

The effects of substrate composition, quantity, and diversity on microbial activity

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Abstract Variation in organic matter inputs caused by differences in plant community composition has been shown to affect microbial activity, although the mechanisms controlling these effects are not entirely understood. In this study we determine the effects of variation in substrate composition, quantity, and diversity on soil extracellular enzyme activity and respiration in laboratory microcosms. Microbial respiration responded predictably to substrate composition and quantity and was maximized by the addition of labile substrates and greater substrate quantity. However, there was no effect of substrate diversity on respiration. Substrate composition significantly affected enzyme activity. Phosphatase activity was maximized with addition of C and N together, supporting the common notion that addition of limiting resources increases investment in enzymes to acquire other

limiting nutrients. Chitinase activity was maximized with the addition of chitin, suggesting that some enzymes may be stimulated by the addition of the substrate they degrade. In contrast, activities of glucosidase and peptidase were maximized by the addition of the products of these enzymes, glucose and alanine, respectively, for reasons that are unclear. Substrate diversity and quantity also stimulated enzyme activity for three and four of the six enzymes assayed, respectively. We found evidence of complementary (i.e., non-additive) effects of additions of different substrates on activity for three of the six enzymes assayed; for the remaining enzymes, effects of adding a greater diversity of substrates appeared to arise from the substrate-specific effects of those substrates included in the high-diversity treatment. Finally, in a comparison of measures of microbial respiration and enzyme activity, we found that labile C and nutrient-acquiring enzymes, not those involved in the degradation of recalcitrant compounds, were the best predictors of respiration rates. These results suggest that while composition, quantity, and diversity of inputs to microbial communities all affect microbial enzyme activity, the mechanisms controlling these relationships are unique for each particular enzyme.

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Introduction

Microbes produce enzymes to acquire energy and nutrients through the breakdown of complex organic substrates, and indeed the activity of extracellular enzymes has been linked to rates of decomposition (Carreiro et al. 2000; but see Keeler et al. 2009) and nutrient mineralization (Allison and Vitousek 2005). Along with abiotic factors, the activity of extracellular enzymes is regulated by the inputs of resources from plants, since detritus, throughfall, and root exudates serve as sources of substrates for enzymatic degradation and provide the energy and elements necessary for enzyme synthesis. Thus, variation in plant inputs to soils, arising from changes in plant productivity, community composition, or resource allocation, have the potential to cause variation in extracellular enzyme activity with implications for ecosystem functioning (Hooper et al. 2000; Waldrop and Firestone 2006).

Most studies examining the effect of substrate composition on enzyme activity have used changes in plant community composition as a proxy for changes in substrate composition. While these studies have demonstrated the importance of plant community composition in influencing enzyme activity (Kourtev et al. 2002; Chung et al. 2007), field studies are unable to distinguish the effects of changes in substrate composition from other coincident changes in inputs (e.g., the total amount or diversity of substrates) that result from changes in plant community composition. Thus, these studies are unable to elucidate the specific mechanisms underlying the effects of changes in plant composition on enzyme activity and their relative importance remains unclear.

There are two main mechanisms by which variation in substrate composition could affect extracellular enzyme activity. Because the production of enzymes requires microbial investment of energy in the form of C, and nutrients, particularly N, they are energetically and nutritionally expensive to produce and thus their activity may increase with increasing availability of labile C and N (e.g., Schimel and Weintraub 2003). Previous studies have shown that increases in C and N availability can increase enzyme activity (Carreiro et al. 2000; Gallo et al. 2004; Waldrop and Firestone 2006), but that the effect of these additions depends on the enzyme of interest and the form of C or N added. This mechanism, hereafter referred to as the

resource limitation model, predicts that additions of a resource will increase enzyme activity, likely of those enzymes that acquire resources that are not otherwise readily available (Schimel and Weintraub 2003) or for which demand is increased by the added resources. For example, previous studies have shown that acid phosphatase activity increases with the addition of inorganic N fertilizer (Olander and Vitousek 2000; Sinsabaugh et al. 2002; Keeler et al. 2009) and that addition of labile C sources stimulates the decomposition of more recalcitrant C (i.e., via the priming effect, Kuzyakov 2002; Fontaine et al. 2004). In the resource limitation model, nutrient additions might inhibit the activity of enzymes involved in acquiring the added nutrient (Sinsabaugh and Moorhead 1994), while increasing the activity of enzymes to acquire other nutrients or other forms of C (Harder and Dijkhuizen 1983).

Alternatively (although not mutually exclusively), enzyme activity simply could be regulated by the presence of the substrate that it degrades (hereafter referred to as the *substrate stimulation model*). This model would predict an enzyme-specific response to substrate additions in which substrate additions would increase the activity of enzymes involved in degrading the added substrate, but have little effect on other enzymes not associated with the added substrate. Indeed, previous studies have shown that chitin additions can stimulate the activity of chitin-degrading enzymes (Smucker and Kim 1987). Stimulation of enzyme activity by the addition of its substrate could arise either from specific microbes increasing their production of a particular enzyme or from a shift in the composition of the microbial community to taxa better able to use the added substrate.

While substrate composition certainly plays a major role in regulating enzyme production, microbial activity could also be influenced by the diversity and quantity of substrates available to microbes. Increasing the number of unique substrates could influence enzyme activity via both mechanisms outlined above. For example, the substrate stimulation model suggests that increasing substrate diversity should increase enzyme activity overall by providing substrate for a greater number of enzymes. This stimulation could occur because greater substrate diversity increases the number of niches available to microbes, supporting a more

diverse microbial community (as hypothesized in Grayston et al. 1998). The resource limitation model predicts that the type and magnitude of effects on enzyme activity depends on the specific composition of the substrates added. For example, under the resource limitation model, synergistic effects could arise from addition of complementary resources, e.g., addition of compounds containing both C and N might increase activity of enzymes more than addition of either C or N alone. Effects should depend on how the diversity of added substrates alters the demand for other elements and thus the activity of enzymes involved in acquisition of those elements.

The quantity of available resources may also affect microbial extracellular enzyme activity. Although we know of no studies to date that have investigated the effect of substrate quantity on enzyme activity directly, differences in substrate concentration has been shown to influence microbial community composition in soil (Griffiths et al. 1998). We expect that increased substrate quantity could influence microbial community composition and result in differences in enzyme production. Under the resource limitation model, activity of enzymes whose production is resource-limited should increase even more with greater resource additions, thereby inducing stronger demand for nutrients in short supply. The addition of more substrate may or may not induce greater activity of the enzyme that breaks it down, depending on whether microbes are able to shift allocation of enzymes towards increased production of that particular enzyme. This shift could occur either because of a shift in enzyme production within the existing community, or because of a change in community composition induced by the addition of substrate (Carney et al. 2007).

In this study, we examine how the composition, quantity, and diversity of inputs to microbial communities can influence the activity of extracellular enzymes. Furthermore, extracellular enzyme activity has been linked to rates of decomposition and nutrient cycling in field studies (Carreiro et al. 2000; Allison and Vitousek 2005), although we know of no direct comparisons of enzyme activity and microbial respiration rates in the same soil. We expect that enzyme activity may provide a mechanistic link by which variation in inputs to soil may influence soil processes such as carbon mineralization.

