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Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss

R.L. SINSABAUGH[1,*], M.M. CARREIRO[2] and D.A. REPERT[3]
*[1]Environmental Science Department, University of Toledo, Toledo, OH 43606, USA; [2]Biology Department, University of Louisville, Louisville, KY 40292, USA; [3]USGS Water Resources Division, Boulder, CO 80303, USA; *Author for correspondence (e-mail: robert.sinsabaugh@utoledo.edu)*

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Abstract. Decomposition of plant material is a complex process that requires interaction among a diversity of microorganisms whose presence and activity is subject to regulation by a wide range of environmental factors. Analysis of extracellular enzyme activity (EEA) provides a way to relate the functional organization of microdecomposer communities to environmental variables. In this study, we examined EEA in relation to litter composition and nitrogen deposition. Mesh bags containing senescent leaves of *Quercus borealis* (red oak), *Acer rubrum* (red maple) and *Cornus florida* (flowering dogwood) were placed on forest floor plots in southeastern New York. One-third of the plots were sprayed monthly with distilled water. The other plots were sprayed monthly with NH_4NO_3 solution at dose rates equivalent to 2 or 8 g N m^{-2} y^{-1}. Mass loss, litter composition, fungal mass, and the activities of eight enzymes were measured on 13 dates for each litter type. Dogwood was followed for one year, maple for two, oak for three. For each litter type and treatment, enzymatic turnover activities were calculated from regressions of LN (%mass remaining) vs. cumulative activity. The decomposition of dogwood litter was more efficient than that of maple and oak. Maple litter had the lowest fungal mass and required the most enzymatic work to decompose, even though its mass loss rate was twice that of oak. Across litter types, N amendment reduced apparent enzymatic efficiencies and shifted EEA away from N acquisition and toward P acquisition, and away from polyphenol oxidation and toward polysaccharide hydrolysis. The effect of these shifts on decomposition rate varied with litter composition: dogwood was stimulated, oak was inhibited and maple showed mixed effects. The results show that relatively small shifts in the activity of one or two critical enzymes can significantly alter decomposition rates.

Introduction

The decomposition of plant litter may be the biosphere's most complex ecological process in that it involves the interactions of a large number of taxa, spanning a wide range of biotic diversity. Though decomposition is typically modeled at an ecosystem scale where rates of carbon flux can be related to temperature, moisture and indices of litter quality (Heal et al. 1997; Moorhead et al. 1996; Paustian et al. 1997), the mechanisms that underlie these relationships operate at the microbial community or biochemical level (Sinsabaugh et al. 1994a). Investigation of these

mechanisms may improve predictions of ecosystem response to large scale changes in climatic and nutrient cycles.

Fungal succession is one of the most studied aspects of microdecomposer ecology. Within a particular habitat, the dominant populations vary more or less predictably through time, selected by their substrate utilization capabilities, their tolerance of inhibitory phenolic compounds and their effective ranges of temperature, water potential, and nitrogen availability (Dix and Webster 1995; Rayner 1995; Dighton 1997; Gessner et al. 1997). Because no population has the enzymic capability to decompose all the constituents of plant litter, unifungal cultures often show little ability to effect mass loss, thus decomposition is a community process, the product of interactions among populations. Despite an apparent need for cooperation, fungal succession is driven by competition for a dwindling and continually changing substrate (Dix and Webster 1995; Chet et al. 1997; Widden 1997). This interplay of enzymatic cooperation and population competition creates a temporally dynamic system that at any particular time is dominated by a relatively small number of populations, whose identity varies with litter type and habitat (Zak and Rabatin 1997).

Because decomposition requires the expression of extracellular enzymes that breakdown the structural components of plant litter and recover organic nitrogen (N) and phosphorus (P), enzyme activities are linked to both community dynamics and the ecosystem perspective. Activity is affected by environmental conditions but also reflects and feeds back on community composition. The result is a successional loop that is highly responsive to environmental changes: alterations in activity affect substrate composition and population dynamics; changes community composition affect enzyme activity. Almost any disturbance is likely to affect more than one element of the loop. One example is atmospheric N deposition.

