



# The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil

K.R. Saiya-Cork<sup>a</sup>, R.L. Sinsabaugh<sup>a,\*</sup>, D.R. Zak<sup>b</sup>

<sup>a</sup>Environmental Sciences Department, University of Toledo, 2801 W. Bancroft Str., Toledo, OH 43606-3390, USA

<sup>b</sup>School of Natural Resources, University of Michigan, Ann Arbor, MI 48109, USA

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## Abstract

Anthropogenic N deposition affects litter decomposition and soil organic matter (SOM) storage through multiple mechanisms. Microbial community responses to long-term N deposition were investigated in a sugar maple-dominated forest in northern Michigan during the 1998–2000 growing seasons. Litter and soil were collected from three fertilized plots (30 kg N ha<sup>-1</sup> y<sup>-1</sup>) and three control plots. The activities of 10 extracellular enzymes (EEA) were assayed. ANOVA and meta-analysis techniques were used to compare treatment responses. EEA responses to N amendment were greater in litter than in soil (litter mean effect size [*d*] = 0.534 std. dev.; soil *d* = 0.308). Urease, acid phosphatase and glycosidase (β-glucosidase, α-glucosidase, cellobiohydrolase, β-xylosidase) activities increased in both soil and litter; mean responses ranged from 7 to 56%. *N*-Acetylglucosaminidase activity increased 14% in soil but decreased 4% in litter. Phenol oxidase activity dropped 40% in soil, but increased 63% in litter. These responses suggest that N deposition has increased litter decomposition rate and depressed SOM decomposition. In previous studies, loss of phenol oxidase activity in response to N deposition has been attributed to suppression of lignin-degrading basidiomycetes. However, the decline of this activity in bacterially-dominated soil suggest that N inhibition of recalcitrant organic matter decomposition may be a more general phenomenon. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** N deposition; Decomposition; Extracellular enzyme activity; Cellulase; Phenol oxidase

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## 1. Introduction

Anthropogenic alteration of the global nitrogen (N) cycle may alter key processes controlling the cycling of C and N in forest ecosystems (Berg and Matzner, 1997). Chronic deposition of nitric and other acids decreases soil pH and base saturation, alters mineral weathering rates, and affects a wide range of biological processes (Rustad et al., 1993). It may also increase organic matter accumulation by fertilizing plant growth and altering microdecomposer activity (Nadelhoffer et al., 1999).

The mechanisms through which N deposition alters decomposition remain unclear (Sinsabaugh et al., 2002). In their reviews, Fog (1988) and Berg and Matzner (1997) emphasize that the effect of N deposition on decomposition changes as decomposition progresses. They suggest that early stages of decomposition are enhanced, while later stages, during which lignin is degraded, are repressed by N

amendments. One way to better understand these effects is to focus on the activity of the extracellular enzymes that mediate microbial decomposition.

Carreiro et al. (2000) found that N-induced attenuations of litter decomposition rates were closely linked to changes in phenol oxidase activity. This finding was consistent with the speculations of Fog (1988) and others that the N amendment may retard the decomposition of lignified litter by repressing the production of lignin-degrading enzymes by white rot Basidiomycetes. The study by Carreiro et al. (2000) only examined litter. It is not clear if the decomposition of soil organic matter (SOM) follows a similar pattern. SOM has a lower C/N ratio than litter and is largely humified. In addition, bacterial activity may more important in SOM degradation than fungal.

The goal of this study was to compare the effects of chronic N deposition on micro-decomposer activities in litter and soil. The study site was a temperate deciduous *Acer saccharum* forest in northern Michigan near the University of Michigan Biological Station in Pellston. In this stand, fine root N concentration, root respiration rates,

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\* Corresponding author. Tel.: +1-419-530-8360; fax: +1-419-530-8399.  
E-mail address: robert.sinsabaugh@utoledo.edu (R.L. Sinsabaugh).

