Ecosystems (2009) 12: 1–15
DOI: 10.1007/s10021-008-9199-z

ECOSYSTEMS

Effects of Long-Term Nitrogen Addition on Microbial Enzyme Activity in Eight Forested and Grassland Sites: Implications for Litter and Soil Organic Matter Decomposition

Bonnie L. Keeler, Sarah E. Hobbie,* and Laurie E. Kellogg

Department of Ecology, Evolution and Behavior, University of Minnesota, 100 Ecology Building, 1987 Upper Buford Circle, Saint Paul, Minnesota 55108, USA

Abstract

Long-term nitrogen (N) addition experiments have found positive, negative, and neutral effects of added N on rates of decomposition. A leading explanation for this variation is differential effects of N on the activity of microbially produced extracellular enzymes involved in decomposition. Specifically, it is hypothesized that adding N to N-limited ecosystems increases activity of cellulose degrading enzymes and decreases that of lignin degrading enzymes, and that shifts in enzyme activity in response to added N explain the decomposition response to N fertilization. We measured litter and soil organic matter (SOM) decomposition and microbial enzyme activity in a long-term N fertilization experiment at eight forested and grassland sites in central Minnesota, USA, to determine (1) variation among sites in enzyme activity, (2) variation in the response of enzymes, litter decomposition, and soil respiration to added N, and (3) whether changes in enzyme activity in response to added N explained variability among sites in the effect of N on litter and SOM decomposition. Site differences in pH, moisture, soil carbon, and microbial biomass explained much of the among-site variation in enzyme activity. Added N generally stimulated activities of cellulose degrading and N- and phosphorus-acquiring enzymes in litter and soil, but had no effect on lignin degrading enzyme activity. In contrast, added N generally had negative or neutral effects on litter and SOM decomposition in the same sites, with no correspondence between effects of N on enzyme activity and decomposition across sites.

Key words: Decomposition; extracellular enzymes; fertilization; litter; microbes; Minnesota; nitrogen; soil respiration.

Received 14 June 2008; accepted 3 September 2008; published online 10 October 2008

B.L.K. and S.E.H. conceived of study; B.L.K., S.E.H., and L.E.K. designed study and performed research; B.L.K. analyzed data and wrote the paper.

**Corresponding author; e-mail:* shobbie@umn.edu

Introduction

Fossil fuel combustion, fertilizer use, and the cultivation of nitrogen (N)-fixing crops have greatly accelerated the formation and deposition of reactive

forms of N (Galloway and others 1995; Vitousek and others 1997). Most temperate terrestrial ecosystems are N limited, therefore increased N deposition has the potential to alter the balance between primary productivity and decomposition with implications for ecosystem carbon (C) storage and nutrient cycling. Fertilization experiments in many terrestrial ecosystems show a positive response of aboveground net primary productivity to N addition (Tilman 1984; Vitousek and Howarth 1991). However, long-term N additions have also resulted in losses of total ecosystem C, increased nitrate leaching, and reduced plant species diversity (Magill and others 1997; Wedin and Tilman 1997; Neff and others 2002; Mack and others 2004). The effects of elevated N availability have been extensively studied revealing a high degree of variability in ecosystem response to N additions. Still, the factors that regulate site-specific responses to N addition are uncertain.

In a recent review of the effects of added N on decomposition, Knorr and others (2005) reported significant positive, negative, and neutral effects of added N on rates of litter decomposition. The most widely cited explanation for variation in the effect of N on decomposition is among-site differences in litter quality, with the decomposition of high-lignin litters typically responding negatively to N addition and more labile litter types responding positively to added N (Fog 1988; Berg and Matzner 1997; Magill and Aber 1998; Carriero and others 2000; Hobbie 2000; Knorr and others 2005; but see Hobbie 2008).

Both abiotic and biotic mechanisms have been proposed to explain observed stimulatory or inhibitory effects of N on decomposition and interactions between added N and lignin. Abiotic reactions between added N and byproducts of lignin decomposition and other polyphenolic compounds are believed to form decay-resistant compounds and slowing rates of decomposition (Fog 1988; Berg and Matzner 1997). Other studies have found that added N reduces the activity of microbial extracellular enzymes responsible for the breakdown of lignin, causing high-lignin litter types to respond more negatively to N additions than more labile litter types (Magill and Aber 1998; Carriero and others 2000; Sinsabaugh and others 2002; DeForest and others 2004; Waldrop and others 2004a, b; Waldrop and Zak 2006).

The hypothesis that changes in enzyme activity explain the effect of added N on decomposition has received support with the publication of several studies finding correlations between enzyme activity and rates of litter and soil organic matter (SOM) decomposition (Alvarez and Guerrero 2000; Carriero and others 2000; Sinsabaugh and others 2002; Allison and Vitousek 2004; Waldrop and others 2004b; Rejmankova and Sirova 2007). In these studies, the best explanation for changes in decay rates following N additions is the effect of N on the activity of the lignin-degrading enzyme, phenol oxidase (Carriero and others 2000; Waldrop and others 2004b). Carriero and others (2000) found that added N inhibited phenol oxidase activity in a high-lignin litter type (where the effect of added N on decomposition was negative), whereas in more labile (that is, lower lignin) litter types, N stimulated or had no effect on phenol oxidase (and the effect of added N on decomposition was positive or neutral). The work of Carriero and others (2000) provided the first evidence that the suppression of lignin-degrading enzyme activity could be an important mechanism explaining the negative effects of added N on decomposition observed in many systems.

In addition to its effects on phenol oxidase activity, added N has been shown to affect C-, N-, and P-acquiring enzyme activity both positively and negatively (Carriero and others 2000; Saiya-Cork and others 2002; Waldrop and others 2004a, b; Henry and others 2005; Sinsabaugh and others 2005; Stursova and others 2006). The effect of added N on enzyme activity is not always consistent across sites. Site-specific variation in the effect of N on enzyme activity has been attributed to differences among sites in nutrient availability (Olander and Vitousek 2000; Treseder and Vitousek 2001), litter lignin content (Carriero and others 2000; Sinsabaugh and others 2002; Frey and others 2004; Waldrop and Zak 2006), litter and soil C:N ratio (Michel and Matzner 2003; Waldrop and others 2004a) and microbial biomass (Ajwa and others 1999).

The results of these studies underscore the complexity of plant–substrate–microbe interactions and highlight the importance of identifying the mechanisms responsible for variation among sites in microbial response to N additions. There is evidence for the importance of enzyme activity in predicting N effects on decomposition and the role of site characteristics in explaining variation in enzyme response to added N; however, the effect of added N on enzyme activity has not been tested simultaneously in litter and soil across a variety of sites that vary in aboveground plant cover and important site characteristics such as litter chemistry, soil C:N, and nutrient availability.

This paper summarizes the effects of long-term N addition on litter decomposition, labile soil C respiration, and microbial extracellular enzyme activity in litter and soil at eight sites that vary in their physical, chemical, and biological properties.

First, we characterized variation in enzyme activity among sites and tested for site characteristics that best explained this variation. Second, we characterized variation in the effect of added N on enzyme activity and determined if variation in enzyme response to added N was related to variation in site characteristics. Our final objective was to determine if the effect of N on litter and soil enzyme activity explained variation in the effect of added N on litter and SOM decomposition.

We hypothesized that added N would stimulate hydrolytic enzyme activity (cellulose degrading and N- and P-acquiring enzymes) and repress lignin-degrading enzyme activity, but that this repression would be stronger in sites dominated by species that produce litter with relatively high-lignin concentrations (for example, oak and pine). Finally, we hypothesized that variation in enzyme response to N would emerge as the best explanation for among-site variability in the effect of added N on litter and SOM decomposition rates.

