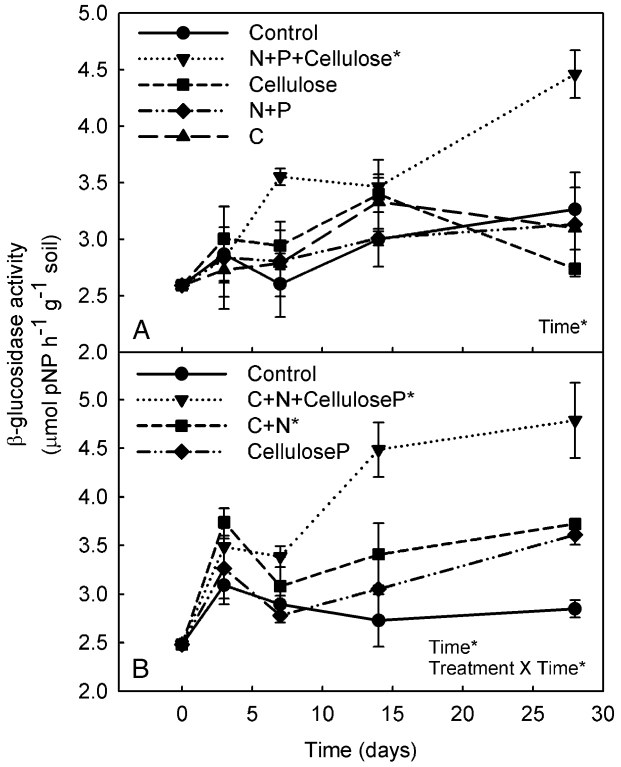

Fig. 1. β-Glucosidase activities from the C sub-experiment (A) and the P sub-experiment (B). Symbols and bars represent means and standard errors of duplicate samples. Asterisks denote significance at $P<0.05$. C, sodium acetate; N, ammonium chloride; P, sodium phosphate; CelluloseP, cellulose phosphate.

alone had no effect on β-glucosidase activities. β-Glucosidase activity also increased significantly in response to cellulose phosphate additions when acetate and ammonium were supplied, but did not respond to cellulose phosphate alone (Fig. 1(B)). β-Glucosidase activities also did not respond significantly to ammonium + phosphate or any other treatments except acetate + ammonium, where activity increased by 30% (1 μmol pNP h^{-1} g^{-1}) over the control (Fig. 1(B)).

In the acetate + phosphate + collagen treatment, glycine aminopeptidase activities increased by ~0.2 μmol pNP h^{-1} g^{-1} over controls after day 7, leading to a significant overall treatment effect (Fig. 2). There was no significant response to collagen alone, or to acetate + phosphate (Fig. 2). Glycine aminopeptidase activity declined significantly relative to the control in response to ammonium addition, ultimately reaching 0.7 μmol pNP h^{-1} g^{-1} (Fig. 2).

Acid phosphatase activities responded strongly to C + N additions, regardless of their form. Acetate + ammonium additions rapidly caused a >25% increase in acid phosphatase activity which was sustained over 28 days (Fig. 3(A)). This increase was mitigated by adding cellulose phosphate, suggesting that available orthophosphate was readily liberated from the polymer (Fig. 3(A)). Activity more than doubled to 71 μmol pNP h^{-1} g^{-1} in response to collagen

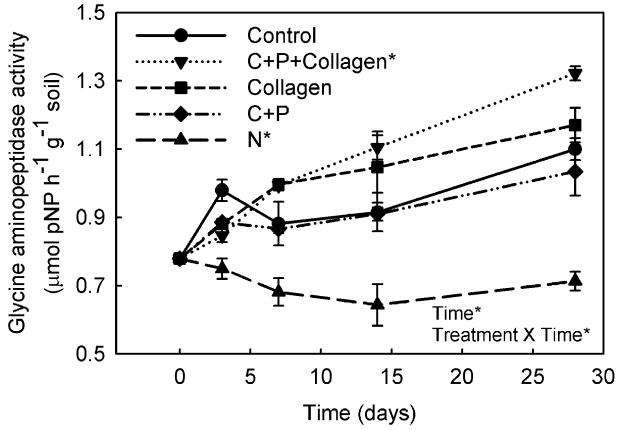

Fig. 2. Glycine aminopeptidase activities from the N sub-experiment. Symbols and bars represent means and standard errors of duplicate samples. Asterisks denote significance at $P<0.05$. C, sodium acetate; N, ammonium chloride; P, sodium phosphate.

additions, although acetate + phosphate + collagen addition suppressed this response as P accumulated in the soil over time (Fig. 3(B)). The remaining treatments, including cellulose phosphate and sodium phosphate (Fig. 3(A)), had no significant effects on phosphatase activity.