N amendment stimulates the degradation of labile, cellulosic organic matter and retards the decomposition of recalcitrant, lignified organic matter (Fog 1988). The negative effects have been attributed to changes in community composition, particularly reductions in basidiomycete decomposers, and the repression of fungal ligninase production (Söderström et al. 1983; Berg 1986; Fog 1988), which is induced by low N availability (Kirk and Farrell 1987; Eriksson et al. 1990; Blanchette 1991; Hammel 1997).

In a previous paper, we reported results from a study of deciduous leaf litter decomposition under three levels of N deposition (Carreiro et al. 2000). Nitrogen amendment was found to stimulate cellulase activity and inhibit phenol oxidase activity. The effect on mass loss varied with litter composition: labile dogwood leaves decomposed more quickly, lignified oak leaves decomposed more slowly, and maple leaves, intermediate in composition, decomposed at about the same rate. In this report, we examine these responses, and others, in detail, focusing on questions of microbial community organization. Specifically, how enzymic resources are allocated among carbon, nitrogen, and phosphorus acquisition and how the efficiency of enzymatic decomposition is affected by litter composition and N deposition.

Methods

Site

The field site for this project was a mixed deciduous woodland located at Fordham University's Louis Calder Center in Armonk NY, approximately 30 km north of New York City. Six plots, 5 × 5 m, separated by at least 20 m were delineated. Each plot was divided into three treatment zones. In one zone, the surface litter was sprayed monthly with distilled water from a backpack sprayer (AMB treatment). In the other two zones, the surface litter was sprayed monthly with a solution of NH_4NO_3 equivalent to 20 kg N ha^{-1} y^{-1} (N1 treatment) or 80 kg N ha^{-1} y^{-1} (N2 treatment). Ambient rates of N deposition were estimated at 10 kg N ha^{-1} y^{-1} (Carreiro et al. 2000).

Sampling

Newly senescent leaves of red oak (*Quercus borealis*), red maple (*Acer rubrum*) and flowering dogwood (*Cornus florida*) were collected in autumn from trees at the Calder Center and air dried. Two litter bags, 2 mm mesh, each containing 10 g of dried litter, were stapled together to form a sampling unit. The oak samples were placed on the surface of the plots on 31 October 1993. Samples were collected at 3–4 month intervals until 13 November 1996. Maple samples were placed on the plots in 14 October 1994 and were sampled bimonthly until 19 November 1996. Dogwood samples were placed in 25 October 1995 and were sampled monthly until 7 November 1996. At the conclusion of the study each litter type had been sampled 13 times. On each sampling date, one sampling unit per treatment was collected from each of the six plots.

Analyses

Sample analysis began immediately after collection. One of the bags in each sampling unit was oven dried at 60 °C. After drying, the contents of the bag were removed and weighed to calculate mass loss. Three subsamples were combusted at 500 °C to determine ash content. The remaining material was milled and subsampled for elemental and fiber analyses. The second bag in each sampling unit was used for microbial and enzyme analyses. The contents of this bag were chopped into $\sim$1 cm pieces and mixed; subsamples were withdrawn for ergosterol and enzyme assays.

Carbon, hydrogen and nitrogen content was measured on 2 mg subsamples using a Perkin-Elmer CHN analyzer. Phosphorus content was determined by combusting 0.1 g subsamples at 500 °C, extracting the ash with H_2SO_4, and assaying the extract using the colorimetric ascorbic acid method (Anonymous 1992).

Fungal mass was estimated as ergosterol. Subsamples (1.0 g) of freshly collected litter were placed in HPLC-grade methanol and stored at 4 °C. Ergosterol was extracted and quantified as described by Sinsabaugh and Findlay (1995). Fungal mass

was estimated using a conversion factor of 5 μg ergosterol per mg hyphae (Antibus and Sinsabaugh 1993; Gessner and Chauvet 1993; Newell 1994).