Study Site

Long-term N fertilization plots (Hobbie 2005; 2008) were established at the Cedar Creek Ecosystem Science Reserve (formerly Natural History Area), Minnesota (latitude 45.40°N, longitude 93.20 W, elevation 270 m) in 1999 in eight sites that vary in aboveground plant cover. Plots are located in two pin oak sites (*Quercus ellipsoidalis*), two white pine sites (*Pinus strobus*), two mixed C3 and C4 grassland sites, one maple-basswood site (*Acer saccharum*, *Tilia americana*, and *Q. ellipsoidalis)*, and one bigtooth aspen site (*Populus grandidentata*). Within each site, each of twelve 2.5 m × 2.5 m plots received either a control treatment (water only) or a fertilization treatment consisting of 10 g N/m^2/year of aqueous NH_4NO_3 ($n = 6$) in three applications per year starting in 1999. Sites are located in close proximity to each other (within 5 km) and share a similar soil type (fine to medium grained sands of the Typic and Alfic Udipsamments). All sites are on sandy, well drained, poorly developed soils, where N limits net primary productivity (Tilman 1984). In earlier papers, soil and litter inorganic N availability, litter pH, and litter chemistry at each site were characterized (Hobbie 2005; 2008).

Methods

Native Litter Decomposition

To assess the effects of N fertilization on leaf litter decomposition in each site, native fine litterfall (leaves, buds, reproductive structures, bark, and twigs and branches <1 cm in diameter) was collected monthly from May–November 1999 prior to initiation of fertilization from 15 randomly located 0.203 m^2 litter traps per site. Fine litterfall was dried (65°C) and sorted into leaf, stem, and reproductive components and leaf litter was sorted by species. Litterbags were assembled from fine litterfall to ensure that different litter components were combined to represent their proportions in annual native fine litterfall. Approximately 6 g air-dried litter were placed in 400 cm^2 litter bags made with fine-mesh polyester (200 μm) bottoms and small-mesh (0.3 mm) nylon tops to exclude sand but not fungi. Litterbags were placed in each plot and decomposed over a 5-year period (1999–2004) as described in Hobbie (2005). Six harvests of litterbags were conducted between 2000 and 2004 (March and October, 2000; October 2001–2004), at which time litter was dried (65°C) and weighed. Decomposition was calculated as the proportion of initial mass remaining over time. The mean mass remaining of all replicates within a treatment was used to develop single decay constants for the control and N-fertilized treatments for each site by fitting the proportion initial mass remaining against time with a single exponential decay model, $X = e_1^{-kt}$, where X is the proportion initial mass remaining at time t and k_l is a litter specific decay constant. The single exponential model was determined to be the best fit for these data based on previous analyses of litter decomposition in these sites (Hobbie 2008).

Soil Labile C Decomposition

To determine the effects of N fertilization on decomposition of SOM, soils were collected for laboratory incubations from six of the eight sites in October of 2004 (excluding Maple and Aspen) and incubated for 9 months in the lab. Three mineral soil cores 0–20 cm in depth (2.5 cm diameter) were removed from each replicate plot, homogenized by plot, and immediately sieved (2 mm). Approximately 200 g of soil from each plot were then placed in Mason jars and covered with polyethylene plastic. We determined the field moisture for the original samples and the incubations were maintained at field moisture for the duration of the study (average percent moisture content of field collected soils was 8.15%). Soil respiration was measured on days 1, 4, 8, 15, 40, 85, and 145 of the incubation by capping the jar with a lid fit with a silicone septum, sampling the headspace immediately and again after 24 h, and measuring

headspace CO_2 concentrations on a gas chromatograph with a thermal conductivity detector and a Poropak N column (Shimadzu GC14A, Shimadzu Scientific Instruments, Columbia, Maryland, USA). Cumulative respiration for each plot was fit to a two-pool model, $C_t = C_1\ (1 - e_s^{-kt}) + ct$, where C_t is the cumulative amount of C respired at time t, C_1 the size of the labile C pool, k_s the decomposition rate constant for the labile pool, and c is the respiration rate of the more recalcitrant C pool (Dijkstra and others 2005).

Site Characterization

We collected soil and litter samples from all eight study sites in May, July, and September of 2005. Randomly located soil samples were collected using a 2-cm diameter × 10-cm deep soil corer. The litter layer (O horizon) overlying each soil core was collected from within a 20 cm × 20 cm sampling frame centered over each soil core. Four litter and four soil samples were collected and composited per plot. Soil samples were mixed and passed through a 2-mm sieve and litter samples were homogenized by mixing and shredding litter by hand prior to subsampling and analysis. Microbial biomass was measured on fresh soils, whereas samples for enzyme assays were frozen at −10°C for 2–12 months until time of analysis. Soil C and N, exchangeable base cations, and soil pH were measured on oven dried (65°C) soils as follows.

We measured soil pH (2:1 water-to-sample ratio) by shaking the sample solution for 30 min, incubating for 1 h, and then measuring pH on an Orion pH meter (Hendershot and others 1993). We determined total soil C and N on finely ground subsamples using dry combustion analysis on a COSTECH ECS 4010 element analyzer (Costech Analytical, Valencia, California, USA). Soil exchangeable base cations (K, Ca, Mg, Na) and aluminum (Al) were measured in 1 M NH_4OAc extracts (3 g soil subsamples, $n = 3$ replicate plots per treatment per site) using inductively coupled plasma atomic emission spectrometry (ICP-AES, Research Analytical Laboratory, University of Minnesota). We determined microbial biomass on soil and litter samples from the May 2005 collection date only using chloroform fumigation extraction as described by Brookes and others (1985). Briefly, soluble C and N were extracted from 10 g soil samples and 1 g litter samples before and after fumigation with a 0.5 M K_2SO_4 solution. Extracts were analyzed for total dissolved organic C and N using a Shimadzu TOC-N analyzer (Shimadzu Corporation, Columbia, Maryland, USA). Conversion factors of 0.45 for C and 0.54 for N were used to convert dissolved organic C and N to microbial biomass (Brookes and others 1985; Beck and others 1997). Soil and litter N availability were assessed in 2003 using ion-exchange resins and reported in Hobbie (2008). Soil and litter moisture were measured gravimetrically (65°C) at each sampling date. Temperature over the growing season at the O–A horizon boundary was measured at all sites in 2006 using temperature-sensing data loggers (ibuttons, Dallas Semiconductors Inc.).

Enzyme Activity

We measured the activity of six extracellular enzymes involved in lignin and cellulose decomposition, N acquisition, and P acquisition in both the litter layer and mineral soil horizons according to methods described in Saiya-Cork and others (2002) and Sinsabaugh and others (1992). The hydrolytic enzymes, β-1,4-glucosidase (BG) (EC 3.2.1.21), cellobiohydrolase (CBH) (EC 3.2.1.91), β-1,4-*N*-acetylglucosaminidase (NAG) (EC 3.1.1.14), and acid phosphatase (AP) (EC 3.1.3.2) were measured flourometrically using methylumbelliferone (MUB) labeled substrates. CBH depolymerizes cellulose into cellobiose and BG hydrolyzes cellobiose to form glucose. The enzyme NAG is involved in the breakdown of chitin and thus the acquisition of organic N. AP hydrolyzes bound organic phosphorus and therefore represents an index of microbial investment in phosphorus acquisition. The oxidative enzymes phenol oxidase (Phenolox) (EC 1.10.3.2) and peroxidase (Perox) (EC 1.11.1.7) were assayed using L-3,4-dihydroxyphenylalanine as the substrate and measured for absorbance on a microplate spectrophotometer. Phenol oxidase and peroxidase degrade polyphenolic compounds such as lignin.

Soil enzyme activity was measured on 1 g subsamples and litter enzyme activity was measured on 0.5 g subsamples ($n = 6$). As is common in other studies, litter and soil samples were frozen prior to analysis (for example, Allison and Vitousek 2004; Hofmockel and others 2007; Waldrop and Zak 2006). Previous work (Sinsabaugh and Linkins 1989) found no consistent directional shift in activity associated with freezing samples and freezing should not differentially affect the activity of enzymes in N-amended versus control treatments, the primary focus of this study. We homogenized samples in 125 ml of acetate buffer (50 mmol/l, pH 5.0) using a Brinkmann Polytron. Sixteen replicate soil or litter suspensions for each sample per assay were then dispensed into 96 well

microplates. Sodium acetate buffer, MUB standard, and labeled substrates were dispensed into plates using a Precision 2000 robotic pipettor (BioTek Instruments). In total, 16 replicate sample wells (sample solution + substrate), eight replicate blank wells (sample solution + buffer), eight negative control wells (substrate + buffer), and eight quench standard wells (standard + sample solution) were used per assay. Prepared plates were incubated in the dark at 20°C for 0.5–20 h depending on the assay. Activity was measured as the fluorescence (hydrolytic enzymes) or absorbance (oxidative enzymes) of the sample wells corrected for negative controls, blanks, and quenching. Enzyme activity was calculated as the nmoles (μmoles for peroxidase and phenol oxidase) of substrate converted per hour per gram sample dry weight.