Cumulative soil respiration responded positively to addition of all C-containing compounds, but negatively to

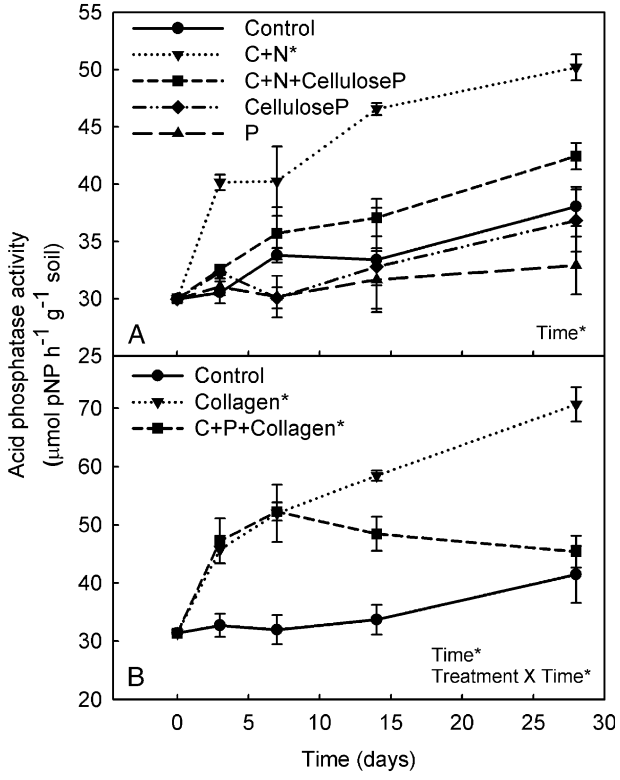

Fig. 3. Acid phosphatase activities from the P sub-experiment (A) and the N sub-experiment (B). Symbols and bars represent means and standard errors of duplicate samples. Asterisks denote significance at $P<0.05$. C, sodium acetate; N, ammonium chloride; P, sodium phosphate; CelluloseP, cellulose phosphate.

Table 2
Fates of C, N, and P in soil incubations

	Soluble C (mg g^{-1})	Microbial biomass C (mg g^{-1})	Cumulative respiration (mg C g^{-1})	Soluble N (μg g^{-1})	Microbial biomass N (μg g^{-1})	Soluble P (μg g^{-1})	Microbial biomass P (μg g^{-1})
C sub-experiment							
C control	0.420±0.005	3.68±0.05	6.40±0.03	9.2±0.2	195.2±3.3	0.39	96.1
Cellulose	0.454±0.030	3.64 ±0.08	**7.31±0.06**	9.4±1.7	189.2±6.3	0.17	72.5
Ammonium + phosphate	0.430±0.003	3.73±0.05	**5.17±0.03**	**222.4±1.2**	**659.4±46.5**	**40.30±2.51**	236.0
Ammonium + phosphate + cellulose	**0.555±0.012**	4.15±0.17	**10.81±0.02**	**219.3±0.2**	**588.7±7.3**	26.57±1.04	145.6
Acetate	**0.970±0.014**	**4.38±0.02**	**11.52±0.07**	11.0±1.4	171.7±6.4	1.02	103.5
N sub-experiment							
N control	0.418±0.001	3.52±0.03	6.54±0.11	9.2±0.5	178.5±1.8	0.76±0.08	87.6±1.0
Collagen	0.422±0.003	3.45±0.07	**8.54±0.16**	**19.7±1.2**	214.3±2.6	0.69±0.08	87.5±3.1
Acetate + phosphate	**1.028±0.019**	**4.15±0.15**	**11.56±0.02**	13.4±0.8	166.1±1.8	**46.78±1.04**	**228.6±4.1**
Acetate + phosphate + collagen	**1.095±0.030**	**4.57±0.02**	**14.16±0.01**	13.9±0.5	**239.7±9.6**	**29.07±3.00**	**240.2±4.5**
Ammonium	0.404±0.005	3.60±0.07	**5.00±0.08**	**205.4±1.7**	**690.7±11.9**	0.70±0.13	89.4±0.1
P sub-experiment							
P control	0.404±0.003	3.45±0.06	6.58±0.07	9.7±0.3	180.2±5.0	0.48±0.08	79.7±0.4
CelluloseP	**0.618±0.001**	3.33±0.04	**8.25±0.13**	12.4±2.3	182.4±6.3	0.71±0.39	107.2±0.8
Acetate + ammonium	0.430±0.022	3.53±0.01	**10.77±0.09**	**183.2±9.1**	**665.1±24.9**	0.28±0.01	99.3±3.9
Acetate + ammonium + celluloseP	0.460±0.014	3.51±0.16	**12.66±0.10**	**178.1±3.0**	**684.7±10.9**	1.63±0.15	**133.3±14.7**
Phosphate	0.465±0.011	3.46±0.04	7.10±0.19	11.0±0.1	173.3±0.4	**28.65±2.02**	**217.2±13.0**