Materials and methods

To distinguish among the effects of the composition, quantity, and diversity of organic inputs on microbial enzyme activity and respiration, we conducted a substrate addition experiment in which nine substrates representing common constituents of plant litter were added singly and in various combinations and quantities to soil microcosms. Substrates were grouped into three “classes”—simple (glucose, xylose, alanine), moderate (cellulose, starch, pectin), and complex (lignin, chitin, tannic acid)—according to their presumed decomposability (based on solubility, size, and previously documented relationships with decomposition rates). Two of the nine substrates added contained N—alanine and chitin. These substrates allowed us to additionally examine the effect of added organic N (in both a labile and recalcitrant form) on microbial activity. Alanine has a low N content, relative to other amino acids, similar to that of chitin (approximately 10% N by mass). Other substrates added contain only C, hydrogen, and oxygen.

Substrates were divided into four treatments (Table 1). Soil microcosms were amended with one substrate (treatment A); three substrates, all from one substrate class (treatment B); three substrates, one randomly selected from each class (treatment C); and all nine substrates added in mixture (treatment D). Treatments A and D were fully replicated at both low and high substrate quantities. Treatments B and C were added only at low quantity. Control microcosms received no substrate additions.

The soil microcosms consisted of 175 1-L mason jars filled with 500 g dry weight of homogenized soil. Soil was collected from Cedar Creek Ecosystem Science Reserve (45° 24' N, 93° 12' W) on July 23, 2004. Soil was taken from a grassland plot in the southeast savanna section of Cedar Creek and collected to a depth of 20 to 30 cm. Soil from this site is sandy and infertile with low soil organic matter (Grigal et al. 1974). Fresh soil was sieved through 2 mm mesh to remove roots and homogenized by mixing it in an electric cement mixer for approximately 30 min. A subsample of soil was used to determine initial moisture content and the corrected fresh weight equivalent was added to each jar. Microcosms were brought up to 10% gravimetric water content (slightly below field capacity of 16%)

Table 1 Experimental design of substrate addition experiment. Quantity refers to the equivalent of $\text{g C m}^{-2} \text{y}^{-1}$. Control jars received only water. The total number of microcosms in each

treatment is equal to the possible substrate-class combinations multiplied by the number of replicates of each treatment

Treatment	Quantity	# Substrates added/ microcosm	# Substrate classes/ microcosm	Possible substrate-class combinations	Replicates	Total number of microcosms/ treatment
A-low	100 g	1	1	9	5	45
A-high	250 g	1	1	9	5	45
B	100 g	3	1	3	5	15
C	100 g	3	3	3	15	45
D-low	100 g	9	3	1	10	10
D-high	250 g	9	3	1	10	10
Control	0 g	0	0	0	5	5

and were covered with clear polyethylene plastic to allow CO_2 and O_2 exchange with the atmosphere, but minimize moisture loss. All jars were stored at room temperature (approximately 22°C) in the dark to eliminate C additions from autotrophic growth.

To maximize the microbial community response to treatments, we waited several weeks before beginning the substrate additions to allow the majority of the native labile C to be respired. We measured microbial respiration (see methods below) three times between July 30 and September 1 to ensure that labile C stocks were significantly reduced before beginning the experiment. We began the substrate additions on September 8 and substrates were added weekly for a period of 36 weeks.

Substrate additions

Substrates were added at the equivalent of $100 \text{ g C m}^{-2} \text{y}^{-1}$ (assuming 20 cm soil depth to calculate volume) for the low quantity treatments and $250 \text{ g C m}^{-2} \text{y}^{-1}$ for the high quantity treatments in 36 equal weekly additions (0.174 and $0.434 \text{ mg C g}^{-1} \text{soil week}^{-1}$, respectively). These amounts were chosen to correspond to relatively low and high levels of average aboveground net primary productivity of grass-dominated communities at Cedar Creek (Reich et al. 2001). The total amount of substrate added differed slightly for each substrate or substrate mixture depending on the % C in each compound.

Soluble substrates (glucose, xylose, alanine, and tannic acid) were added in solution while all other substrates were added in dry powder form. All microcosms (including the control treatment) received

1 ml of water per week either with substrates dissolved in the water or 1 ml deionized water dripped on top of the dry substrates. This amount of water was sufficient to replace water lost through evaporation and keep the microcosms at 8–10% gravimetric water content. Soils within jars (including the control treatment) were gently mixed once every 4 weeks to better incorporate added substrates into the soil.

Microbial respiration

We measured microbial respiration on each microcosm three times during the experiment: 1 day before substrate additions began and 1, 4, and 36 weeks after the substrate additions began. To standardize the timing following substrate additions, measures of microbial respiration were initiated 24 h following one of the weekly additions. The randomization of jars minimized any potential effects of sample timing on our results. At each measurement, the plastic wrap covers on the jars were removed, vented with ambient air, and replaced with airtight canning lids fitted with silicone septa. Respiration was measured by taking a 7-ml subsample of headspace gas immediately after capping the jars and again 4–5 h later. Samples were injected into a gas chromatograph (Shimadzu GC-14A) with a thermal conductivity detector. To standardize measurements for the duration of the experiment and account for the relative contribution of initial soil organic matter to total microbial respiration, all data are presented relative to average microbial respiration of the control treatment at each time point.

Enzyme assays

We measured enzyme activity 3 days following the final substrate addition, assaying six enzymes important in catalyzing the breakdown of the substrates we added or that play a role in microbial acquisition of N or P: α -1,4-glucosidase (α GLU), β -1,4-glucosidase (β GLU), L-leucine aminopeptidase (LAP), β -1,4-N-acetylglucosaminidase (NAG), acid phosphatase (PHOS), and peroxidase (PER). Note that α GLU and β GLU catalyze the hydrolysis of starch and cellobiose (a cellulose oligomer), respectively. Also, LAP catalyzes the hydrolysis of amino acids, NAG is involved in the breakdown of chitin, and PHOS mineralizes organic P into phosphate. Finally, PER functions to catalyze the oxidation of lignin (Weintraub et al. 2007).

Enzyme assays were performed according to the methods of Saiya-Cork et al. (2002). Sample suspensions were created by adding 1 g soil to 125 ml pH 5 sodium acetate buffer, homogenizing using a Polytron Homogenizer (Brinkmann Instruments, Inc., Westbury, NY), and pouring into a Petri dish. The suspension was stirred while 200 μ l was added to 96-well microplates using an 8-channel pipettor, with 16 replicate wells per sample. Except for the peroxidase assay, a 7-amino-4-methyl coumarin (for LAP) or 4 methylumbelliferone (MUB)-linked substrate was added to the sample wells. To account for quenching by soil particles a MUB or methyl coumarin standard was added to sample solutions in eight replicate control wells. To control for quenching by the substrate and buffer alone, there were also eight replicates per microplate of each of the following: blank wells (buffer and soil solution), reference standard wells (standard and acetate buffer), and negative control wells (substrate and buffer). Microplates were incubated at room temperature in the dark for 1–7 h, depending on the assay. Enzyme activity was determined by measuring fluorescence on a microplate reader (BioTek Instruments, Winooski, VT) with excitation set to 365 nm and emission set to 460 nm. PER activity was measured using L-3,4-dihydroxyphenylalanine (L-DOPA) plus 0.3% H₂O₂ as the substrate. There were 16 replicate wells for each soil and 8 replicates of blank wells (sample, H₂O₂, and acetate buffer) and negative control wells (L-DOPA, H₂O₂, and acetate buffer). PER activity was determined spectrophotometrically at 460 nm.