Table 1

Extracellular enzymes assayed in soil and litter collected from Pellston, MI, their enzyme commission number (EC), corresponding substrate, and the abbreviation used in this study (DOPA = L-3,4-dihydroxyphenylalanine; 4-MUB = 4-methylumbelliferyl)

Enzyme	Abbreviation	EC	Substrate
Urease	Urease	3.5.1.5	Urea
Phenol Oxidase	PhOx	1.10.3.2	L-DOPA
Peroxidase	Perox	1.11.1.7	L-DOPA
Acid phosphatase	aP	3.1.3.2	4-MUB-phosphate
$\beta$ -1,4-glucosidase	$\beta$ G	3.2.1.21	4-MUB- $\beta$ -D-glucoside
Cellobiohydrolase	CBH	3.2.1.91	4-MUB- $\beta$ -D-cellobioside
$\beta$ -1,4-N-acetylglucosaminidase	NAG	3.1.6.1	4-MUB-N-acetyl- $\beta$ -D-glucosaminide
$\beta$ -1,4-xylosidase	$\beta$ X	3.2.1.37	4-MUB- $\beta$ -D-xyloside
$\alpha$ -1,4-glucosidase	$\alpha$ G	3.2.1.20	4-MUB- $\alpha$ -D-glucoside
L-leucine aminopeptidase	LAP	3.4.11.1	L-Leucine-7-amino-4-methylcoumarin

and fine-root life spans have increased with N amendment treatments while fine root biomass has decreased (Zogg et al., 1996; Burton et al., 1997, 1998; Pregitzer et al., 1998). Our working hypothesis was that soil extracellular enzyme activities (EEA) had responded to N amendments to the same degree and direction as those in litter.

## 2. Materials and methods

### 2.1. Study site and experimental design

The study site was a temperate hardwood forest stand located near Pellston, Michigan, at latitude 45°33'N and longitude 84°51'W. The stand is one of a series of matched experimental sites arrayed along a climatic and nitrogen (N) deposition gradient extending from southern to northern Michigan in which the northern most sites receive the smallest amount of anthropogenic NO<sub>x</sub> and have the coldest temperatures. These sites are part of a long-term experiment to understand the effects of nitrate deposition on hardwood forests. The stands are similar in age, basal area, species composition, and soil development (Rothstein et al., 1996; Burton et al., 1991). The canopy vegetation is dominated by *A. saccharum* Marshall. The soils are classified as sandy, mixed, frigid Spodosols, closely related to Typic Haplorthods and Alfic Haplorthods subgroups (Randlett et al., 1996; MacDonald et al., 1995). The Pellston site was chosen for this study because it has been the most intensively studied.

The Pellston site includes six 30 m<sup>2</sup> plots, each surrounded by a 10 m wide buffer strip (Rothstein et al., 1996); three plots are controls and three are treated with sodium nitrate. Sodium nitrate is applied in pellet form six times per year at 5-week intervals from May to November. The total dose is equivalent to 30 kg N ha<sup>-1</sup>y<sup>-1</sup>.

### 2.2. Sampling, storage and preparation

Soil samples were collected in May, July and September 1998, September 1999, and May, July and September 2000.

The soil samples consisted of six composited 2 cm diameter × 10 cm deep cores from each plot, for a total mass of approximately 340 g/plot. Litter samples from each plot were a composite of six grab samples collected from the area directly above the soil core collection points. The samples were put on ice in a cooler and transported to the University of Toledo immediately following collection. At the lab, they were kept refrigerated at 4 °C for no longer than 2 days before processing.

The composite soil samples for each plot were mixed by passing them through 4 and 2 mm sieves before subsampling for EEA assays. Litter samples were prepared for subsampling by cutting them into approximately 1 cm<sup>2</sup> pieces with a Hamilton Beach blender. Moisture content for soil and litter was determined gravimetrically by drying at 60 °C.