Values represent means (±standard error) after 28 days of incubation with C and nutrient substrates ($N=2$). Means significantly different from their respective sub-experiment control within columns are denoted in bold text ($P<0.05$, corrected for multiple comparisons). $N=1$ for values without standard errors due to missing data.

ammonium addition. Cumulative basal respiration was ~6.5 mg C g^{-1} soil over 28 days, and increased to 7.3 mg C g^{-1} soil in the cellulose addition treatment (Table 2), an increase equivalent to less than 10% of the added cellulose. When ammonium and phosphate were also present, respiration increased to 10.8 mg C g^{-1} soil, equivalent to 50% of the added cellulose. By contrast, ammonium additions significantly reduced cumulative respiration to 5 mg C g^{-1} soil, whether alone or in combination with phosphate (Table 2). Other C-containing substrates, such as acetate, collagen, and cellulose phosphate also significantly increased cumulative respiration during the incubations (Table 2).

Soluble and microbial biomass C pools responded only slightly to substrate additions, with acetate causing increases in both pools except when added with ammonium (Table 2). Cellulose additions increased soluble C by 0.13 mg g^{-1} soil and microbial biomass C by 0.5 mg g^{-1} soil when ammonium and phosphate were present, although the biomass change was not statistically significant (Table 2). Cellulose phosphate addition also resulted in a slight but significant increase in soluble C, but there was no evidence that additional C was incorporated into microbial biomass (Table 2).

Collagen additions affected soil N pools most strongly when acetate and phosphate were also added. Microbial biomass N increased significantly from 178.5 to 239.7 μg g^{-1} soil in the combined treatment, but only increased to 214.3 μg g^{-1} soil in the collagen treatment (Table 2). The collagen treatment resulted in a doubling of soluble N, and a total increase of 46.3 μg N g^{-1} soil in the soluble + microbial pool. However, this combined pool contained 65.9 μg N g^{-1} soil with acetate and phosphate present. These changes corresponded to respiration increases of 2000 and 2600 μg C g^{-1} soil, respectively (Table 2, with respiration in acetate treatment subtracted out).

Some form of N addition was required to increase soluble and microbial N pools; without collagen present, acetate + phosphate addition had no effect on measured N pools (Table 2). As expected, ammonium additions caused large increases in soluble and microbial biomass N pools, although more than 50% of the 1.6 mg N g^{-1} soil added was not recovered in these pools.

Analogous to the collagen treatments, soluble and microbial P pools responded to cellulose phosphate addition most strongly when labile C and N were present. In the cellulose phosphate only treatment, soluble + microbial P increased by 27.7 μg P g^{-1} soil, in contrast to

54.7 μg P g^{-1} soil in the acetate + ammonium + cellulose phosphate treatment (Table 2). These changes were associated with respiration increases of 1670 and 1890 μg C g^{-1} soil, respectively. As expected, phosphate additions caused dramatic increases in soluble and microbial biomass P, and generally >50% of added P was recovered in these pools.

4. Discussion

By increasing the supply of complex substrates in the soil environment, we expected to induce the microbial community to produce extracellular enzymes that could exploit these new resources. This process would allow enzyme producers to convert the complex substrates into usable products that could be assimilated and used for biomass growth. However, we found that adding a complex substrate alone never significantly increased the activity of the focal enzyme or the microbial biomass (Table 2). Cellulose addition had no effect on β-glucosidase activity, and the increase in respiration we observed represented only 10% of the amount of C added. Similarly, collagen and cellulose phosphate additions did not increase glycine aminopeptidase or acid phosphatase activities, although constitutive enzyme levels may have allowed for some degradation and assimilation of these substrates. Thus in this soil, the presence of a potential resource is not by itself sufficient to stimulate microbial enzyme production or increased microbial biomass.

Adding simple and complex resources in combination revealed that enzyme production probably depends most strongly on microbial demand and the availability of C and N for enzyme synthesis. By adding ammonium and phosphate with cellulose, we apparently made C limiting relative to available N and P, thereby providing a strong incentive for microbes to invest in C acquisition. At the same time, because N was abundant, N-rich enzymes may have become a relatively inexpensive means of accessing the C substrates in the soil. Similarly, adding acetate and phosphate with collagen appeared to make N limiting relative to C and P, thereby stimulating glycine aminopeptidase activity. This response is somewhat paradoxical because N was potentially limiting, yet microbes responded by producing N-rich enzymes. However, pre-existing or constitutively produced enzymes are probably able to stimulate enough collagen breakdown to supply N for the initial synthesis of aminopeptidases. Once microbes produce a small amount of new enzyme, collagen degradation can proceed more rapidly, further increasing the supply of N.