Statistical analysis

All statistical analyses were performed using JMP version 6.0.3 (SAS Institute Inc.). In our analyses we excluded outliers if their value was greater than ± 2 standard deviations of the group mean. All cases where outliers were removed from the data set are noted in the tables.

To confirm our a priori classification of substrates into classes based on complexity, we compared microbial respiration among microcosms receiving the three different classes of substrates with repeated-measures ANOVA. Within treatment A, we used a repeated-measures ANOVA to compare substrate quantity and substrate composition across sampling dates. Within treatment B, we compared substrate classes using repeated-measures ANOVA with substrate class as the main effect.

We examined effects of substrate quantity on respiration additionally within treatment D (all nine substrates) across dates using a repeated-measures ANOVA with substrate quantity as the main effect across sampling dates. We analyzed the effect of substrate class by comparing treatments B (three substrates, all from one class) and C (three substrates, one from each class) using repeated-measures ANOVA. We also analyzed the effect of substrate richness on respiration by comparing treatments C and D (all 9 substrates). Finally, to determine the effect of substrate N on respiration, we compared microbial respiration on the final sampling date in treatment C microcosms that received alanine ($n=14$, eight of which also contained chitin) to those that did not ($n=31$, six of which contained chitin) using t -test. We performed the same analysis for chitin, again using treatment C alone.

To determine the effect of substrate composition on enzyme activity, we compared each level of treatment A (single-substrate additions) and treatment D-low (all substrates added, low addition rate) with the control (no substrates added) using a Bonferroni-corrected alpha level to determine significance ($\alpha=0.0056$). To test for effects of substrate quantity, we performed a two-way ANOVA with addition rate and substrate composition as main effects in treatment A and a t -test comparing D-low and D-high. To determine effects of N addition on enzyme activity, we compared microcosms that received N as alanine

or chitin with those that did not, as described previously for respiration.

Effects of both class diversity and substrate diversity on enzyme activity were determined using a one-way ANOVA with each enzyme fit separately. To test for the effects of increasing the number of substrate classes we compared treatment B (three substrates, all from one class) to treatment C (three substrates, one from each class) using a *t*-test. Effects of substrate diversity were determined by comparing treatment C to treatment D-low.

We tested the combined relationship between all enzymes assayed and microbial respiration on the final sampling date using backward stepwise regression. We also tested the relationship between the activity of individual enzymes and microbial respiration using simple linear regression. For this test, the activity of each enzyme was compared to relative microbial respiration on the final sampling date.

Results

Microbial respiration

Measures of microbial respiration confirmed our a priori classifications of substrate classes, although simple substrates were more distinct from moderate and complex substrates than moderate and complex substrates were from each other, at least initially. In treatment A, there was a significant effect of substrate class ($P < 0.001$), quantity ($P = 0.008$), and time ($P <$

0.001) and a substrate class by time interaction ($P < 0.001$) (Table 2, repeated-measures ANOVA). After 1 week and 1 month, simple substrates added singly showed significantly higher respiration rates than moderate and complex substrates. By the end of the experiment, respiration at all three levels of substrate complexity—simple, moderate, and complex—were significantly different from one another, with more labile substrates inducing higher rates of microbial respiration. The pattern was similar for treatment B (three substrates, all from a single class): there was a significant interaction between substrate class composition and time (repeated-measures ANOVA, $P < 0.001$), as all three substrate classes did not become distinct from one another until the final sampling date (Table 2).

Substrate quantity also had a significant effect on respiration rates in treatment D, which included all nine substrates, and there was a significant quantity by date interaction (Table 2, repeated-measures ANOVA, $P = 0.031$). By the final sampling date, increased substrate quantity increased relative microbial respiration rates by 60% (Table 2). The diversity (within or among classes) of substrates added to soil microcosms did not affect respiration rates. Average relative microbial respiration in treatment B (three substrates, all from a single class, 7.39 ± 0.97) was not significantly different from that of treatment C (three substrates, three classes, 6.29 ± 0.31) (Table 2; repeated-measures ANOVA, $P = 0.167$), although relative respiration rates increased over time ($P < 0.001$). Similarly, increasing substrate diversity from three

Table 2 Relative microbial respiration (treatment/control) for three sampling dates in all treatments. Values are means (standard errors). The number of replicates for each treatment is given in Table 1

Treatment	Substrate class	Relative respiration 1 week	Relative respiration 4 weeks	Relative respiration 36 weeks
A-low	Simple	3.60 (0.21)	2.25 (0.14)	11.02 (0.85)
	Moderate	1.21 (0.12)	1.80 (0.18)	6.94 (1.09)
	Complex	1.19 (0.07)	1.76 (0.11)	4.91 (0.69)
A-high	Simple	4.73 (0.36)	2.69 (0.16)	13.10 (0.97)
	Moderate	1.25 (0.12)	2.40 (0.26)	10.37 (1.37)
	Complex	1.25 (0.08)	1.84 (0.17)	5.63 (0.77)
B	Simple	3.21 (0.10)	2.01 (0.07)	11.74 (1.45)
	Moderate	1.33 (0.06)	2.57 (0.09)	6.07 (0.49)
	Complex	1.26 (0.01)	1.88 (0.06)	4.37 (0.33)
C	Mix	1.91 (0.06)	1.97 (0.06)	6.29 (0.31)
D-low	Mix	2.03 (0.07)	2.04 (0.04)	5.72 (0.57)
D-high	Mix	2.35 (0.06)	2.62 (0.04)	9.51 (0.84)

substrates to nine (treatment C vs. D-low) while maintaining a constant number of substrate classes did not affect relative microbial respiration (two-way ANOVA, $P=0.744$), but there was again a significant effect of date ($P<0.001$).

Additions of N-containing substrates (alanine or chitin) in mixtures did not affect rates of microbial respiration differently from additions of mixtures without N-containing substrates in treatment C microcosms (3 substrates, 3 classes). The results were similar (i.e., no significant effect of N additions) when using jars that had only one N-containing substrate present when compared with jars that received no form of N (t -test, $P>0.05$).

Enzyme assays

In treatment A (substrates added singly), there was a significant effect of substrate composition ($P<0.05$) for all enzymes assayed except PER (Table 3, two-way ANOVA, with substrate and quantity as main effects). However, there was no effect of substrate quantity in treatment A and no significant interactions between substrate composition and quantity for any of the enzymes assayed ($P>0.05$). Therefore we pooled the high and low quantity treatments for our presentation of treatment A (Table 3). Increasing the quantity of substrate added

in the 9-substrate treatment (t -test, treatment D-high vs. D-low) increased enzyme activity significantly for three of the six enzymes, β GLU, NAG, and PHOS (Fig. 1, $P<0.05$). Increased quantity marginally increased α GLU activity ($P=0.086$) and had no effect in the other two enzymes. Therefore, to compare with treatment A, we include only data from the low quantity treatment of treatment D (D-low) in Table 3.