### 2.3. Extracellular enzyme activity assays

Enzyme assays began within 48 h of sample collection. Sample suspensions were prepared by adding 1 g soil or 0.5 g litter to 125 ml of 50 mM, pH 5.0, acetate buffer and homogenizing for 1 min with a Brinkmann Polytron. The resulting suspensions were continuously stirred using a magnetic stir plate while 200  $\mu$ l aliquots were dispensed into 96-well microplates: 16 replicate wells per sample per assay. The soil and litter suspensions were assayed for the activity of 10 enzymes involved in the degradation of plant litter or cycling of organic nitrogen and phosphorus (Table 1).

All the assays except phenol oxidase, peroxidase and amidohydrolase (urease) were fluorimetric. Fifty microliters of 200  $\mu$ M substrate solution (Table 1) were added to each sample well. Blank wells received 50  $\mu$ l of acetate buffer plus 200  $\mu$ l of sample suspension. Negative control wells received 50  $\mu$ l substrate solution plus 200  $\mu$ l of acetate buffer. Quench standard wells received 50  $\mu$ l of standard (10  $\mu$ M 4 methylumbelliferone, or 7-amino-4-methyl coumarin in the case of leucine aminopeptidase) + 200  $\mu$ l sample suspension. Reference standard wells received 50  $\mu$ l of standard plus 200  $\mu$ l acetate buffer. There were eight replicate wells for each blank, negative control, and quench

standard. The microplates were incubated in the dark at 20 °C for up to 4 h, depending on the assay. To stop the reaction, a 10 µl aliquot of 1.0 M NaOH was added to each well. Fluorescence was measured using a microplate fluorometer with 365 nm excitation and 450 nm emission filters. After correcting for negative controls and quenching, activities were expressed in units of nmol h<sup>-1</sup> g<sup>-1</sup>.

Phenol oxidase and peroxidase activities were measured spectrophotometrically using L-3,4-dihydroxyphenylalanine (DOPA) as the substrate. For phenol oxidase, 50 µl of 25 mM DOPA was added to each sample well. Peroxidase assays received 50 µl of 25 mM DOPA plus 10 µl of 0.3% H<sub>2</sub>O<sub>2</sub>. Negative control wells for phenol oxidase contained 200 µl of acetate buffer and 50 µl of DOPA solution; blank wells contained 200 µl of sample suspension and 50 µl of acetate buffer. For peroxidase, negative control and blank wells also contained 10 µl of H<sub>2</sub>O<sub>2</sub>. There were 16 replicate sample wells for each assay and eight replicate wells for blanks and controls. The microplates are incubated in the dark at 20 °C for up to 18 h. Activity was quantified by measuring absorbance at 450 nm using a microplate spectrophotometer and expressed in units of nmol h<sup>-1</sup> g<sup>-1</sup>.

Amidohydrolase (urease) activity was measured spectrophotometrically (610 nm) using urea as a substrate. The microplate configuration was similar to that described for the phenol oxidase assay. The concentration of urea in the assay wells was 20 mM. The plates were incubated at 20 °C for approximately 18 h. Ammonium released by the reaction was quantified using colorimetric reagent packets from Hach (Sinsabaugh et al., 2000). Activity was expressed as nanomole of ammonium released per hour per gram of soil (nmol h<sup>-1</sup> g<sup>-1</sup>). A list of the enzymes assayed, their substrates and abbreviations is presented in Table 1.

#### 2.4. Data analysis

The SAS Univariate statistical procedure was used to evaluate data distribution prior to analysis of variance (ANOVA; SAS 1998). Upon finding data to be non-parametric, ln(x + 1) transformations were applied. Two-way ANOVA was used to evaluate statistical differences by treatment and season using Statsgraphics Plus 4.1 (Manugistics, 1999). Correlation patterns were examined by principal components analysis (PCA) which was performed using Statistica 4.0 (1994).