Data Analysis

We used two-way analysis of variance (ANOVA) to determine the effect of treatment (control and N fertilized) and site on litter decomposition (k_l) and soil labile C decay (k_s). For soil and litter enzymes assayed on multiple dates, we used repeated-measures ANOVA to determine the effect of sampling date (May, July, September), site, and treatment (control and N added) on enzyme activity. We found no significant date by treatment interactions using repeated-measures ANOVA so we averaged across dates to calculate a mean value for enzyme activity for each replicate of each treatment at each site for further analyses.

To determine if among-site variation in activity of each enzyme was related to measured site characteristics, we used analysis of covariance (ANCOVA) with treatment as the main effect and site characteristics as covariates, including soil chemistry (pH), SOM characteristics (soil C and C:N), soil N availability, soil microbial biomass, and soil physical properties (% moisture and temperature). Similarly, we used ANCOVA to analyze the relationship between litter enzyme activity and litter pH, litter layer N availability (assessed using ion-exchange resins), litter microbial biomass, and litter physical properties (% moisture and temperature). Initial ANCOVA models included interactions between covariates and treatment to test for homogeneity of slopes required by ANCOVA. Where interactions between treatments and covariates were significant, we analyzed bivariate regressions for control and N fertilized plots separately. Additionally, for litter characteristics that were only measured at the site level (prior to the initiation of the fertilization treatment), we used simple linear regression (instead of ANCOVA) to test for relationships between litter enzyme activity and litter % lignin and % N.

We also examined relationships among leaf litter decomposition, soil labile C decay, and enzyme activity (in control plots only) using simple linear regression. We first calculated mean litter decay constants and mean labile soil C decay constants from control plots for each site as described above, and then regressed decay constants for each site against mean litter or soil enzyme activity in control plots at each site for each enzyme separately.

Linear regression was also used to determine if the response of enzyme activity to added N was related to the effect of added N on litter and SOM decomposition. We first analyzed relationships between the effect of added N on enzyme activity and the effect of added N on native litter decomposition (calculated as the difference in decay constants for N fertilized and control plots for each site). Finally, we analyzed relationships between the effect of added N on enzyme activity and the effect of added N on labile C decay constants (calculated as the difference in decay constants, k_s, for N fertilized and control plots). Unless otherwise indicated, results are reported as significant when $P < 0.05$. All statistical analyses were done using JMP (JMP 6.0, SAS Institute).

Results

Litter and SOM Decomposition

We found a significant effect of site on both leaf litter decomposition ($P < 0.0001$) and labile soil C ($P = 0.0093$) decay rates (Figure 1). The effect of added N on litter decomposition rate was generally negative ($P = 0.0595$). For litter decomposition rate, there was a marginally significant site by treatment interaction ($P = 0.0537$), such that N decreased litter decay rate in the Oak, Maple, and Pine sites, but stimulated decomposition relative to control plots in the Aspen site. Similarly, there was a positive effect of N on the proportion of initial litter mass remaining at the final harvest, with the N-amended treatment having on average 31.1% (SE = 1.2) initial mass and the control treatment having on average 26.3% (SE = 1.2) initial mass (two-way ANOVA with site and treatment as main effects: treatment $P = 0.0067$, site $P < 0.0001$, site × treatment $P = 0.1492$, data not shown). For the effect of added N on soil labile C decomposition rate there was a significant site by treatment interaction ($P = 0.0093$). In Field 1 and Oak 1,

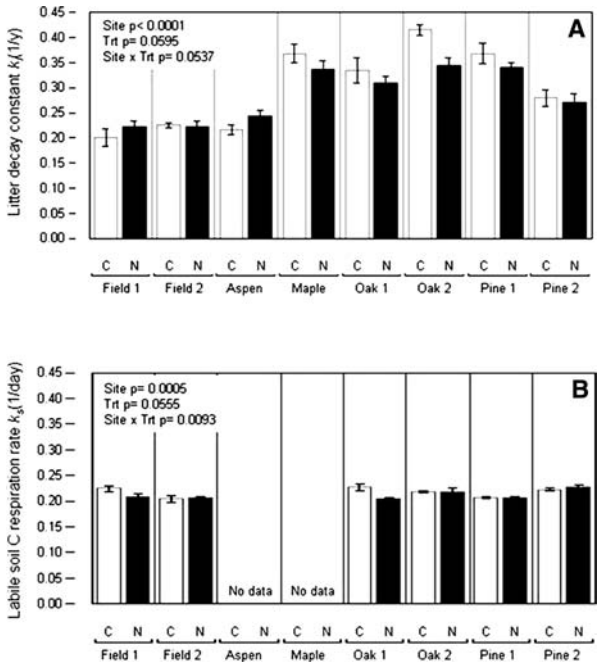

Figure 1. The effect of N and control (C) treatments across sites on **A** litter decomposition rates k_l, and **B** soil labile C decomposition rate, k_s. *P*-values are results of two-way ANOVA with site and treatment (Trt) as main effects.

added N decreased labile soil C decay rate, whereas in Pine 2 added N increased soil labile C decomposition rate. There was a significant positive effect of added N on the decay rate of the more recalcitrant C pool ($P < 0.001$, data not shown). The effect of added N on litter decomposition among sites was not correlated with the effect of added N on soil labile C decomposition.

Site Characteristics

Added N did not change total soil C concentrations or soil C:N (Table 1). There was a significant effect of site ($P = 0.0041$), treatment ($P < 0.0001$), and a significant site by treatment interaction ($P = 0.0219$) for the effect of added N on the size of the labile C pool as measured using laboratory incubations (Table 1). In the two pine sites, added N had no effect on the size of the labile C pool. In the two grassland sites and the two oak sites, added N decreased the size of the labile C pool. Added N significantly decreased soil pH (Table 2, $P < 0.0001$), but had no effect on litter pH (Hobbie 2008). Added N significantly increased exchangeable Al ($P = 0.006$) and decreased exchangeable Mg ($P = 0.0011$) concentrations, but had no effect on soil exchangeable K concentrations. A significant site by treatment interaction was observed for Ca ($P = 0.0063$) as exchangeable Ca concentrations declined with added N in most sites, but by differing amounts.

There was no effect of added N on total microbial biomass in litter or soil for the May sampling date (Table 1). However, added N increased microbial biomass C:N in soil ($P = 0.0002$) and decreased microbial biomass C:N in litter ($P = 0.0367$) (Table 1).

Enzyme Activity Among Sites

Activities of all enzymes in both soil and litter differed significantly among sites (Tables 3 and 4). Phenol oxidase activity was measured in all plots and across all dates, but was undetectable in litter and was detectable in soil samples only on the May sampling date, therefore where presented, phenol oxidase activity refers to May activity values only. For soil enzymes, soil moisture was positively related to the activity of AP ($P = 0.0015$, $R^2 = 0.57$), BG ($P < 0.0001$, $R^2 = 0.82$), CBH ($P < 0.0001$, $R^2 = 0.89$), NAG ($P < 0.0528$, $R^2 = 0.30$); this relationship was marginally significant for phenol oxidase ($P < 0.0891$, $R^2 = 0.31$) (Table 5). The activities of the oxidative enzymes, peroxidase, and phenol oxidase were negatively correlated with soil pH ($P = 0.0301$, $R^2 = 0.32$; $P = 0.0113$, $R^2 = 0.50$). These negative relationships held even when excluding the two grassland sites from our analysis (Fields 1 and 2 are drier and have higher pH than the forested sites). Soil C was positively related to activity of both cellulose degrading enzymes (BG and CBH) and chitinase (NAG). Soil microbial biomass was positively related to both soil cellulose degrading enzymes and AP activity. Pairwise correlations among site characteristics revealed significant positive correlations between soil moisture and soil C ($P = 0.0212$, $R^2 = 0.57$) and soil microbial biomass C ($P < 0.001$, $R^2 = 0.85$), likely explaining why these variables are all positively related to the activity of several enzymes. Field temperature at the O–A horizon boundary was negatively correlated with soil cellulose-degrading and peroxidase enzyme activity, but this relationship is likely driven by differences in soil moisture as the warmer grassland sites also tended to be drier (note that all assays were conducted at a constant temperature in the lab). Soil microbial biomass C:N and soil N availability were unrelated to soil enzyme activity.