The activities of β-1,4-glucosidase (βG), β-1,4-endoglucanase (EG), cellobiohydrolase (CBH), acid phosphatase (AP), glycine aminopeptidase (GAP), β-1,4-N-acetylglucosaminidase (NAG), phenol oxidase (PhOx), and peroxidase (Perox) were assayed as described by Sinsabaugh et al. (1999), using p-nitrophenyl (pNP)-glucopyranoside, carboxymethyl cellulose, pNP-β-D-cellobioside, pNP-phosphate, glycine p-nitroanilide, pNP-β-N-acetylglucosaminide, L-3,4-dihydroxyphenylalanine (DOPA), and DOPA + H_2O_2 as substrates, respectively. Briefly, litter subsamples, approximately 1.0 g fresh mass, were homogenized in 125 ml of 50 mM acetate buffer, pH 5. 2.0 ml of sample homogenate and 2.0 ml of substrate solution were combined in a 5 ml polypropylene tube. For each assay, there were four analytical replicates plus negative controls for both sample and substrate color. The tubes were tumbled from 1–5 h at 20 °C and centrifuged. The supernatants, except for EG, were analyzed colorimetrically. The EG assay was viscometric with viscosity calculated from fall velocities in small bore pipette. Results were calculated as μmol of substrate converted per hour per g litter dry mass (μmol h^{-1} g^{-1}), except for the EG results which were reported as units h^{-1} g^{-1}.

Fiber analyses were conducted following a modified Van Soest procedure (Van Soest et al. 1991) to estimate the initial composition of the litter. Additional analyses were conducted when samples reached 40% mass loss. From the fiber data, the lignocellulose index (LCI), the fraction of plant fiber that is 'lignin' was calculated as: acid insoluble matter/(acid insoluble matter + acid hydrolyzed polysaccharide).

LCI is a measure of how accessible plant fiber polysaccharides are to enzymatic hydrolysis (Melillo et al. 1989). As LCI approaches 0.7, mass loss rates decline to levels difficult to measure by the litter bag technique.

Models

To compare EEA allocation among litter types and treatments, we converted our EEAs, originally calculated as hourly rates, to daily rates, then integrated activity over exposure time (in days). The result was an estimate of cumulative EEA, which can be tallied as activity-days, analogous to the degree-days used to quantify the cumulative influence of temperature. For all enzymes except endoglucanase, cumulative EEA has units of moles of substrate converted per g of litter (mol g^{-1}). For endoglucanase, cumulative activity was expressed as GU g^{-1}, with a GU defined as 10^9 units.

To generate measures of decomposition efficiency, relationships between cumulative EEAs and litter mass loss were modeled by regressions of LN (% mass remaining) as a function of cumulative EEA. The slope of these first-order exponential decay models is a rate constant (k_a [g mol^{-1}]: the fractional increment in mass loss per activity-day) analogous to the first order rate constant k_t used to compare decomposition rates as a function of time. k_a can also be interpreted as a measure of apparent enzymatic efficiency when comparing systems. The inverse of k_a is the turnover interval for the litter expressed in terms of enzyme activity-days or

mol g^{-1}. Like turnover times, turnover activities are a useful metric for comparisons among litter types and treatments.

Relationships among enzymes were interpreted in the context of the MARCIE (Microbial Allocation of Resources among Community Indicator Enzymes) model (Sinsabaugh and Moorhead 1994, 1997). The model is based on the premises that extracellular enzymatic breakdown of complex molecules is the rate-limiting step in decomposition; that the expression of extracellular enzymes is linked to environmental nutrient availabilities; and that, at the community level, the distribution of extracellular enzyme activity can be interpreted as an optimal resource allocation strategy.

In the model, enzyme activities are grouped into three categories, those principally involved in carbon acquisition (E_C), those involved in nitrogen acquisition (E_N) and those involved in phosphorus acquisition (E_P). Mass loss rates from plant detritus are assumed to be proportional to E_C:

$$dM/dt = k_C E_C$$

However, the expression of C-acquiring enzymes is constrained by the need to enzymatically acquire N and P; thus E_C can also be expressed as a fraction of total extracellular enzyme production ($E_T = \Sigma E_C, E_N, E_P$) whose value is dependent on N and P availability (see Sinsabaugh and Moorhead (1994) for derivation):

$$dM/dt = k_C E_T/(1 + E_N/E_C + E_P/E_C)$$

In this formulation, the ratios of E_N: E_C and E_P: E_C become indices of relative N and P limitation.

Recent experience with the model (Tank et al. 1998; Carreiro et al. 2000) has shown that the lumping of cellulases with polyphenol oxidative enzymes in an E_C term is problematic because the two enzyme classes are differentially regulated in relation to N. Consequently, it is useful to parse E_C into two terms, one for enzymes involved in the breakdown of holocellulose, E_{Cel}, and one for enzymes involved in the oxidation of lignin and other polyphenols, E_{ox}.