Meta-analysis techniques were used to assess whether microbial community function, as measured by 10 enzyme activities, was altered in response to N amendment. Meta-analysis (MA) typically has been used to measure the magnitude of difference between treatment and control variables in multiple independent studies (Gurevitch and Hedges 1999; Bender et al., 1998; Osenberg et al., 1999), but MA also can be used to compare multiple independent variables within a single study (Hedges and Olkin, 1985). In this study, each soil enzyme activity represents a different

measure of microbial response. These responses can be statistically integrated to address a null hypothesis that microdecomposer function is unaffected by N amendments.

The first step in the meta-analysis was to calculate an effect size ( $d_i$ ) for each enzyme by subtracting the mean activity of the treatment plots (Xt) from the mean activity of the control plots (Xc) and dividing the difference by the control plot standard deviation (SDc)

$$d_i = (Xc - Xt)/SDc. \quad (1)$$

The grand effect mean ( $d_g$ ) was calculated by averaging the absolute values of  $d_i$  for all variables ( $n$ ).

$$d_g = (\sum |d_i|)/n \quad (2)$$

The variance in effect size for each enzyme variable ( $s_d^2$ ) was calculated as twice the total number of independent samples in treatment and control plots ( $n + n$ ) divided by the square of the number of samples ( $n^2$ ), added to the square of the mean effect divided by the degrees of freedom ( $2n - 2$ ).

$$s_d^2 = [(n + n)/n^2] + [d_i/2^*(2n - 2)] \quad (3)$$

The variance in grand effect size ( $s_{gd}^2$ ) was calculated by summing the inverse variances for all enzyme variables, then inverting the result.

$$1/s_{gd}^2 = \sum (1/s_d^2) \quad (4)$$

Ninety-five percent confidence intervals (95% CI) were calculated from  $d_g$  and  $s_{gd}^2$ .

$$95\%CI = d_g \pm (2 * \sqrt{s_{gd}^2}) \quad (5)$$

Alpha values associated with  $d_g$  were determined from a table of normal distributions (Hayter, 1996).

### 3. Results

Most soil and litter enzyme activities increased when N was added (Tables 2–3; Fig. 1). The glycosidases (βG, αG, CBH, and βX) showed increased activity in both soil and litter; mean responses ranged from 7 to 56%. Acid phosphatase (aP) activity increased in litter and soil, but the degree of change was substantially greater in litter than in soil (52 versus 17%, respectively). Urease activity increased in both soil (14%) and litter (29%). NAG activity increased 14% in soil plots but decreased 4% in litter plots. The PhOx and LAP responses also varied with soil horizon. In soil, PhOx activity decreased 40% while Perox activity increased 7% with N addition. The opposite results were found in litter, where PhOx increased by 63% and Perox decreased by 7%.

Soil LAP activity ( $p = 0.016$ ) was significantly repressed by N amendments (ANOVA, Table 2). For litter (Table 3), βG ( $p = 0.020$ ), αG ( $p = 0.043$ ), aP ( $p = 0.058$ ), and LAP ( $p = 0.051$ ) activities were significantly enhanced by N amendments. All enzyme activities except for litter

Table 2

Soil EEA responses to N amendment. Treatment effects were determined by two-way ANOVA performed on  $\ln(x + 1)$  transformed data. Only LAP activity showed a significant treatment response. All soil enzyme activities decreased significantly through the course of the experiment. There were no interaction effects. LAP was assayed only on the last five collection dates; urease was assayed only on the final four collection dates

Enzyme	<i>n</i>	% Change	Treatment		Sampling date		Interaction	
			<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
βG	42	+12	1.87	0.18	6.33	0.000*	1.28	0.297
αG	42	+51	1.65	0.21	5.74	0.000*	1.60	0.185
aP	42	+17	1.91	0.18	15.39	0.000*	0.32	0.920
NAG	42	+14	1.34	0.26	13.40	0.000*	1.20	0.338
CBH	42	+13	0.61	0.44	5.80	0.000*	1.19	0.338
βX	42	+32	0.59	0.45	3.03	0.018*	0.06	0.999
LAP	30	-47*	6.43	0.02*	10.14	0.000*	1.7	0.184
PhOx	42	-40	2.26	0.14	14.96	0.000*	1.74	0.149
Perox	42	+7	0.25	0.62	640.38	0.000*	0.81	0.574
Urease	24	+14	0.40	0.53	164.68	0.000*	0.32	0.810

PhOx and Urease showed statistically significant declines over the sampling period. There were no interactive effects between sampling date and treatments.