Microcosms receiving glucose additions showed significantly higher activity of both α GLU and β GLU than the control treatment (Table 3). Xylose also significantly increased α GLU activity. LAP activity was increased by alanine addition, and alanine also increased activity of β GLU, NAG and PHOS relative to controls. Taken together, these results support the resource limitation model of enzyme production, as enzyme activity was generally stimulated by addition of simple (i.e., relatively labile) compounds. In contrast, chitin additions maximized enzyme activity for NAG, following the substrate stimulation model. Although chitin also maximized the activity of PER, activity for this enzyme was extremely low and no treatment had higher activity than the control. Microcosms in treatment D consistently showed high enzyme activity that was significantly greater than the control treatment for five of the six enzymes assayed. In only one case was there evidence for strong inhibition of enzyme production

Table 3 Enzyme activities of individual substrates in treatment A (averaged among high and low quantity treatments; $n=10$ per substrate) and treatment D (low quantity treatment only; $n=10$). See text for abbreviations. All values are expressed as $\mu\text{mol/g soil/hr}$

with standard errors given in parentheses. Values in **bold** denote the treatment that induced maximum activity for a particular enzyme. Columns with more than one bold term had multiple treatments that were not significantly different from one another

Treatment	Substrate	α GLU	β GLU	LAP	NAG	PHOS	PER
A	Glucose	8.42 (1.37)^a	94.20 (9.70)^{a,b}	5.74 (1.26)	48.66 (9.25)	183.35 (11.34)	0.059 (0.005)
A	Xylose	3.46 (0.44) ^a	45.46 (1.45)	8.41 (2.01)	43.32 (10.61)	188.25 (9.85) ^a	0.054 (0.004)
A	Alanine	3.41 (0.97)	60.70 (2.53) ^{a,b}	16.87 (3.68)^{a,b}	77.57 (7.83) ^a	257.72 (10.91) ^a	0.048 (0.004)
A	Cellulose	2.91 (0.72) ^b	57.62 (2.99)	5.26 (1.60)	54.56 (8.79)	188.31 (10.10) ^a	0.055 (0.007)
A	Starch	2.86 (0.44)	76.98 (6.56) ^a	4.63 (1.23)	49.66 (6.85)	164.00 (7.52)	0.053 (0.004)
A	Pectin	3.88 (0.85)	43.88 (1.36)	5.25 (1.33)	52.90 (8.29)	179.30 (9.09) ^a	0.047 (0.004)
A	Lignin	2.06 (0.44)	46.52 (1.43)	0.003 (0.003) ^a	38.40 (2.09)	101.59 (5.83)	0.044 (0.006)
A	Chitin	3.06 (0.81)	57.58 (3.60)	7.63 (2.65)	118.04 (15.56)^a	185.98 (8.44)	0.070 (0.007)
A	Tannic acid	2.80 (0.35)	69.25 (2.58) ^a	2.58 (0.84)	40.17 (5.15)	131.77 (6.27)	0.060 (0.003)
D-low	All	3.47 (0.39) ^a	103.00 (3.16)^a	11.29 (0.83)^a	66.89 (5.93) ^a	459.12 (25.73)^a	0.055 (0.004)
Control	None	1.16 (0.10)	43.09 (2.91)	5.57 (0.53)	29.39 (2.06)	129.88 (4.80)	0.050 (0.005)

^a Activity differed significantly from the control treatment ($P<0.0056$, experiment-wise error: $\alpha=0.05$)

^b One outlier was removed

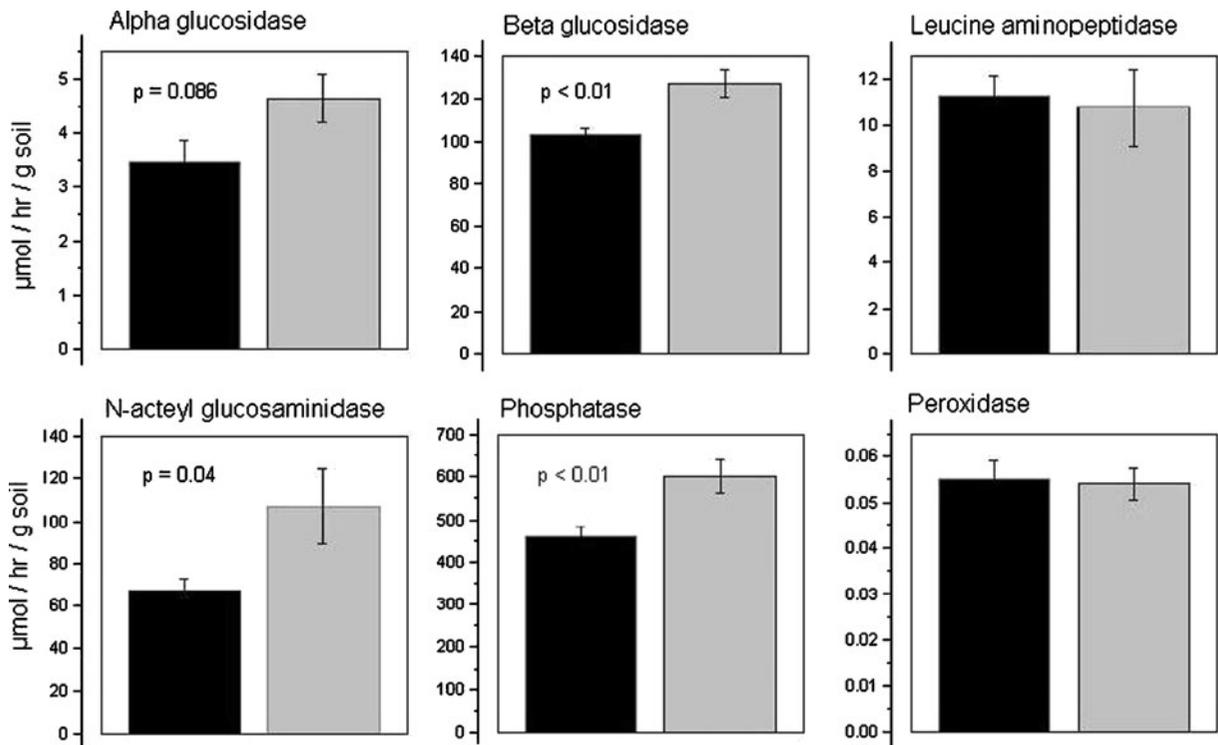


Fig. 1 The effect of substrate quantity on enzyme activity in treatment D (all nine substrates added in mixture). Means and standard errors are given for low quantity (black bars, $n=10$)

and high quantity (gray bars, $n=10$) treatments (one-way ANOVA, each enzyme fit separately)

due to the addition of a particular substrate: in the LAP assay, activity was significantly inhibited by lignin addition. We found no evidence that the products of any enzyme inhibited that enzyme's activity.