E terms are calculated on a relative basis because of the different scalar ranges of the assays and because assay methodology varies widely. The activity of each enzyme is standardized to a 0–1 scale by dividing each value by the maximum value for that enzyme in the data set. For an E term that includes multiple enzymes activities, the standardized values of each constituent enzyme are averaged. In this study, E_P was the standardized activity of AP; E_N was the mean standardized activity of GAP and NAG; E_C was the mean standardized activity of βG, CBH, EG, PhOx and Perox; E_{cel} was the mean standardized activity of βG, CBH and EG; E_{ox} was the standardized activity of PhOx and Perox.

Table 1. Sampling and mass loss rate data. The litter types were senescent leaves of flowering dogwood (*Cornus florida*), red maple (*Acer rubrum*) and red oak (*Quercus borealis*). One-third of the forest floor plots received only ambient N deposition (AMB); one-third received supplemental N in monthly doses equivalent to 20 kg ha^{-1} y^{-1} (N1); one-third received supplemental N equivalent to 80 kg ha^{-1} y^{-1} (N2). Each litter type was collected 13 times over the sampling period. k_t is a first-order rate constant calculated as the slope of the regression LN (% mass remaining) vs. time. Turnover time (T_t) is $k_t - 1$

Litter	Treatment	Sampling period (d)	Cumulative mass loss (%)	k_t (d^{-1}) $\times 10^{-4}$	T_t (d)	r^2
Dogwood	AMB	371	68	−25.0	400	0.92
	N1	371	77	−36.3*	275	0.89
	N2	371	79	−38.2*	262	0.88
Maple	AMB	763	70	−12.2	818	0.95
	N1	763	70	−12.2	823	0.95
	N2	763	62	−9.5*	1060	0.93
Oak	AMB	1108	54	−7.5	1340	0.98
	N1	1108	43	−5.9*	1700	0.92
	N2	1108	41	−5.3*	1890	0.92

* rate is significantly different from that of the AMB treatment.

Results

Mass loss

Oak litter was sampled for three years, maple for two and dogwood for one. Mass loss followed a first-order exponential decay model (Table 1). The estimated turnover time for dogwood litter was 0.7–1.1 y, for maple 2.2–2.9 y, and for oak 3.7–5.2 y. The effect of the N1 and N2 treatments varied by litter type. The decomposition rate of dogwood litter increased by 45% and 53% as the N deposition rate increased. The rate of mass loss from maple litter was unaffected by the N1 treatment, but declined by 22% in the N2 treatment. Oak litter decomposition declined by 21% and 27% as the N deposition rate increased.

Elemental composition

The initial C:N ratios for the dogwood, maple and oak litter were 80, 69 and 64, respectively. Values declined exponentially with time reaching 25–36 by the end of the sampling period (Figure 1). The application of supplemental N increased immobilization, especially during the early stages of decomposition, but overall the treatment effect was not statistically significant.

The initial C:P ratios for dogwood, maple and oak litter were 1110, 1160 and 900, respectively (Figure 1). Unlike the C:N ratios, there was no pattern in relation to mass loss. Dogwood and oak litters appeared to show a seasonal trend with higher ratios in the autumn and lower ones in the summer. There were no significant differences in P abundance among litter types (mean C:P values over the sam-