Results from several other treatments also indicate that sufficient supplies of C and N are a prerequisite for microbial enzyme production. Of all the simple nutrient treatments, only acetate + ammonium caused significant increases in enzyme production, for both β-glucosidase and acid phosphatase. Complex C + N in the form of collagen caused even more dramatic increases in phosphatase activity (Fig. 3(B)), possibly because converting collagen into enzyme protein avoids the cost of amino acid synthesis (Akashi and Gojobori, 2004). In contrast to other soils where labile C alone may stimulate enzyme activities and N mineralization, (Asmar et al., 1994), we did not observe any enzyme response unless the treatment included some form of added C and N. This difference is reasonable because our soils are N-poor (Harrington et al., 2001), and microbes probably do not have access to a readily available N source to build enzymes.

Given the low N concentrations in our soil, we were surprised to find that ammonium-only additions actually reduced soil respiration and did not stimulate enzyme activity. Reduced respiration is a common response to mineral N fertilization, and this has been suggested to reflect microbial reallocation of C to biomass or enzyme production (Schimel and Weintraub, 2003). We measured both of these parameters and found no increases, suggesting that microbes are mineralizing less C from protein sources due to reduced protease production (as in Fig. 2), or that ammonium has a direct negative affect on microbial respiration rates. Although enzyme synthesis requires that both C and N be available, neither resource alone stimulated enzyme production or vigorous microbial growth in our soils.

Even when resources were available to increase enzyme production, nutrient mineralization was minor unless a complex substrate was added to the soil. This pattern was evident for phosphatase activity in response to collagen and acetate + ammonium treatments—with C and N inexpensive and P in demand, microbes shuttled resources into phosphatase production but mobilized only small amounts of P from the soil (Table 2). Only when a complex P substrate was added did enzymatic catalysis allow for significant P uptake and immobilization (Table 2). Despite their polymeric form, the substrates we added were probably more physically and chemically accessible to enzymes than compounds native to the soil.

While many of our results conformed to predictions based on resource allocation theory, some of our results suggest that microbes do not regulate the entire pool of soil enzyme activity on short time scales. A substantial fraction of soil enzyme activity could be constitutively produced (Koroljova-Skorobogatko et al., 1998) or stabilized on mineral surfaces and thus decoupled from microbial regulation (Burns, 1982). For example, adding available C and N with cellulose phosphate increased P availability without significantly increasing acid phosphatase activity (Table 2, Fig. 3(A)). This result contrasts with the acetate + ammonium (Fig. 3(A)) and collagen (Fig. 3(B)) treatments, which both caused significant increases in phosphatase activity due to potentially

greater P demand. When cellulose phosphate was added in combination with available C+N, increased P demand may have been met through the action of constitutive or mineral stabilized phosphatases on cellulose phosphate, thereby making additional phosphatase synthesis unnecessary. Pre-existing soil phosphatases also appeared to increase P availability slightly in the cellulose phosphate only treatment, although the increase was not statistically significant (Table 2).

The persistence of some enzymes even under high nutrient availability provides additional evidence for mineral stabilization and/or constitutive enzyme production. For example, β-glucosidase did not decline after acetate addition (Fig. 1(A)), and acid phosphatase did not decline significantly after phosphate addition (Fig. 3(A)). In contrast, the recently-produced phosphatase activity associated with collagen addition may have been more dependent on sustained enzyme production, and more responsive to nutrient availability, as evidenced by the drop in activity as we added available P (Fig. 3(B), compare dotted and dashed lines). Elevated glycine aminopeptidase activity in the acetate + phosphate + collagen treatment (Fig. 2) could have also accelerated loss from this newly-produced, labile phosphatase pool because glycine aminopeptidase catalyzes protein degradation.

Overall, our results support the hypothesis that enzyme-dependent C and nutrient mineralization is constrained by 'microbial economics'. When assimilable resources are available, microbes may decrease the production of enzymes that degrade complex substrates, consistent with the hypothesis that abundant labile nutrients inhibit the decomposition of more recalcitrant soil compounds (Schimel et al., 1992; Moorhead and Linkins, 1997). However, constitutive enzyme production and stabilization mechanisms may prevent enzyme activities from falling below basal levels. When a resource is limiting, microbes may benefit from producing enzymes to obtain it, but could be constrained by the availability of C and N required for enzyme synthesis. If C and N are available, even in complex forms, investment in enzymes becomes an inexpensive means of extracting additional resources from the soil. However, this investment does not necessarily increase nutrient availability unless complex substrates are abundant in the soil.

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