PCA was used to examine relationships among soil enzyme activities (Fig. 2). PCA was not done for litter because of the smaller sample size. For both control and treatment plots, most of the variance in enzyme activities could be attributed to two factors. Factor loadings were similar in control plots ( $s_{\text{factor1}}^2 = 0.43$ ;  $s_{\text{factor2}}^2 = 0.34$ ) and treatment plots ( $s_{\text{factor1}}^2 = 0.50$ ;  $s_{\text{factor2}}^2 = 0.24$ ). In general, N amendments tended to strengthen the correlation among glycosidase activities and attenuate relationships with oxidative, N-acquiring, and P-acquiring enzymes.

MA showed that soil EEA increased ( $d = 0.308$ ,  $\alpha = 0.0015$ ) in response to the N treatment (Table 3). For individual enzymes, the largest negative effects were found for soil phenol oxidase ( $d = -0.423$ ) and leucine aminopeptidase ( $d = -0.704$ ) activities. The largest positive effect was for α-glucosidase ( $d = 1.529$ ). Cellobiohydrolase ( $d = 0.272$ ) and acid phosphatase ( $d = 0.256$ ) activities showed small positive changes,

while fertilization had almost no effect on peroxidase ( $d = 0.063$ ) activity (Fig. 1).

MA indicated that the litter EEA response ( $d = 0.534$ ,  $\alpha = 0.0003$ ) was larger than that of soil (Table 4). Although peroxidase ( $d = -0.022$ ) and *N*-acetyl glucosaminidase ( $d = -0.091$ ) had negligible responses, cellobiohydrolase ( $d = 1.072$ ), β-glucosidase ( $d = 1.053$ ), acid phosphatase ( $d = 0.819$ ), and leucine aminopeptidase ( $d = 0.773$ ) activities increased markedly. The overall pattern is shown in Fig. 1.

#### 4. Discussion

The purpose of this study was to compare N deposition effects on microdecomposer activity in the soil and litter of a temperate hardwood forest. To our knowledge, there have been no studies in which litter and soil EEA responses have been measured concurrently. Because of differences in organic matter abundance and quality, and in the biomass of fungi and bacteria, concurrent comparisons of litter and soil

Table 3

Litter EEA responses to N amendment. Treatment effects were determined by two-way ANOVA performed on  $\ln(x + 1)$  transformed data. βG, aP, and LAP activities were significantly increased by N amendments. All litter enzyme activities decreased significantly through the course of the experiment except for PhOx and urease. There were no interaction effects

Enzyme	<i>n</i>	% Change	Treatment		Sampling date		Interaction	
			<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
βG	18	+56*	6.96	0.020*	11.17	0.001*	0.18	0.837
αG	18	+25*	3.96	0.043*	3.96	0.043*	1.06	0.378
aP	18	+52*	4.26	0.058*	10.45	0.002*	2.32	0.141
NAG	18	-4	0.04	0.841	11.66	0.001*	0.98	0.405
CBH	18	+44	2.15	0.165	3.56	0.056*	2.29	0.144
βX	18	+7	0.13	0.740	4.33	0.034*	1.57	0.248
LAP	18	+124*	4.54	0.051*	41.73	0.000*	2.37	0.136
PhOx	18	+63	1.64	0.221	0.51	0.614	2.27	0.145
Perox	18	-7	0.00	0.956	33.98	0.000*	0.23	0.796
Urease	18	+29	0.14	0.714	1.83	0.197	0.22	0.805