There was a significant effect of substrate class on enzyme activity for five of the six enzymes assayed in treatment B, with simple substrates generally exhibiting the highest activity (one-way ANOVA, Tukey's HSD, $P<0.05$, Table 4). This effect was possibly driven by presence of one

substrate that maximized activity for a particular enzyme, as the activity of the simple substrate class mixture never exceeded that of the single substrate addition with the highest activity (Table 3).

The diversity of inputs added influenced potential enzyme activity for some of the enzymes assayed. β GLU, LAP, and PHOS activity increased significantly (t -test, $P<0.05$) with an increase in the number of substrates (C vs. D-low; Fig. 2). However, for the other three enzymes, there was no effect of substrate diversity on activity. Substrate class diversity (treat-

Table 4 The effect of substrate class on enzyme activity (see text for abbreviations). Values represent the mean activity for microcosms in treatment B (3 substrates, 1 substrate class; $n=5$ per class) and are expressed as $\mu\text{mol/g soil/hr}$. Standard errors

Treatment	Substrate class	α GLU	β GLU	LAP	NAG	PHOS	PER
B	Simple	4.66 (1.71) ^a	100.78 (3.85) ^a	14.83 (3.70) ^a	52.08 (3.65) ^a	274.08 (19.18) ^a	0.058 (0.005) ^a
B	Moderate	2.19 (0.18) ^{ab}	57.88 (5.65) ^b	7.79 (1.34) ^{ab}	35.89 (3.51) ^a	167.00 (9.65) ^b	0.047 (0.004) ^{ab}
B	Complex	1.45 (0.24) ^b	55.46 (13.56) ^b	5.73 (2.12) ^b	37.34 (10.14) ^a	143.59 (11.26) ^b	0.042 (0.006) ^b

are given in parentheses. Different superscript letters indicate significant differences between substrate classes within each enzyme (one-way ANOVA with Tukey's HSD, $\alpha=0.05$)

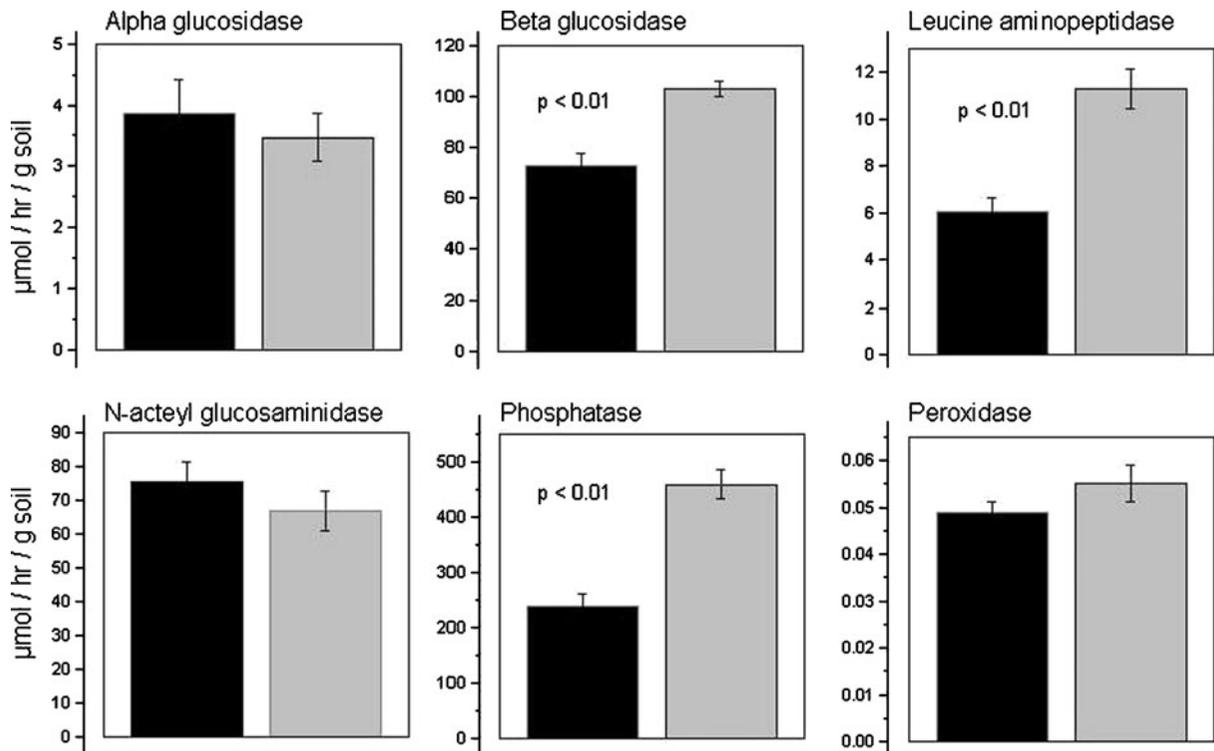


Fig. 2 Effect of substrate diversity on enzyme activity. Means and standard errors are given for treatment C (3 substrates, 3 classes; *black bars*, $n=45$) and treatment D (all substrates; *gray*

bars, $n=10$). Only microcosms receiving the low quantity additions were used for the analysis of treatment D (one-way ANOVA, each enzyme fit separately)

ment B vs. C) only affected LAP activity (t -test, $p=0.032$, data not shown), which decreased with increased class diversity.

The effect of N-containing substrates on enzyme activity depended on the form in which N was added. For microcosms from treatment C (3 substrates, 3 classes), jars receiving alanine ($n=14$, eight of which also contained chitin) had higher enzyme activity than jars that did not ($n=31$, six of which contained chitin) (Fig. 3). This effect was significant for β GLU ($P=0.04$), PHOS ($P<0.01$), and PER ($P=0.02$), and was marginally significant for LAP ($P=0.09$; one-way ANOVA). When we removed microcosms receiving chitin from our analysis the results varied slightly. Alanine additions increased enzyme activity for α GLU ($P=0.02$), β GLU ($P<0.01$), PHOS ($P<0.001$), but there was no significant effect for the other enzymes (data not shown).

Microcosms receiving chitin ($n=14$, eight of which also contained alanine), the other N-containing substrate, exhibited increased activity of only two enzymes—LAP and NAG (Fig. 3; one-way ANOVA,

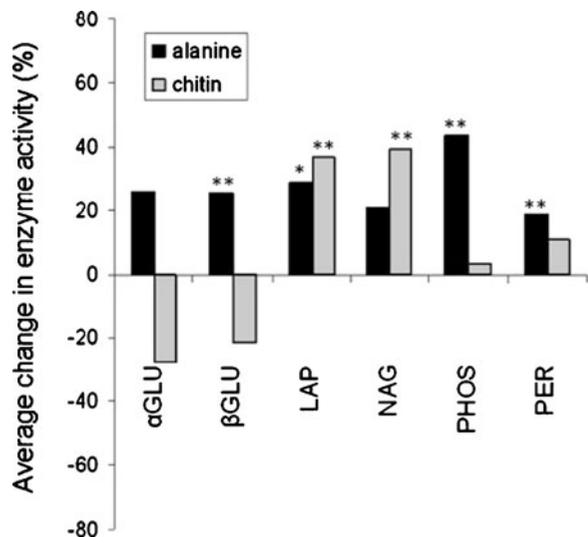


Fig. 3 The effect of substrate N additions on enzyme activity (see text for abbreviations). Values are the average percent change in enzyme activity in response to the presence of alanine (*black bars*) or chitin (*gray bars*) in treatment C (three substrates, 3 classes). Asterisks denote those values that are significantly different than zero ($*=P<0.10$, $**=P<0.05$)

$P < 0.01$). When we removed microcosms receiving alanine from our analysis, NAG and LAP remained significant and PHOS activity was also significantly increased under these conditions ($P < 0.01$; data not shown).