Consistent with results from other studies, litter enzyme activity was significantly higher than soil enzyme activity on average (Table 3; Saiya-Cork and others 2002; Finzi and others 2006). As in soil, litter microbial biomass C was positively correlated with the activity of litter AP ($P = 0.0231$,

Table 1. Litter and Soil Characteristics of Study Sites

	Field 1		Field 2		Aspen		Maple		Oak 1		Oak 2		Pine 1		Pine 2	
	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N
Litter																
Litter % N	0.43	–	0.30	–	0.64	–	1.03	–	1.14	–	1.09	–	0.58	–	0.63	–
Litter % lignin	3.81	–	4.27	–	16.59	–	17.83	–	17.24	–	17.54	–	19.22	–	19.79	–
Litter gravimetric moisture (%)	11.0 (1.0)	16.1 (1.5)	8.5 (0.4)	10.0 (1.8)	27.8 (1.5)	27.1 (2.0)	24.5 (1.0)	23.6 (1.3)	32.5 (1.4)	30.8 (1.1)	34.8 (0.6)	35.2 (1.1)	28.9 (1.6)	30.4 (0.9)	32.5 (0.9)	33.9 (2.3)
Litter microbial biomass C (μg C/g litter)	1697 (136)	1676 (196)	1082 (371)	1491 (288)	1492 (146)	1133 (153)	747 (168)	382 (87)	1032 (170)	959 (138)	1018 (207)	1230 (224)	647 (83)	550 (101)	422 (84)	320 (48)
Litter microbial biomass C:N	6.24 (0.25)	6.70 (0.33)	7.05 (0.41)	6.78 (0.23)	10.23 (0.75)	7.95 (0.87)	5.49 (1.15)	6.68 (0.26)	7.33 (0.79)	5.65 (0.51)	6.38 (0.45)	5.42 (0.43)	6.04 (0.46)	4.89 (0.49)	4.00 (0.61)	3.31 (0.48)
Soil																
Soil C(mg C/g soil)	16.13 (2.93)	16.08 (2.24)	6.44 (0.58)	5.53 (0.56)	14.38 (0.52)	14.99 (0.90)	18.71 (0.37)	19.16 (1.50)	12.24 (1.96)	13.38 (1.77)	10.76 (1.47)	10.69 (0.53)	8.21 (0.84)	8.51 (0.85)	6.13 (0.47)	10.68 (3.24)
Soil labile C (*u*g C/g soil)	146.9 (3.9)	137.9 (3.1)	142.9 (2.7)	132.9 (1.4)	–	–	–	–	150.7 (3.9)	137.6 (1.2)	144.8 (1.8)	141.9 (1.5)	139.4 (0.9)	139.0 (0.8)	146.6 (1.3)	146.6 (2.0)
Soil C:N	17.0 (0.4)	17.56 (0.70)	14.1 (0.5)	12.77 (0.40)	16.2 (0.1)	16.13 (0.27)	18.3 (0.5)	19.72 (0.48)	14.8 (0.4)	15.76 (0.20)	16.0 (0.6)	15.82 (0.26)	16.7 (0.4)	16.18 (0.51)	11.5 (0.1)	12.05 (0.92)
Soil gravimetric moisture (%)	7.6 (0.2)	7.7 (0.4)	7.0 (0.7)	5.8 (0.8)	12.0 (0.2)	11.6 (0.5)	15.1 (0.4)	13.8 (0.5)	13.0 (0.5)	11.8 (0.6)	12.2 (0.3)	10.8 (0.2)	8.2 (0.2)	7.7 (0.3)	10.5 (0.7)	12.0 (0.3)
Soil microbial biomass C (μg C/g soil)	41.6 (3.7)	33.0 (3.4)	35.3 (3.2)	23.3 (1.5)	41.3 (3.2)	47.9 (3.6)	80.3 (5.2)	70.2 (3.7)	59.0 (2.6)	43.6 (5.7)	45.5 (2.4)	45.3 (4.5)	35.4 (5.0)	30.7 (3.1)	64.3 (1.6)	61.2 (7.1)
Soil microbial biomass C:N	5.8 (0.1)	6.5 (0.4)	5.4 (0.2)	6.6 (0.3)	5.1 (0.2)	5.2 (0.3)	6.2 (0.1)	6.9 (0.2)	5.8 (0.2)	7.1 (0.4)	5.2 (0.1)	5.8 (0.2)	4.8 (0.5)	4.8 (0.2)	5.4 (0.2)	4.6 (0.4)
Temperature (°C)	19.5 (0.2)	20.0 (0.2)	20.9 (0.2)	20.9 (0.3)	16.5 (0.1)	16.9 (0.1)	16.0 (0.1)	15.8 (0.1)	15.7 (0.1)	16.1 (0.1)	15.5 (0.1)	15.5 (0.1)	15.5 (0.1)	15.7 (0.1)	15.3 (0.1)	15.5 (0.1)

Values are means
(SE) for six replicate control
(C) and N fertilized plots
(N) averaged across three sampling dates, with the following exceptions:
(1) microbial biomass was measured on soil and litter collected from the May sampling date only,
(2) litter chemistry was analyzed on litterfall collected May to November, 1999, prior to the initiation of fertilization, and
(3) temperature at the O–A horizon boundary is averaged over May to October, 2006.

Table 2. Soil Exchangeable Base Cations (Potassium, Calcium, Magnesium, and Sodium), Aluminum, and Soil pH_{water} from N Fertilized and Control Plots

	Field 1		Field 2		Aspen		Maple		Oak 1		Oak 2		Pine 1		Pine 2	
	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N
Al	0.029	0.041	0.034	0.057	0.021	0.017	0.034	0.039	0.074	0.081	0.047	0.064	0.013	0.021	0.032	0.034
	(0.002)	(0.006)	(0.004)	(0.007)	(0.001)	(0.001)	(0.006)	(0.006)	(0.003)	(0.009)	(0.010)	(0.007)	(0.0001)	(0.001)	(0.009)	(0.009)
K	0.092	0.090	0.113	0.096	0.115	0.111	0.086	0.081	0.107	0.105	0.099	0.119	0.072	0.070	0.122	0.126
	(0.007)	(0.008)	(0.008)	(0.003)	(0.012)	(0.014)	(0.006)	(0.006)	(0.008)	(0.009)	(0.006)	(0.003)	(0.015)	(0.013)	(0.010)	(0.016)
Ca	1.514	1.389	1.436	0.970	1.954	1.812	2.800	1.440	0.959	0.677	0.809	0.936	1.843	1.405	2.622	3.893
	(0.087)	(0.116)	(0.011)	(0.064)	(0.043)	(0.239)	(0.627)	(0.082)	(0.271)	(0.102)	(0.196)	(0.130)	(0.479)	(0.287)	(0.486)	(0.207)
Mg	0.538	0.407	0.359	0.215	0.575	0.543	0.668	0.374	0.381	0.257	0.345	0.303	0.501	0.343	0.445	0.521
	(0.068)	(0.035)	(0.011)	(0.040)	(0.009)	(0.052)	(0.088)	(0.044)	(0.075)	(0.023)	(0.062)	(0.034)	(0.108)	(0.068)	(0.057)	(0.070)
Na	0.012	0.012	0.014	0.011	0.018	0.029	0.026	0.020	0.025	0.025	0.019	0.008	0.008	0.019	0.012	0.009
	(0.001)	(0.001)	(0.003)	(0.001)	(0.003)	(0.007)	(0.004)	(0.004)	(0.002)	(0.006)	(0.002)	(0.000)	(0.000)	(0.002)	(0.003)	(0.001)
pH	4.73	4.44	4.66	4.15	4.27	3.98	4.25	4.30	3.76	3.64	3.91	3.78	4.57	4.30	4.46	4.55
	(0.08)	(0.10)	(0.04)	(0.09)	(0.14)	(0.02)	(0.05)	(0.09)	(0.03)	(0.05)	(0.11)	(0.03)	(0.17)	(0.10)	(0.19)	(0.13)

Soil base cations and soil pH were measured once (May 2005) in three of the six replicate plots per treatment (n = 3). Values for base cations are means reported in cmole (+)/kg soil (SE).