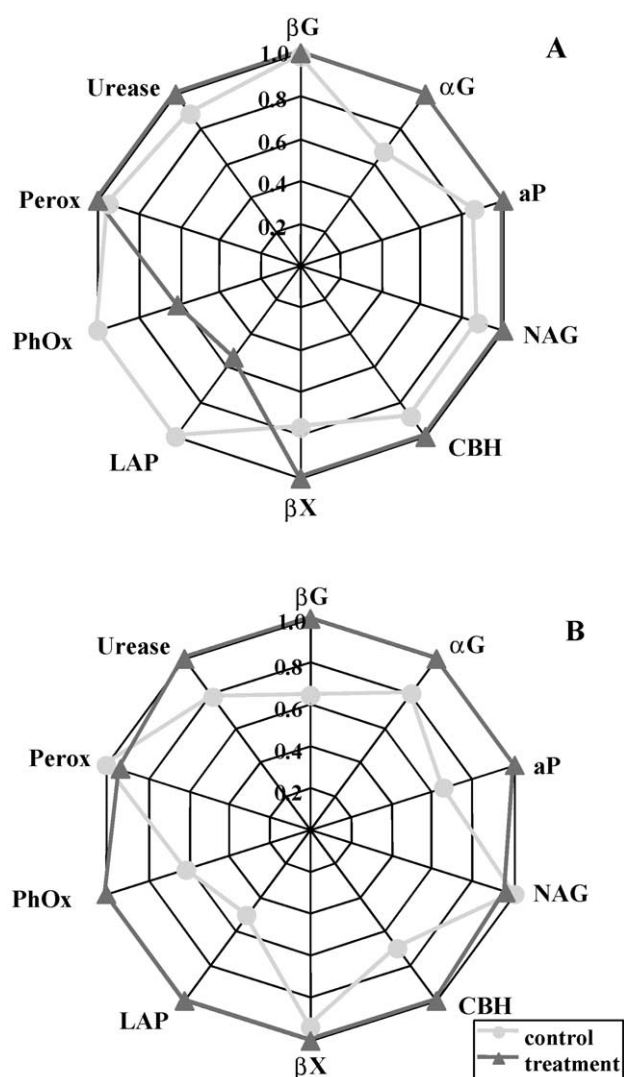


Fig. 1. Radar graphs illustrating the relative response of soil (A) and litter (B) extracellular enzyme activity to N deposition. Enzyme abbreviations are listed in Table 1.

responses should generate further insights into N effects on decomposer activity. Past studies in grassland ecosystems found that soil EEA was enhanced by N amendments (Johnson et al., 1998 [phosphomonoesterase]; Ajwa et al., 1999 [ $\beta$ -glucosidase, acid phosphatase, alkaline phosphatase, arylsulfatase, deaminase, dehydrogenase]). Ajwa et al. (1999) reported repression of urease activity by fertilization. For litter, Carreiro et al. (2000) reported that N amendment increased cellulolytic activity for three types of deciduous leaf litter while phenol oxidase responses varied with litter composition: increasing for labile dogwood and declining for lignified red oak.

Of the enzymes investigated in this study, glycosidases had the most consistent treatment responses. The ANOVA showed that  $\beta$ G, and  $\alpha$ G activities were significantly higher in litter from N-amended plots. PCA showed that glycosidase activities were more closely correlated in the treatment