Interactions between microbial respiration and enzyme activity

Enzyme activity overall was a significant predictor of microbial respiration, although no single enzyme was strongly correlated with respiration rates. Using stepwise regression, four enzymes were included in the model ($r^2 = 0.19$) and positively correlated with respiration— α GLU ($P < 0.01$), β GLU ($P < 0.01$), LAP ($P = 0.02$), and PHOS ($P < 0.01$). For five of the six enzymes assayed, there was no relationship between enzyme activity and microbial respiration using simple linear regression, although LAP activity had a weak positive effect ($P < 0.001$, $r^2 = 0.070$; data not shown).

Discussion

Enzyme activity response to substrate additions

No single mechanism explained the effects of substrate composition, quantity and diversity on activity of six soil enzymes involved in the acquisition of C, N, and P. Rather, we found evidence for enzyme-specific responses to substrate additions that were consistent with both the resource limitation and substrate stimulation models of substrate effects on enzyme activity, while some enzymes (α GLU, β GLU, and LAP) appeared to be maximized by the addition of their products, a mechanism we had not previously considered.

Treatment effects on α GLU and β GLU activity were partially consistent with the predictions of the resource limitation model. Labile C inputs maximized activity of both glucosidases, suggesting C-limitation, with α GLU activity stimulated by glucose and xylose and β GLU stimulated by glucose and alanine. However, we expected that if glucosidase activity was indeed C-limited, then other labile C additions such as xylose (for β GLU) and alanine (for α GLU) would also increase enzyme activity. Although xylose addition increased activity of α GLU relative to the

control treatments, glucose stimulated activity by twice as much. In the β GLU assay, both C and N additions increased enzyme activity, but activity in microcosms receiving glucose was 30% greater than in the other microcosms in treatment A, and activity in xylose-amended microcosms was not significantly greater than in the control treatment.

Low soil C content may make the microbial communities in these soils strongly C-limited (Paul and Clark 1996). Although previous studies examining enzyme activity showed no effect of labile C additions (Allison and Vitousek 2005), soils from our study site are relatively low in C (Johnston et al. 1996). In addition, we incubated the soils for several weeks before adding the substrates to minimize availability from C already in the soil. Therefore, low soil C content may explain the strong response of glucosidase enzymes to C additions, although this effect was not seen in the other enzymes we assayed. However, that the activity of enzymes involved in hydrolysis of molecules to form glucose would be maximized by addition of glucose for 9 months seems counterintuitive.

Given the complexity of soil microbial communities (Paul and Clark 1996), the short response time of microbes to labile C availability (Melillo et al. 2002; McLauchlan and Hobbie 2004), and the potential competitive interactions occurring between microbial groups (Wardle 2006), the increase in glucosidase activity in response to glucose additions may be a result of glucose effects on microbial community dynamics rather than direct effects on enzyme activity of substrate additions per se. The observed increase in glucosidase activity in response to glucose additions may be caused by glucose stimulating microbial biomass turnover as a result of a rapid increase in microbial biomass, and thus increasing microbial demand for labile C, or shifting competitive interactions in favor of cellulose degraders. Note that the response of the microbial community to labile C additions was the production of enzymes to mineralize C, suggesting that the resource limitation model may not predict the functional response of intact microbial communities under all circumstances.

Treatment effects on aminopeptidase activity were only partially consistent with the predictions of the conceptual models, as aminopeptidase activity was also maximized by the addition of its product.

Contrary to our expectations, and those of theoretical studies (Sinsabaugh and Moorhead 1994), alanine additions maximized LAP, even though LAP releases N in the form of amino acids (including alanine) through the breakdown of proteins. According to the resource limitation model, increased N supply should have neutral effects on activity of this enzyme. One explanation for the increase in LAP activity is that the combined addition of labile C and N within one substrate stimulated LAP activity according to the resource limitation model. However, additions of other C sources did not significantly increase LAP activity compared to the control treatment.

In contrast to the glucosidase and LAP enzymes, PHOS activity closely followed the predictions of the resource limitation model of controls on enzyme production. Similar to other studies (Allison and Vitousek 2005; Elfstrand et al. 2007; Keeler et al. 2009), PHOS activity was elevated in alanine-amended soils and was maximized when C and N were added in combination. This suggests N addition both increased demand for P and increased availability of N for investment in P-acquiring enzymes, so microbes became energy limited and thus PHOS activity was maximized when both C and N were available.

The response of NAG activity to the treatments was consistent with the substrate stimulation model, as NAG was maximized by the addition of chitin, the compound it degrades. Although chitin also contains both C and N, it is a complex compound that is difficult to decompose (Olander and Vitousek 2000). Therefore, it is unlikely that chitin represents a readily available C or N source for microbes. We hypothesize that the effect of chitin additions on NAG activity likely resulted from changes in microbial community composition toward greater fungal abundance, as fungi are the dominant producers of NAG in soils (Olander and Vitousek 2000).

Effects of N addition on enzyme activity

The addition of chitin and alanine, both N-containing substrates, allowed us to compare the addition of C compounds with and without N, and our results contrast those of other studies. Addition of N has been shown to inhibit the activity of both oxidative and N-acquiring enzymes, including aminopeptidase (Allison and Vitousek 2005; Stursova et al. 2006),

peroxidase (Carreiro et al. 2000; Gallo et al. 2004; Sinsabaugh et al. 2005) and chitinase (Olander and Vitousek 2000). However, similar to the results of a recent study (Keeler et al. 2009), we found no evidence for the inhibition of any enzyme from N added as alanine or chitin. In fact, in treatment C (three substrates, three classes) alanine additions significantly increased the activity of several enzymes including LAP and PER, and in treatment A (one substrate), alanine increased NAG activity.

One important contrast between our study and previous studies is that we added organic N (rather than inorganic N). It is possible that organic and inorganic N additions have contrasting effects on enzyme activity, although studies using inorganic N have found a stimulatory effect in some of the enzymes mentioned above (Saiya-Cork et al. 2002; Waldrop and Firestone 2004; Keeler et al. 2009). Recent studies have found that the presence of N-fixing trees increases the activity of enzymes involved in the degradation of organic N compounds (Selmants et al. 2005; Allison et al. 2006), perhaps because of the response of microbes to complex inputs of organic N. Complex N compounds require enzymatic breakdown before they can be assimilated by microbes (in contrast to inorganic N that can be taken up by microbes directly), resulting in the increase in enzyme activity seen in these studies. Our results suggest that aminopeptidase activities also may increase with the addition of both simple and complex forms of organic N. Given the prevalence of studies examining the effects of simulated N deposition on enzyme activity (Caldwell 2005; Sinsabaugh et al. 2005), and the importance of N as the primary limiting nutrient in many ecosystems, this apparent contrast in the effects of organic and inorganic N should be further investigated.