$R^2 = 0.39$), BG ($P = 0.0043$, $R^2 = 0.48$), and CBH ($P = 0.002$, $R^2 = 0.68$) (Table 5). Litter pH was positively related to the activity of both cellulose-degrading enzymes, but did not correlate negatively with peroxidase as it did in soil. Litter N availability was negatively related to the activity of both cellulose-degrading enzymes and peroxidase. In each case, there was an interaction with treatment and litter N availability such that the relationship between enzyme activity and N availability was only significant in control plots, although the sign of the relationship was still negative in N-fertilized plots.

In comparisons of mean litter enzyme activity at each site (control plots only, eight points per regression) against litter % lignin and N (Table 5), CBH and AP were negatively correlated with % lignin ($P = 0.0018$, $R^2 = 0.54$ and $P = 0.0129$, $R^2 = 0.44$, respectively). Peroxidase activity was positively related to litter % N ($P = 0.0152$, $R^2 = 0.38$). Pairwise correlations among litter characteristics from control plots revealed significant positive correlations between litter pH and litter microbial biomass ($P = 0.0200$, $R^2 = 0.79$) and a marginally significant negative correlation between litter lignin content and microbial biomass ($P = 0.0798$, $R^2 = 0.65$). There was no relationship between litter % N and litter microbial biomass.

Among sites, the activity of CBH and BG were significantly positively correlated in both litter and soil (Pearson's correlation coefficients, 0.86 and 0.95, respectively).

Enzyme Activity and Decomposition Among Sites

Litter decomposition was unrelated to the activity of any litter enzyme. For example, the Aspen and grassland sites (Field 1 and 2) had slower rates of litter decomposition relative to other sites (Figure 1), but higher cellulose-degrading enzyme activity on average and did not differ from other sites in the activity of peroxidase and NAG. The soil labile C decay constant (k_s) was positively related to cellulose-degrading enzyme activity (the combined activity of CBH and BG, $R^2 = 0.82$, $P = 0.0130$), but was unrelated to lignin-degrading enzyme activity.

The Effect of Added N on Enzyme Activity Among Sites

N fertilization had significant positive, neutral, and negative effects on litter and soil enzyme activity, depending on the site and on the enzyme assayed (Tables 3–5). In soil, added N increased the activity

Table 3. Soil and Litter Enzyme Activity in Control (C) and N Fertilized (N) Plots

	Field 1		Field 2		Aspen		Maple		Oak 1		Oak 2		Pine 1		Pine 2	
	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N
Litter																
AP	10003.4	9123.1	7548.2	9556.0	4893.6	5450.5	5735.2	6749.1	8097.7	8808.1	7807.7	9766.9	4468.3	6154.7	6147.3	6883.1
	(753.1)	(820.0)	(537.4)	(663.0)	(267.1)	(256.2)	(469.0)	(968.8)	(443.6)	(708.9)	(396.2)	(809.1)	(157.2)	(711.2)	(560.9)	(591.2)
BG	4018.7	4026.9	3568.2	3998.5	4758.1	4703.0	3619.7	3432.2	4037.0	3619.7	3523.4	3700.7	2492.6	2972.0	3000.7	3510.3
	(255.6)	(198.0	(115.3)	(195.7)	(213.0)	(270.2)	(177.9)	(278.3)	(402.7)	(368.7)	(103.4)	(236.1)	(249.1)	(277.3)	(147.7)	(332.8)
NAG	2510.2	2439.5	2333.7	2611.1	1161.9	1350.4	1793.6	1855.9	2660.5	2611.5	2810.8	2305.9	1858.3	2043.0	2220.3	1871.0
	(250.8)	(136.8	(98.7)	(240.7)	(96.5)	(125.9)	(177.0)	(216.1)	(134.1)	(204.1)	(130.8)	(145.4)	(113.9)	(200.5)	(197.8)	(112.1)
CBH	2416.0	2187.7	1924.9	2297.7	2125.4	2140.0	1660.4	1646.2	1905.8	1532.9	1408.7	1760.9	926.5	1214.3	1250.6	1541.3
	(110.3)	(113.7)	(67.9)	(126.9)	(125.3)	(103.8)	(99.2)	(188.5)	(183.6)	(236.0)	(40.8)	(135.1)	(125.4)	(140.5)	(85.1)	(168.4)
Perox	0.12	0.20	*	*	0.33	0.32	0.76	0.42	0.79	0.42	0.32	0.57	*	*	*	*
	(0.11)	(0.06)			(0.27)	(0.22)	(0.18)	(0.30)	(0.16)	(0.22)	(0.20)	(0.51)				
Phenolox	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Soil																
AP	141.3	177.3	142.1	139.7	205.7	242.0	190.9	240.9	187.7	224.6	271.8	230.6	79.8	107.4	227.4	261.8
	(16.4)	(9.6)	(15.6)	(8.2)	(11.7)	(18.4)	(7.3)	(12.2)	(15.2)	(18.2)	(17.7)	(16.7)	(3.9)	(5.0)	(29.0)	(27.6)
BG	112.2	115.3	91.2	80.7	145.4	158.8	150.6	155.4	140.4	135.6	118.4	128.8	89.9	110.1	118.9	155.7
	(6.0)	(12.4)	(3.1)	(7.5)	(14.4)	(15.9)	(14.9)	(14.5)	(6.4)	(11.7)	(7.2)	(14.1)	(4.5)	(6.3)	(16.9)	(13.4)
NAG	40.7	40.5	43.1	35.6	66.4	69.6	60.5	73.1	47.1	50.7	66.7	58.4	37.1	76.9	33.3	41.6
	(1.5)	(1.3)	(2.3)	(2.6)	(2.9)	(4.1)	(5.3)	(7.5)	(2.5)	(7.8)	(3.6)	(3.1)	(4.4)	(5.4)	(4.3)	(3.9)
CBH	19.8	20.8	13.5	11.8	29.7	37.1	34.6	43.0	33.0	29.0	24.7	30.7	15.2	24.7	27.9	35.0
	(1.3)	(2.9)	(1.5)	(1.6)	(4.5)	(6.7)	(4.8)	(4.0)	(2.1)	(2.7)	(2.7)	(5.0)	(1.6)	(2.0)	(6.0)	(4.1)
Perox	1.13	1.27	1.45	1.53	1.74	1.80	1.77	1.75	1.99	2.21	1.50	1.65	1.72	2.02	1.77	1.40
	(0.05)	(0.10)	(0.08)	(0.06)	(0.05)	(0.05)	(0.09)	(0.09)	(0.11)	(0.04)	(0.10)	(0.06)	(0.07)	(0.08)	(0.15)	(0.09)
Phenolox[1]	0.07	0.06	0.05	0.07	0.36	0.39	0.20	0.12	0.43	0.46	0.42	0.39	0.14	0.17	0.17	0.08
	(0.03)	(0.03)	(0.00)	(0.05)	(0.10)	(0.06)	(0.09)	(0.04)	(0.13)	(0.06)	(0.07)	(0.07)	(0.06)	(0.07)	(0.08)	(0.02)

**Sites in which enzyme activity was undetectable.*
[1]Soil phenol oxidase activity was only detectable in May soils—values are means for May sampling date only.
Values are means (SE) calculated by taking the average of the three sampling dates for each of six replicate plots per site. Values for AP, BG, NAG, and CBH are presented in nmoles/h/g. Values for Perox and Phenolox are given in μmoles/h/g.