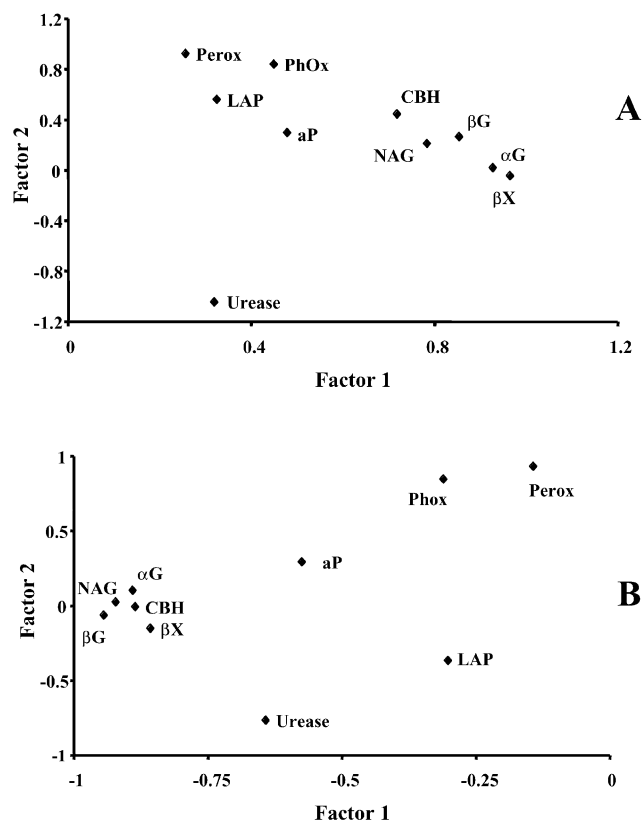


Fig. 2. Extracellular enzyme activity PCA factor loadings for soil control (A) and treatment (B) plots. For the control plots, factor 1 accounted for 43% of the variance, factor 2 accounted for 34%. In the treatment plots, factor 1 accounted for 50% of the variance, factor 2 accounted for 24%. Enzyme abbreviations are listed in Table 1.

plots than in the control plots. MA showed N amendment had medium to large effects on most individual glycosidase enzymes. Glycosidases also showed similar responses to N fertilization in both soil and litter.

Our results are consistent with litter  $\beta$ G responses in an urban forest (Carreiro et al., 2000), tall grass prairie soil  $\beta$ G responses in Manhattan, KS (Ajwa et al., 1999), and soil heathland and grasslands in North Wales (Johnson et al., 1998). At all sites, fertilized samples showed increased  $\beta$ G activity. The Pellston CBH responses were also similar to those in urban forest in which litter from fertilized plots showed increased CBH activity (Carreiro et al., 2000). Collectively these results suggest that glycosidase activities, associated with the decomposition of cellulose, chitin, and storage carbohydrate, are generally enhanced by N amendments in both soil and litter. Contrary to the findings from these natural systems, Bandick and Dick (1999) found no change in soil  $\alpha$ G or  $\beta$ G activities when nitrogen was added to agricultural plots. All previously mentioned experiments applied at least the same amount or more N fertilizer to treatment plots.

The N-acquiring enzymes showed mixed responses. As urease, LAP, and NAG attack different classes of substrates, this was not unexpected. Although these enzymes contribute



Table 4

Meta-analysis of soil and litter enzyme activity responses to N amendments. The effect size ( $d$ ) is expressed in units of standard deviation,  $n$  is the number of samples collected per treatment,  $\text{Var}(d)$  is the effect size variance,  $\alpha$  is the probability that microbial activity did not respond to the treatment.

Metric	$d$	$n$	$\text{Var}(d)$	$\alpha$
<i>Soil: enzyme activity</i>				
$\beta$ G	0.444	21	0.098	
$\alpha$ G	1.529	21	0.124	
Ap	0.256	21	0.096	
NAG	0.368	21	0.097	
CBH	0.272	21	0.096	
$\beta$ X	0.683	21	0.101	
LAP	-0.704	15	0.142	
PhenOx	-0.423	21	0.097	
Perox	0.063	21	0.095	
Urease	0.597	12	0.175	
Mean	0.308		0.011	0.0015
<i>Litter: enzyme activity</i>				
$\beta$ G	1.053	9	0.257	
$\alpha$ G	0.583	9	0.233	
aP	0.816	9	0.243	
NAG	-0.091	9	0.222	
CBH	1.072	9	0.258	
$\beta$ X	0.171	9	0.223	
LAP	0.773	9	0.241	
PhenOx	0.486	9	0.230	
Perox	-0.022	9	0.222	
Urease	0.506	9	0.230	
Mean	0.534		0.024	0.0003