The only case where we found inhibition of an enzyme was for LAP activity in response to lignin additions. It is not clear why lignin would inhibit LAP activity and this response to high lignin substrates has not been previously reported in the literature. Other studies have suggested that high amounts of polyphenolic compounds in soil can inhibit enzyme activity by binding to enzymes and making them inactive (Kraus et al. 2003; Allison 2006). However, lignin additions did not inhibit any other enzyme we assayed. While tannins have also been shown to inhibit enzyme activity (Benoit and Starkey 1968,

Scalbert 1991), we found no evidence for inhibition of any enzyme from the addition of tannic acid.

Interactions between enzyme activity and microbial respiration

We found evidence for a positive relationship between the activity of C and nutrient-acquiring enzymes and microbial respiration rates, while enzymes involved in the degradation of more recalcitrant compounds showed no relationship with microbial respiration. The relationship between microbial respiration and enzyme activity is not surprising given that both responded similarly to substrate class in treatment B (three substrates, all from one substrate class), with microbial respiration and five of the six enzymes showing their highest response to the simple substrate class. However, the amount of variation explained by enzyme activities was small ($r^2=0.19$) and no single enzyme was strongly correlated with microbial respiration rates.

Given the mechanistic links between the activity of extracellular enzymes and C mineralization, we would expect a high degree of correlation between these two measures of microbial activity, particularly in direct measures from the same soil. In this study, we measured only a small number of the suite of total enzymes present in soil. It is likely that respiration rates are more dependent on the total activity of all enzymes in soil than on a subset of enzymes or any specific enzyme per se. In addition, little is known about the complex interactions that influence enzyme activity in natural environments, including microbial community dynamics, the controls on the production and efficiency of enzymes, and the effectiveness of enzymes in the soil matrix—all of which may potentially influence the correlation between enzyme activity and rates of C mineralization (Keeler et al. 2009).

Effects of substrate diversity and quantity

In contrast to our hypothesis that increased substrate diversity would increase enzyme activity overall, a combination of all substrates (treatment D) significantly increased enzyme activity only for PHOS when compared to the one-substrate treatment (treatment A) and for PHOS, β GLU, and LAP when compared to the three-substrate treatment (treatment C). For the

other enzymes, activity for treatment D was equal to or less than that for the substrate inducing maximum activity in treatment A (Table 3). We had expected that activity of all enzymes would be maximized in microcosms receiving all 9 substrates compared to single- or 3-substrate microcosms. Our expectation was based on the notion of resource “complementarity” (sensu Loreau 1999), since the high-diversity treatment included the addition of several labile C and N sources that might stimulate enzyme activity more together than individually. However, complementary effects were only apparent for PHOS activity, which was much higher in the high-diversity than in the lower diversity treatments. For β GLU and LAP, potential enzyme activities in high diversity treatments were not significantly less than the single substrate inducing maximum activity, suggesting resource complementarity may have played a role in these enzymes as well. For α GLU and NAG, we found no evidence of complementary effects operating in the high-diversity treatment. Effects of diversity for these enzymes appear to be related more to the “sampling effect” (Loreau 1999) that occurred because the 9-substrate treatment included all nine substrates and therefore reflected the substrate-specific effects on the different enzymes that were largely apparent from the single-substrate additions.

In a study on the effects of resource input diversity on substrate induced microbial respiration, Orwin et al. (2006) found that the effects of substrate diversity saturated at low numbers of substrates (i.e., there was no effect of adding more than two substrates). In the present study, we found mixed results, with a significant effect of substrate diversity at relatively high levels for some enzymes assayed and neutral effects for the other enzymes as well as for microbial respiration. This latter finding supports the results of Orwin et al. (2006) and the theoretical model of Loreau (2001) which suggests that diverse substrate mixtures are not likely to increase rates of microbial functions. This could be because microbes are generalists with respect to function and thus rates of microbial processes saturate at low levels of input diversity or because some substrates are poorly decomposed by microbes and thus increases in substrate diversity decrease the amount of utilizable substrate and have a negative effect on microbial processes (Loreau 2001).

The lack of an effect of substrate diversity on microbial respiration is in contrast to a recent study which found that chemical diversity of plant litter correlated with both soil respiration and N mineralization rates (Meier and Bowman 2008). This discrepancy may be due to differences in measures of substrate diversity used in each study. Meier and Bowman (2008) defined chemical diversity based on chemical traits of the litter (e.g., acid soluble fraction, acid insoluble fraction, condensed tannins, etc.) while our study used a more narrow definition, using the number of specific chemical compounds as our measure of diversity. It is possible that broad-scale measures of microbial activity, such as microbial respiration rates, are more likely to be influenced by the diversity of chemical traits present in complex substrates, while more narrow measures, such as enzyme activity, are influenced by the composition and diversity of specific compounds. Nevertheless, the chemical composition, not richness, of substrates is consistently the strongest predictor of microbial activity in soil (Orwin et al. 2006; Meier and Bowman 2008; present study).

Substrate quantity had a significant effect on four of the six enzymes assayed and neutral effects on the others. We expected that low additions of substrate would induce enzyme activity and that higher additions of substrate would cause further increases or neutral effects, depending on the availability of resources to invest in further enzyme production. However, competition among microbes might reduce the number of enzymes exhibiting positive effects of increased substrate quantity. For example, in plant communities, increases in a limiting resource generally decrease diversity (Tilman 1987; Suding et al. 2005). If we consider enzyme activity to be a measure of microbial functional diversity (i.e., considering the rate of enzyme activity overall as a proxy for the diversity of functional abilities of microbial communities; Caldwell 2005) we would expect that increasing substrate quantity would decrease the enzyme activity of most enzymes, as microbes expressing particular enzymes become dominant and outcompete others. Contrary to this hypothesis, increasing substrate quantity increased enzyme activity overall. Relative microbial respiration was also greater in high quantity treatments. However, the effect of substrate quantity was only apparent in treatment D where any individual substrate was added at very low

quantities, suggesting resource limitation of enzyme production at low substrate quantities. In treatment A, there was a marginally significant effect of substrate quantity on enzyme activity. Therefore, this effect may saturate at relatively low levels of substrate addition.

This study highlights the importance of variation in inputs in regulating microbial activity in soil. Substrate composition, quantity, and diversity all had a significant effect on microbial community function. However, this study shows that microbial function may be regulated by different mechanisms depending on the composition of substrates added to soil. We also suggest that although enzyme activity and microbial respiration are certainly related, respiration is not dependent on the activity of any single enzyme. Rather, it is likely that enzyme activity influences C mineralization only through the summative effects of all enzymes produced by the microbial community. In addition, we demonstrate that the effects of N addition on microbial activity may depend on the form of N added, as the organic N forms added in this study differed from one another in their effects, and from effects of inorganic N additions reported in some previous studies. Taken together, these results suggest that the role of the microbial community in the decomposition of organic matter is highly dependent on the composition and complexity of inputs to soil.