Table 4. Significance of Repeated-Measures ANOVA for Enzyme Activity Measured Three Times Over the Growing Season with Site and Treatment (Control or N-fertilized) as Independent Variables

Source of variation	AP	BG	CBH	NAG	Perox
Litter					
Between subjects					
Intercept	***	***	***	***	***
Site	***	***	***	***	**
Trt	** (+)	ns	ns	ns	ns
Site × Trt	ns	ns	ns	ns	ns
Within subjects					
Date	***	***	*	***	***
Date × site	***	***	***	***	**
Date × Trt	ns	ns	ns	ns	ns
Date × site × Trt	ns	ns	ns	*	ns
Soil					
Between subjects					
Intercept	***	***	***	***	***
Site	***	***	***	***	**
Trt	* (+)	ns	* (+)	** (+)	ns
Site × Trt	ns	ns	ns	***	**
Within subjects					
Date	***	***	*	ns	***
Date × site	***	***	***	***	**
Date × Trt	ns	ns	ns	ns	ns
Date × site × Trt	*	ns	ns	**	ns

Where significant, the sign of the effect of added N on enzyme activity is indicated in parenthesis.
** $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.*

of AP by 13% on average ($P = 0.0221$), of CBH by 17% ($P = 0.0286$), and of NAG by 18% ($P = 0.0031$). N fertilization significantly increased soil BG activity when adjusted for soil moisture using ANCOVA ($P < 0.001$). For soil NAG activity, added N increased activity at all sites except Oak 1 and Field 2 (site by treatment interaction, $P < 0.0001$). There was a significant site by treatment interaction for soil peroxidase activity. Peroxidase activity increased with N fertilization in both oak sites and one pine site (Pine 1), but decreased in the other pine site (Pine 2). The effect of N fertilization on soil phenol oxidase activity was not significant (two-way ANOVA with site and N fertilization as main effects; for site, $P < 0.0001$, for treatment, $P = 0.4894$).

The effect of N on litter enzyme activity was largely neutral. Only one enzyme, AP, responded positively to the N treatment (average increase 16%, $P = 0.0020$). Among different sites and enzymes, there was no relationship between the effect of added N on soil enzyme activity and the effect of added N on litter enzyme activity.

The effect of N on litter cellulose-degrading enzyme activity was significantly positively correlated with litter N availability ($R^2 = 0.56$, $P = 0.0323$), however the effect of N on litter enzyme activity was unrelated to litter % N or lignin. In soil, the effect of N on cellulose-degrading enzyme activity was also positively correlated (albeit weakly) with soil N availability ($R^2 = 0.44$, $P = 0.0708$).

The Effect of N on Enzyme Activity and Decomposition

Site-specific responses of enzyme activity to added N did not correlate with effects of N on litter decomposition or labile soil C decomposition. Backwards stepwise regressions of the effect of N on enzyme activity against the effect of N on litter decomposition did not yield any significant relationships based on the litter enzymes we measured (analyses not shown). Similarly, the effect of added N on labile soil C decomposition did not correlate with the effect of added N on soil enzyme activity. Notably, the effect of N on litter peroxidase activity was positive, though not significantly so, in several sites where the overall effect of N on litter decomposition was negative, suggesting that among these sites, shifts in lignin-degrading enzyme activity do not explain variation in the effect of added N on litter decomposition (analyses not shown).

Discussion

Enzyme Activity Among Sites

Previous investigations have found large differences in enzyme activity among ecosystems (Waldrop and others 2004a; Zeglin and others 2007), and attributed these differences to variation in litter chemistry (specifically lignin content), microbial community composition, or edaphic factors. We measured enzyme activity in both soil and litter simultaneously across multiple sites that vary in their aboveground plant cover, organic matter content and chemistry, and environmental characteristics. Among our eight sites, the best predictor of soil enzyme activity was soil moisture, which was also significantly positively correlated with soil C and soil microbial biomass. Teasing apart causal relationships is not possible using regression approaches, but higher moisture per se likely was not responsible for higher enzyme activity because all enzyme assays were run under standard moisture conditions. However, more C-rich soils with higher water holding capacity and more available C likely created more favorable conditions for microbial growth leading to greater production of enzymes.

Table 5. Significance of Relationships Between Enzyme Activities and Site Characteristics (Averaged Across Dates and Replicate Plots, Except for Phenol Oxidase Where Values are for May Sampling Date Only)

	AP	BG	CBH	NAG	Perox	Phenolox
Litter						
Litter % moisture	ns	ns	0.43 (−)**	ns	ns	–
Litter pH	ns	0.34 (+)*	0.36 (+)*	ns	ns	–
Litter % lignin	0.44 (−)*	ns	0.54 (−)**	ns	ns	–
Litter % N	ns	ns	ns	ns	0.38 (+)*	–
Litter microbial biomass (μg C/g litter)	0.39 (+)*	0.48 (+)**	0.68 (+)**	ns	ns	–
Litter microbial biomass C:N	ns	0.55 (+)**	0.36 (+)*	ns	0.26 (+)†	–
Surface resin DIN (μg N/g resin)	ns	inx (−)	inx (−)	ns	inx (−)	–
Temperature (°C)	0.31 (+)†	ns	0.50 (+)**	ns	ns	–
Soil						
Soil % moisture	0.57 (+)**	**0.82(+)*** **	**0.89(+)*** **	0.30 (+)†	ns	0.31 (+)†
Soil pH	ns	ns	ns	ns	0.32 (−)*	0.50 (−)*
Soil C (mg C/g soil)	ns	0.49 (+)**	0.40 (+)*	0.25 (+)†	ns	ns
Soil C:N	ns	ns	ns	0.38 (+)*	ns	ns
Soil microbial biomass (μg C/g soil)	0.41 (+)*	0.64 (+)***	**0.77(+)*** **	ns	ns	ns
Soil microbial biomass C:N	ns	ns	ns	ns	ns	ns
Soil resin DIN (μg N/g resin)	ns	ns	ns	ns	ns	ns
Temperature (°C)	ns	0.36 (−)*	0.51 (−)**	ns	0.36 (−)*	ns

Where site characteristics were measured in both control and N fertilized plots we used ANCOVA (see section ''Methods'' for details on analysis). For litter chemistry that was only measured in control conditions, we used simple linear regression. For ANCOVA, main effects of treatment and treatment by predictor interactions were not significant except where noted. When significant, the R^2 value, significance, and sign of the relationship (in parentheses) with enzyme activity are indicated. Bold terms indicate instances where the main effect of treatment was significant ($P < 0.05$). Models in which the interaction between treatment and covariates was significant (and therefore violated the assumptions of ANCOVA) are indicated by ''inx.'' These relationships were run as individual linear regressions for each treatment. In all three instances the sign of the relationship was consistent for both treatments, but only the control plots were significant ($P < 0.05$).
**$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, †$P < 0.10$, ns, not significant, dashes indicate lack of enzyme activity so analyses were not done.*

In litter, the activity of phosphatase and cellulase-degrading enzymes was positively correlated with litter microbial biomass, which was in turn correlated with litter pH and (negatively) with litter lignin. These results suggest that enzyme activities of both litter- and soil-dwelling microbial communities are influenced by microbial biomass, and thus indirectly by factors such as C availability and quality and environmental conditions.

Effect of Added N on Site Characteristics and Enzyme Activity

After 7 years of N fertilization, added N decreased soil pH and exchangeable base cations, and increased exchangeable Al. Added N did not change total microbial biomass, but did shift microbial biomass C:N ratios in both the soil and litter in a direction consistent with a more bacteria-dominated community (with lower overall C:N) in the litter layer, and a more fungus-dominated community (with higher C:N) in the soil (Paul and Clark 1996). In previous N addition experiments in both grasslands and forests fungal to bacteria ratios declined in N-amended soils (Hogberg and others 2007; Bradley and others 2006; Frey and others 2004; Bardgett and Shine 1999), consistent with our litter results, but opposite our soil results, although we measured microbial biomass at only one sampling date and repeated sampling on a finer temporal scale may have produced different results.

Consistent with our hypothesis, across all sites, added N stimulated soil and litter AP activity, soil CBH activity, and soil NAG activity. The stimulation of enzyme activity by N addition, particularly phosphatase and cellulose-degrading enzymes, has been observed in multiple N fertilization studies (Ajwa and others 1999; Olander and Vitousek 2000; Saiya-Cork and others 2002; Henry and others 2005; Sturstova and others 2006). Our results, in combination with previous studies, suggest that in N-limited systems, the addition of N stimulates microbial activity and increases the demand for both P and C, leading to increases in C and P acquiring enzymes. Enzymes are N-rich compounds, therefore their production is tightly regulated by the availability of N.