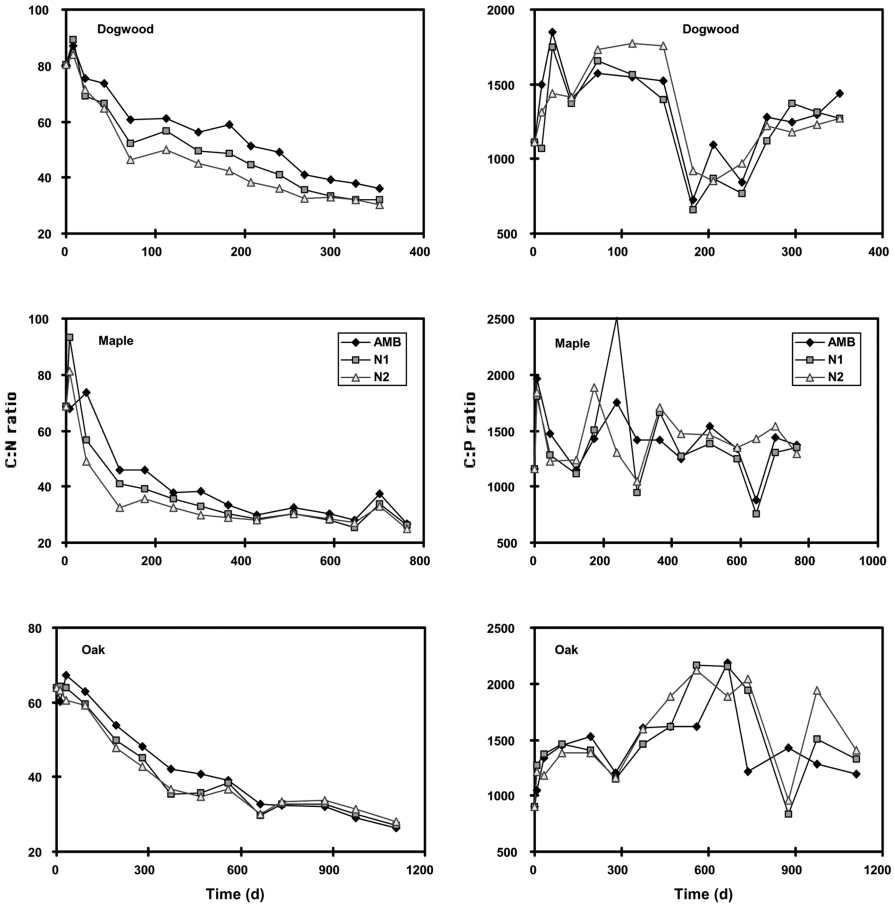

Figure 1. Molar C:N and C:P ratios during the decomposition of flowering dogwood (*Cornus florida*), red maple (*Acer rubrum*) and red oak (*Quercus borealis*) leaf litter. The litter was placed on forest floor plots that received 0 (AMB), 2 (N1), or 8 (N2) g supplemental N m^{-2} y^{-1} in monthly doses.

pling period: dogwood 1280, maple 1400, oak 1460) and the N amendment treatments had no apparent effect on P immobilization.

Fungal mass

Fungal mass associated with dogwood litter increased steadily through time, peaking at about 70% mass loss (Figure 2). For maple and oak, peak fungal biomass occurred earlier in decomposition ($\sim$40% and $\sim$25% mass loss, respectively) and maxima were well below that of dogwood (dogwood maximum $\sim$100 mg g^{-1}; maple $\sim$45 mg g^{-1}; oak $\sim$60 mg g^{-1}). N amendment increased fungal mass across all litter types by 10–20%; however the effect was not statistically significant.