to N uptake, their responses depend on substrate availability, which can be altered by fertilization. Urease activity displayed a modest increase in both soil (14%;  $d = 0.597$ ) and litter (29%;  $d = 0.506$ ) plots in response to fertilization treatments, but this effect was not statistically significant (ANOVA). An increase in urease activity suggests potential for an increase in gross N mineralization rates. However, Zogg et al. (2000) reported that after five years of treatment, there were no significant changes in net N mineralization or root tissue N concentration in these plots. The urease responses in this study are inconsistent with the results reported for a tall grass prairie by Ajwa et al. (1999); they found that N fertilization suppressed soil urease activity by approximately 15%.

Soil LAP activity decreased dramatically in response to N addition (-47%;  $d = -0.704$ ). Interestingly, litter LAP activity also showed a large significant result (124%;  $d = 0.773$ ), but in the opposite direction. Changes in NAG activity were small but showed a different pattern: it was greater in N-amended soil and lower in the litter. At present, it is not clear whether alterations in substrate availability or community composition underlie these differential responses. It also is possible that mechanisms of response differ between litter and soil.

For oxidative enzymes, the magnitude of response to our N treatment was similar in litter and soil. Peroxidase activity

was unresponsive in both soil and litter (soil  $d = 0.063$ ; litter  $d = -0.022$ ). Similar findings were reported by Sinsabaugh et al. (2002) for three types of deciduous leaf litter. In contrast, phenol oxidase activity was repressed by N amendment in soil ( $d = -0.423$ ) but it increased in litter ( $d = 0.486$ ). This pattern is consistent with reports by Carreiro et al. (2000) and Sinsabaugh et al. (2002). They found that mass loss rates from litter were strongly related to phenol oxidase response. When phenol oxidase activity associated with cellulosic litter increased, so did mass loss rates. For lignified litter, losses in phenol oxidase activity paralleled declines in mass loss rate. Based on these results, it appears that litter decomposition rate has increased at Pellston. Such a response for relatively labile sugar maple litter is also consistent with the observation by Fog (1988) that decomposition rates for cellulosic material generally increases with N addition.

Phosphatase activity increased in both soil and litter, but not to the same magnitude. The small increase in soil activity ( $d = 0.256$ ) was not significant ( $p = 0.78$ ). N fertilization in litter, however, had a large significant effect on phosphatase activity ( $d = 0.816$ ;  $p = 0.04$ ). These results are not unexpected because litter has more microbial activity than soil and N addition stimulates polysaccharide decomposition. These findings are consistent with those Ajwa et al. (1999); tall grass prairie ecosystem and Johnson et al., 1998; grassland and heathland ecosystem) who also found increases in phosphatase activity with N additions. Carreiro et al. (2000) found that N amendments increased phosphatase activity in litter. Sinsabaugh et al. (2002) concluded that one effect of N deposition was to shift EEA toward phosphorus acquisition and away from N acquisition.

Sinsabaugh et al. (2002) reported that N deposition affected litter decomposition by increasing the degradation of cellulose, which is a process generally considered N-limited; repressing the oxidative activities associated with recalcitrant litter, presumably because the microdecomposers doing the work are generally adapted to low N conditions; and shifting EEA away from N acquisition and towards P acquisition. In this study, we have corroborated these trends and extended them to SOM. This extension of these predictions from litter to soil is significant because litter and SOM are very different in structure and host different types of microdecomposer communities. Not a lot is known about the oxidation of lignin and humus by various groups of microbes. In the well studied basidiomycete system, ligninase expression is known to be N-induced. Because of the abundance of basidiomycetes in litter, it has been widely assumed that suppression of basidiomycetes accounted for the slower decomposition of recalcitrant organic material. Our results suggest that N deposition affects EEA in predictable ways across diverse microbial communities and that N inhibition may be a more general phenomena than previously thought.

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