In contrast to our hypothesis, we found little effect of N fertilization on oxidative enzymes. These results differ from some previous studies that have found significant negative effects of added N on lignin-degrading enzyme activity (Carriero and

others 2000; DeForest and others 2004; Frey and others 2004). Low levels of phenol oxidase activity precluded many tests of N effects on its activity. Average rates of soil phenol oxidase activity reported here are similar to those measured in other temperate forests (for example, Sinsabaugh and others 2005), and other studies have reported similarly low or undetectable levels of phenol oxidase, particularly in litter (Sinsabaugh and others 2005, 2008; Finzi and others 2006). The reasons for such low or undectable phenol oxidase activity are unclear, and elucidating them will likely require further more temporally intensive sampling. Nevertheless, when detectable, phenol oxidase activity exhibited little evidence for N suppression. Activity of the oxidative enzyme peroxidase showed inconsistent responses to added N in litter and soil—N increased peroxidase activity in soil in some sites, whereas decreasing it in others, but had no effect on litter peroxidase activity. Repression of lignin-degrading enzyme activity with N additions has gained considerable attention as one of the mechanisms that may explain negative effects of added N on decomposition. However, as shown here, the negative response of oxidative enzyme activity to N addition is not universal. And indeed, there are numerous examples from other studies also showing neutral or positive effects of N on oxidative enzyme activity (Saiya-Cork and others 2002; Michel and Matzner 2003; Henry and others 2005; Stursova and others 2006; Bragazza and others 2007; Zeglin and others 2007).

Our results contrast those of other studies showing that site variation in N effects on enzyme activity was related to variation in litter chemistry, with N reducing lignolytic activity in high-lignin litter but stimulating it in more labile litter (Carriero and others 2000; Sinsabaugh and others 2005; Waldrop and Zak 2006). Additional studies have hypothesized that variation in the effect of added N on enzyme activity is related to litter C:N ratio (Michel and Matzner 2003; Waldrop and others 2004a). In contrast, in this study, sites with similar plant species composition differed as much as sites with different plant cover in enzyme response to added N. For example, soil peroxidase activity increased with added N in one pine site (Pine 1), but decreased with added N in the other pine site (Pine 2). Similarly, added N increased NAG activity in one oak site (Oak 2), and decreased it in the other (Oak 1). Thus, even among sites with the same plant cover (and hence litter chemistry), soil conditions and/or communities appear to differ sufficiently to cause opposite responses to N addition in terms of enzyme activity.

Enzyme Activity as Predictor of Rates of Decomposition

Among sites (in control plots), litter enzyme activity was not related to the decomposition of fine litter. These results contrast those of studies that found a significant relationship between enzyme activity and decomposition rates in both terrestrial and aquatic environments (Sinsabaugh and others 1992; Sinsabaugh and Findlay 1995; Alvarez and Guerrero 2000; Rejmankova and Sirova 2007). Additional work has linked the effect of added N on enzyme activity to the effect of added N on litter mass loss (Carriero and others 2000) and soil C loss (Waldrop and others 2004b). Here, the response of soil and litter enzymes to N additions was unrelated to the effect of added N on rates of litter decomposition and soil respiration among sites. The overall effect of added N on litter decomposition was negative or neutral, whereas the overall effect of N on litter and soil enzymes was neutral, positive, or negative. Specifically, in the Aspen site where N increased litter decomposition rates (by 12%), N had a slight negative effect on the activity of cellulose and lignin-degrading enzymes. In Oak 2, added N repressed litter decomposition rates by 18%, whereas the effect of N on peroxidase and both cellulose-degrading enzymes was positive. Similarly, we found no relationship between N effects on soil enzymes and N effects on soil labile C decay constants. For example, added N repressed soil labile C decay constants in Field 1 relative to controls, however in this same site, added N increased the activities of cellulose-degrading enzymes and both lignin-degrading enzymes.

There are several reasons why enzyme activity may not correlate with rates of decomposition. Decomposition relies on a complex suite of enzymes, only a small number of which were measured in this study. N additions may have numerous and complex effects on ecosystems, including alteration of microbial community composition (Fog 1988), decomposer efficiency (Agren and others 2001), and/or increasing the chemical recalcitrance of the substrate making enzymes less effective in their environment (Berg and Matzner 1997). Additionally, what we know about extracellular enzymes suggests that they respond quickly to pulse events in moisture or resource availability and that sampling thrice over the course of one growing season (as done in this study) may not capture the seasonal and inter-annual dynamics of enzyme activities (Sinsabaugh and others 2008). Therefore, the high-temporal heterogeneity of

enzyme activity may hinder our ability to detect significant relationships between enzyme activity and the integrated process of decomposition that occurs over much longer timescales. Finally, efforts to link microbial community composition with the abundance of specific enzyme-producing genes and enzyme activity have thus far yielded only weak correlations in some sites (Blackwood and others 2007; Hofmockel and others 2007). Little is known about the functional diversity of enzyme activity in natural environments, specifically the groups of organisms responsible for the production of different types of enzymes, how production and efficiency are regulated among different groups of organisms, and what happens to the effectiveness of extracellular enzymes once they are released into the heterogeneous soil matrix (Nannipieri and others 2002).

Although negative effects of N on white rot basidiomycete fungi and their production of lignin-degrading enzymes have been highlighted in the literature as an explanation for negative effects of added N on decomposition, the production of lignin-degrading enzymes is likely regulated by many different types of microbes and may respond to many other environmental signals in addition to resource availability. These potential interactions among resource availability, microbial community composition, and extracellular enzyme production need further research to fully elucidate the role of microbial communities in mediating N effects on ecosystem processes like decomposition. What is clear from our work is that the assumed linkages between enzyme activity and rates of decomposition that have been observed in previous studies are not evident over the timescale of this study. The lack of a relationship between the effect of N on enzyme activity and N effects on decomposition suggests that either N effects on enzyme (particularly oxidative enzyme) activity occur in sufficiently short time scales that they were undetectable with our temporal sampling intensity, or that other mechanisms besides N effects on extracellular enzyme activity are responsible for among-site variation in decomposition response to long-term N additions.

Conclusion

Our research adds to growing evidence that added N stimulates activities of both cellulose-degrading and phosphorus-acquiring enzymes. However, as we did not find consistent negative effects of added N on lignin-degrading enzyme activity, our results along with those of others, suggest that such effects vary among sites and are not universal. Across sites, activities of cellulose-degrading and phosphorus (and to some extent N) acquiring enzymes were enhanced in sites with high microbial biomass, both in litter and soil, suggesting that biomass, rather than abiotic conditions, was the most important proximate control on microbial enzyme activity.

Enzyme activity and decomposition were unrelated among sites. Nor was the effect of added N on enzyme activity related to the effect of added N on decomposition. We also provide evidence against the hypothesis that the effect of added N on lignin degrading enzyme activity is the best predictor of the effect of N on decomposition. These results suggest that patterns of N effects on enzyme activity observed in studies of N addition in a relatively small number of sites, may not apply broadly across all systems.

Understanding the mechanisms that regulate the long-term effects of N deposition on litter and SOM decomposition is critical. Recent efforts have been made to incorporate microbial parameters into decomposition models (Schimel and Weintraub 2003; Moorhead and Sinsabaugh 2006). More research on finer temporal scales is needed before the effects of N on microbial enzyme activity across sites can be generalized. It is not clear from this study that N effects on enzyme activity alone explain the variation in N effects on decomposition and other explanations such as shifts in community composition, resource use efficiency, and abiotic reactions with N and phenolic compounds need further evaluation.

Acknowledgements

We gratefully thank William Eddy, Jeff Eickhoff, Daniel Hernandez, Adrienne Keller, Tiffany Miley, Angie Moline, Chinelo Njaka, Megan Ogdahl, and Krista Ristanen for assistance with field and lab work. This material is based upon work supported by the National Science Foundation under Grants DEB-0080382 and DEB-0347103 a National Science Foundation Graduate Research Fellowship to BLK, a Grant-in-Aid from the University of Minnesota Graduate School, and a McKnight Land Grant Professorship to SEH.