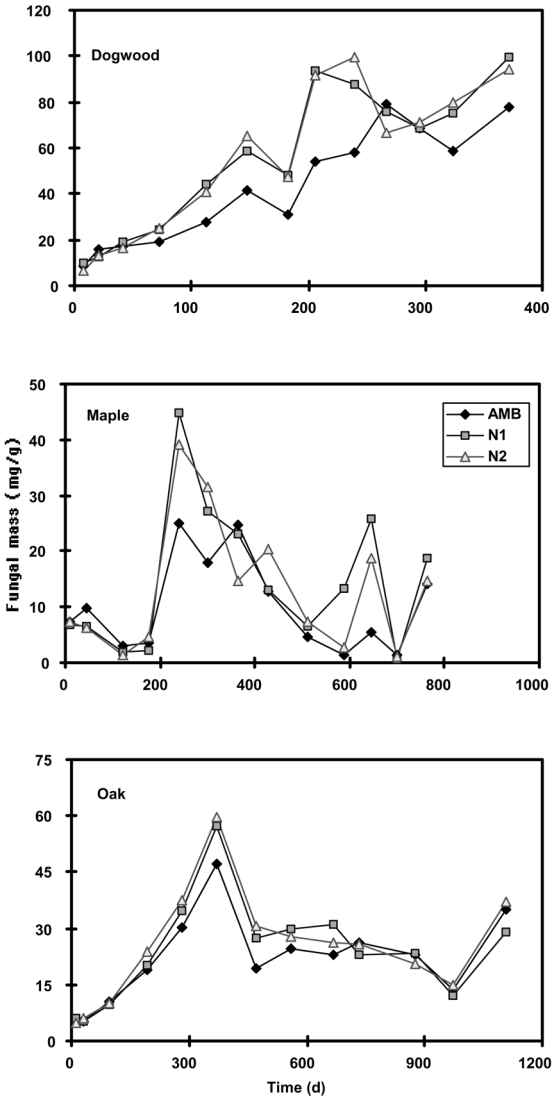

Figure 2. Fungal mass during the decomposition of dogwood, maple and oak leaf litter exposed to three levels of N deposition. Fungal mass was calculated from ergosterol concentration using a conversion factor of 5 μg/mg hyphae.

EEA dynamics

β-glucosidase activity showed varying temporal trends (Figure 3). Activity tended to increase with time for dogwood litter, decrease through time for oak litter, and follow a unimodal trajectory for maple. For all litter types, activity generally in-

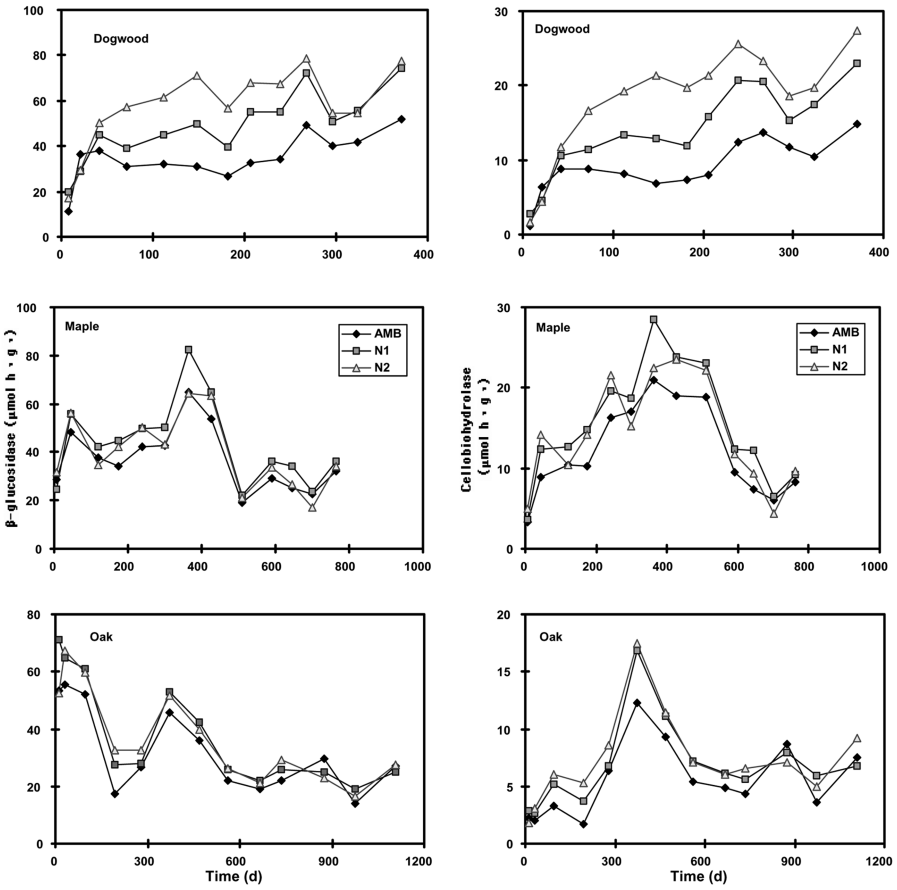

Figure 3. β-glucosidase and cellobiohydrolase activities during the decomposition of dogwood, maple and oak leaf litter exposed to three levels of N deposition.

creased with N amendment although the effect was most pronounced for dogwood (Table 2). Cellobiohydrolase activity showed similar patterns (Figure 3). Across litter types and treatments, the two enzyme activities were strongly correlated (r = 0.72, n = 117). Both also correlated with fungal mass (βG r = 0.40, CBH r = 0.53).