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References

- Allison SD (2006) Soil minerals and humic acids alter enzyme stability: implications for ecosystem processes. *Biogeochem* 81:361–373
- Allison SD, Vitousek P (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biol Biochem* 37:937–944
- Allison SD, Nielsen C, Hughes R (2006) Elevated enzyme activities in soils under the invasive nitrogen-fixing tree *Falcataria moluccana*. *Soil Biol Biochem* 38:1537–1544
- Benoit RE, Starkey R (1968) Enzyme inactivation as a factor in the inhibition of decomposition of organic matter by tannins. *Soil Sci* 105:203–208
- Caldwell BA (2005) Enzyme activities as a component of soil biodiversity: a review. *Pedobiologia* 49:637–644
- Carney KM, Hungate B, Drake B, Megonigal J (2007) Altered soil microbial community at elevated CO₂ leads to loss of soil carbon. *Proc Natl Acad Sci* 104:4990–4995

- Carreiro MM, Sinsabaugh R, Repert D, Parkhurst D (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* 81:2359–2365
- Chung H, Zak D, Reich P, Ellsworth D (2007) Plant species richness, elevated CO₂, and atmospheric nitrogen deposition alter soil microbial community composition and function. *Glob Chang Biol* 13:980–989
- Elfstrand S, Hedlund K, Martensson A (2007) Soil enzyme activities, microbial community composition and function after 47 years of continuous green manuring. *Appl Soil Ecol* 35:610–621
- Fontaine S, Bardoux G, Benest D, Verdier B, Mariotti A, Abbadie L (2004) Mechanisms of the priming effect in a savannah soil amended with cellulose. *Soil Sci Soc Am J* 68:125–131
- Gallo M, Amonette R, Lauber C, Sinsabaugh R, Zak D (2004) Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. *Microb Ecol* 48:218–229
- Grayston SJ, Wang S, Campbell C, Edwards A (1998) Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biol Biochem* 30:369–378
- Griffiths BS, Ritz K, Ebbelwhite N, Dobson G (1998) Soil microbial community structure: effects of substrate loading rate. *Soil Biol Biochem* 31:145–153
- Grigal DF, Chamberlain L, Finney H, Wroblewski V, Gross E (1974) Soils of the Cedar Creek natural history area. Miscellaneous report 123. University of Minnesota Agricultural Experiment Station, St. Paul
- Harder W, Dijkhuizen L (1983) Physiological-responses to nutrient limitation. *Ann Rev Microbiol* 37:1–23
- Hooper DU, Bignell D, Brown V, Brussaard L, Dangerfield J, Wall D, Wardle D, Coleman D, Giller K, Lavelle P, Van der Putten W, De Ruiter P, Rusek J, Silver W, Tiedje J, Wolters V (2000) Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms, and feedbacks. *Bioscience* 50:1049–1061
- Johnston MH, Homann P, Engstrom J, Grigal D (1996) Changes in ecosystem carbon storage over 40 years on an old-field forest landscape in east-central Minnesota. *For Ecol Manag* 83:17–26
- Keeler BL, Hobbie S, Kellogg L (2009) Effects of long-term nitrogen addition on microbial enzyme activity in eight forested and grassland sites: implications for litter and soil organic matter decomposition. *Ecosyst* 12:1–15
- Kourtev PS, Ehrenfeld J, Huang W (2002) Enzyme activities during litter decomposition of two exotic and two native plant species in hardwood forests of New Jersey. *Soil Biol Biochem* 34:1207–1218
- Kraus TEC, Dahlgren R, Zasoski R (2003) Tannins in nutrient dynamics of forest ecosystems—a review. *Plant Soil* 256:41–66
- Kuz'yakov Y (2002) Review: factors affecting rhizosphere priming effects. *J Plant Nutr Soil Sci* 165:382–396
- Loreau M (1999) Separating sampling and other effects in biodiversity experiments. *Oikos* 92:600–602
- Loreau M (2001) Microbial diversity, producer-decomposer interactions and ecosystem processes: a theoretical model. *Proc Roy Soc Lon, Series B, Biol Sci* 268:303–309
- McLaughlan KK, Hobbie S (2004) Comparison of labile soil organic matter fractionation techniques. *Soil Sci Soc Am J* 68:1616–1625
- Meier CL, Bowman W (2008) Links between plant litter chemistry, species diversity, and belowground ecosystem function. *Proc Natl Acad Sci* 105:19780–19785
- Melillo JM, Steudler P, Aber J, Newkirk K, Lux H, Bowles F, Catricala C, Magill A, Ahrens T, Morrisseau S (2002) Soil warming and carbon-cycle feedbacks to the climate system. *Science* 298:2173–2176
- Olander LP, Vitousek P (2000) Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochem* 49:175–190
- Orwin KH, Wardle D, Greenfield L (2006) Ecological consequences of carbon substrate identity and diversity in a laboratory study. *Ecology* 87:580–593
- Paul EA, Clark FE (1996) *Soil microbiology and biochemistry*, 2nd edn. Academic, San Diego
- Reich PB, Peterson D, Wedin D, Wrage K (2001) Fire and vegetation effects on productivity and nitrogen cycling across a forest-grassland continuum. *Ecology* 82:1703–1719
- Saiya-Cork KR, Sinsabaugh R, Zak D (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biol Biochem* 34:1309–1315
- Scalbert A (1991) Antimicrobial properties of tannins. *Phytochem* 30:3875–3883
- Schimel JP, Weintraub M (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biol Biochem* 35:549–563
- Selmants PC, Hart S, Boyle S, Stark J (2005) Red alder (*Alnus rubra*) alters community-level soil microbial function in conifer forests of the Pacific Northwest, USA. *Soil Biol Biochem* 37:1860–1868
- Sinsabaugh RL, Moorhead D (1994) Resource-allocation to extracellular enzyme production—a model for nitrogen and phosphorous control of litter decomposition. *Soil Biol Biochem* 26:1305–1311
- Sinsabaugh RL, Carreiro M, Repert D (2002) Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochem* 60:1–24
- Sinsabaugh RL, Gallo M, Lauber C, Waldrop M, Zak D (2005) Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. *Biogeochem* 75:201–215
- Smucker RA, Kim D (1987) Chitinase induction in an estuarine ecosystem. In: Llewellyn G, O'Rear C (eds) *Biodeterioration research*. Plenum, New York
- Stursova M, Crenshaw C, Sinsabaugh R (2006) Microbial responses to long-term N deposition in semiarid grassland. *Microb Ecol* 51:90–98
- Suding KN, Collins S, Gough L, Clark C, Cleland E, Gross K, Milchunas D, Pennings S (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proc Natl Acad Sci* 102:4387–4392
- Tilman D (1987) Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. *Ecol Monogr* 57:189–214

- Waldrop MP, Firestone M (2004) Microbial community utilization of recalcitrant and simple carbon compounds: impact of woodland plant communities. *Oecologia* 138:275–284
- Waldrop MP, Firestone M (2006) Response of microbial community composition and function to soil climate change. *Microb Ecol* 52:716–724
- Wardle D (2006) The influence of biotic interactions on soil biodiversity. *Ecol Lett* 9:870–886
- Weintraub MN, Scott-Denton L, Schmidt S, Monson R (2007) The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in a subalpine forest ecosystem. *Oecologia* 154:327–338