References

Agren G, Bosatta E, Magill AH. 2001. Combining theory and experiment to understand effects of inorganic nitrogen on litter decomposition. Oecologia 128:464.

Ajwa HA, Dell CJ, Rice CW. 1999. Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. Soil Biol Biochem 31:769–77.

Allison SD, Vitousek PM. 2004. Extracellular enzyme activity and carbon chemistry as drivers of tropical plant litter decomposition. Biotropica 36:285–96.

Alvarez S, Guerrero MC. 2000. Enzymatic activities associated with decomposition of particulate organic matter in two shallow ponds. Soil Biol Biochem 32:1941–51.

Bardgett RD, Shine A. 1999. Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biol Biochem 31:317–21.

Beck T, Joergensen RG, Kandeler E, Makeschin F, Nuss E, Oberholzer HR, Scheu S. 1997. An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biol Biochem 29:1023–32.

Berg B, Matzner E. 1997. Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. Environ Rev 5:1–25.

Blackwood CB, Waldrop MP, Zak DR, Sinsabaugh RL. 2007. Molecular analysis of fungal communities and laccase genes in decomposing litter reveals differences among forest types but no impact of nitrogen deposition. Environ Microbiol 9:1306–16.

Bradley K, Drijber RA, Knops J. 2006. Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. Soil Biol Biochem 38:1583–95.

Bragazza L, Freeman C, Jones T, Rydind H, Limpense J, Fennerc N, Ellisc T, Gerdol R, Hajek M, Hajek T, Iacumin P, Kutnar L, Tahvanainen T, Toberman H. 2007. Atmospheric nitrogen deposition promotes carbon loss from peat bogs. Proc Natl Acad Sci 103:19386–9.

Brookes PC, Landman A, Pruden G, Jenkinson DS. 1985. Chloroform fumigation and the release of soil-nitrogen—a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–42.

Carreiro MM, Sinsabaugh RL, Repert DA, Parkhurst DF. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81:2359–65.

DeForest JL, Zak DR, Pregitzer KS. 2004. Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. Soil Biol Biochem 36:965–71.

Dijkstra FA, Hobbie SE, Reich PB, Knops J. 2005. Divergent effects of elevated CO2, N fertilization, and plant diversity on soil C and N dynamics in a grassland field experiment. Plant Soil 272:41–52.

Finzi AC, Sinsabaugh RL, Long TM, Osgood MP. 2006. Microbial community responses to atmospheric carbon dioxide enrichment in a warm-temperate forest. Ecosystems 9:215–26.

Fog K. 1988. The effect of added nitrogen on the rate of decomposition of organic-matter. Biol Rev Camb Philos Soc 63:433–62.

Frey SD, Knorr M, Parrent JL, Simpson RT. 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. For Ecol Manage 196:159–71.

Galloway JN, Schlesinger WH, Levy H, Michaels A, Schnoor JL. 1995. Nitrogen-fixation—anthropogenic enhancement-environmental response. Global Biogeochem Cycles 9:235–52.

Hendershot WH, Lalande H, Duquette M. 1993. Soil reaction and exchangeable acidity. In: Carter MR, Ed. Soil sampling and methods of analysis. Boca Raton: Lewis Publishers. p 167–76.

Henry HA, Juarez JD, Field CB, Vitousek PM. 2005. Interactive effects of elevated CO2, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. Glob Change Biol 11:1808–15.

Hobbie SE. 2000. Interactions between litter lignin and soil nitrogen availability during leaf litter decomposition in a Hawaiian montane forest. Ecosystems 3:484–94.

Hobbie SE. 2005. Contrasting effects of substrate and fertilizer nitrogen on the early stages of litter decomposition. Ecosystems 8:644–56.

Hobbie SE. 2008. Nitrogen effects on litter decomposition: a five-year experiment in eight temperate grassland and forest sites. Ecology 89:2633–44.

Hofmockel KS, Zak Blackwood DR CB. 2007. Does atmospheric NO3- deposition alter the abundance and activity of lignino-lytic fungi in forest soils? Ecosystems 10:1278–86.

Hogberg MN, Hogberg P, Myrold DD. 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150:590–601.

Knorr M, Frey SD, Curtis PS. 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86:3252–7.

Mack MC, Schuur EAG, Bret-Harte MS, Shaver GR, Chapin FS. 2004. Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. Nature 431:440–3.

Magill AH, Aber JD. 1998. Long-term effects of experimental nitrogen additions on foliar litter decay and humus formation in forest ecosystems. Plant Soil 203:301–11.

Magill AH, Aber JD, Hendricks JJ, Bowden RD, Melillo JM, Steudler PA. 1997. Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecol Appl 2:402–15.

Michel K, Matzner E. 2003. Response of enzyme activities to nitrogen addition in forest floors of different C-to-N ratios. Biol Fertil Soils 38:102–9.

Moorhead DL, Sinsabaugh RL. 2006. A theoretical model of litter decay and microbial interaction. Ecol Monogr 76:151–74.

Nannipieri P, Kandeler E, Ruggiero P. 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Burns RG, Dick RP, Eds. Enzymes in the environment. New York: Marcel Dekker. p 1–33.

Neff JC, Townsend AR, Gleixner G, Lehman SJ, Turnbull J, Bowman WD. 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419:915–7.

Olander LP, Vitousek PM. 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry 49:175–90.

Paul EA, Clark FE. 1996. Soil microbiology and biochemistry, 2nd edition. San Diego, CA: Academic Press, p 340.

Rejmankova E, Sirova D. 2007. Wetland macrophyte decomposition under different nutrient conditions: relationships between decomposition rate, enzyme activities and microbial biomass. Soil Biol Biochem 39:526–38.

Saiya-Cork KR, Sinsabaugh RL, Zak DR. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biol Biochem 34:1309–15.

Schimel JP, Weintraub MN. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35:549–63.

Sinsabaugh RL, Antibus RK, Linkins AE, Mcclaugherty CA, Rayburn L, Repert D, Weiland T. 1992. Wood decomposition

over a 1st-order watershed—mass-loss as a function of lignocellulase activity. Soil Biol Biochem 24:743–9.

Sinsabaugh RL, Carreiro MM, Repert DA. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60:1–24.

Sinsabaugh RL, Findlay S. 1995. Microbial-production, enzyme-activity, and carbon turnover in surface sediments of the Hudson River estuary. Microb Ecol 30:127–41.

Sinsabaugh RL, Gallo ME, Lauber C, Waldrop MP, Zak DR. 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75:201–15.

Sinsabaugh RL, Lauber C, Weintraub M, Ahmed B, Allison S, Crenshaw C, Contosta A, Cusack D, Frey S, Gallo M, Gartner T, Hobbie S, Holland K, Keeler B, Powers J, Stursova M, Takacs-Vesbach C, Waldrop M, Wallenstein M, Zak D, Zeglin L. 2008. Stoichiometry of soil enzyme activity at global scale. Ecol Lett 11:1–13.

Sinsabaugh RL, Linkins AE. 1989. Natural disturbance and the activity of *trichoderma viride* cellulase complexes. Soil Biol Biochem 21:835–9.

Stursova M, Crenshaw CL, Sinsabaugh RL. 2006. Microbial responses to long-term N deposition in a semiarid grassland. Microb Ecol 51:90–8.

Tilman D. 1984. Plant dominance along an experimental nutrient gradient. Ecology 65:1445–53.

Treseder KK, Vitousek PM. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. Ecology 82:946–54.

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7:737–50.

Vitousek PM, Howarth RW. 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13:87–115.

Waldrop MP, Zak DR, Sinsabaugh RL. 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biol Biochem 36:1443–51.

Waldrop MP, Zak DR, Sinsabaugh RL, Gallo M, Lauber C. 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. Ecol Appl 14:1172–7.

Waldrop MP, Zak DR. 2006. Response of oxidative enzyme activities to nitrogen deposition affects soil concentrations of dissolved organic carbon. Ecosystems 9:921–33.

Wedin DA, Tilman D. 1997. Atmospheric nitrogen deposition—response. Science 275:740–74.

Zeglin LH, Stursova M, Sinsabaugh RL, Collins SL. 2007. Microbial responses to nitrogen addition in three contrasting grassland ecosystems. Oecologia 154:349–59.