The activity of the third cellulolytic enzyme, β-1,4-endoglucanase, increased rapidly during the early stages of decomposition peaking at mass loss values of 30%, 40% and 25%, respectively, for dogwood, maple and oak litter (Figure 4). After declining, activity increased again during the later stages of decomposition. N amendment increased the activity associated with dogwood litter, but had little effect on maple and oak activities (Table 2). Endoglucanase activity was moderately correlated with cellobiohydrolase activity (r = 0.50, n = 117) and fungal mass (r = 0.43), but only weakly with β-glucosidase activity (r = 0.21).

Table 2. Cumulative enzyme activities for decomposing dogwood, maple and oak leaves over the sampling periods listed in Table 1. Cumulative activity was calculated by integrating enzyme activity over time; the results were expressed in units of moles of substrate converted per g of litter (mol g^{-1}), except for endoglucanse which has units of GU g^{-1} (GU = 10^9 unit). The effects of supplemental N (N1 and N2 treatments) on cumulative enzyme activity are shown as (% change) relative to the ambient (AMB) treatment plots

Enzyme	Dogwood			Maple			Oak		
	AMB	N1	N2	AMB	N1	N2	AMB	N1	N2
β-1,4-glucosidase	0.316	0.440	0.528	0.680	0.813	0.733	0.767	0.877	0.882
	–	(39.3)	(67.3)	–	(20.3)	(8.1)	–	(14.3)	(14.9)
Cellobiohydrolase	0.084	0.129	0.168	0.236	0.300	0.276	0.156	0.194	0.206
	–	(54.2)	(101)	–	(28.3)	(16.3)	–	(24.0)	(31.8)
β-1,4-endoglucanase	0.123	0.157	0.173	0.348	0.331	0.354	0.558	0.497	0.567
	–	(27.6)	(40.5)	–	(–4.1)	(3.2)	–	(–10.9)	(1.6)
Phenol oxidase	0.111	0.155	0.164	0.227	0.252	0.235	0.705	0.446	0.325
	–	(39.6)	(47.7)	–	(11.3)	(6.1)	–	(–36.6)	(–53.9)
Peroxidase	0.031	0.027	0.028	0.115	0.111	0.110	0.052	0.061	0.065
	–	(–12.9)	(–9.7)	–	(–3.5)	(–4.3)	–	(17.3)	(25.0)
β-1,4-NAGase*	0.101	0.107	0.118	0.133	0.139	0.143	0.059	0.054	0.054
	–	(5.4)	(17.0)	–	(4.4)	(7.7)	–	(–9.5)	(–8.8)
Gly-aminopeptidase	0.047	0.048	0.053	0.087	0.089	0.090	0.036	0.034	0.032
	–	(2.1)	(10.5)	–	(2.2)	(3.8)	–	(–5.6)	(–9.7)
Acid phosphatase	0.425	0.546	0.740	1.267	1.889	1.945	1.304	1.278	1.257
	–	(28.8)	(74.3)	–	(49.0)	(53.5)	–	(–2.0)	(–3.7)

* NAGase = N-acetylglucosaminidase.

Phenol oxidase activity also peaked early in decomposition, coincident with endoglucanase, with additional peaks later in the process (Figure 5). The effects of N deposition varied with litter type. Dogwood activity increased by 40% and 48% in the N1 and N2 treatments (Table 2); maple was little affected; and oak activity declined by 37% and 54% as the rate of N deposition increased. Overall, phenol oxidase activity was correlated with endoglucanase (r = 0.35, n = 117), cellobiohydrolase (r = 0.26), and fungal mass (r = 0.27).

Except for maple litter, peroxidase activity was only a small fraction of total oxidative activity (Figure 5). The temporal trends were similar to those of phenol oxidase and endoglucanase, though across litter types activity was only readily measured during autumn. Interestingly, peroxidase responses to N amendment were inverted relative to those of phenol oxidase. Compared to the AMB treatment, dogwood activity was depressed by 13% and 10% in the N1 and N2 treatments; maple was little affected; and oak activity increased by 17% and 25% as the rate of N deposition increased (Table 2). Peroxidase activity was correlated with cellobiohydrolase (r = 0.26, n = 117) and endoglucanase (r = 0.33), negatively correlated with fungal mass (r = −0.28), and had no relationship with phenol oxidase (r = 0.06).

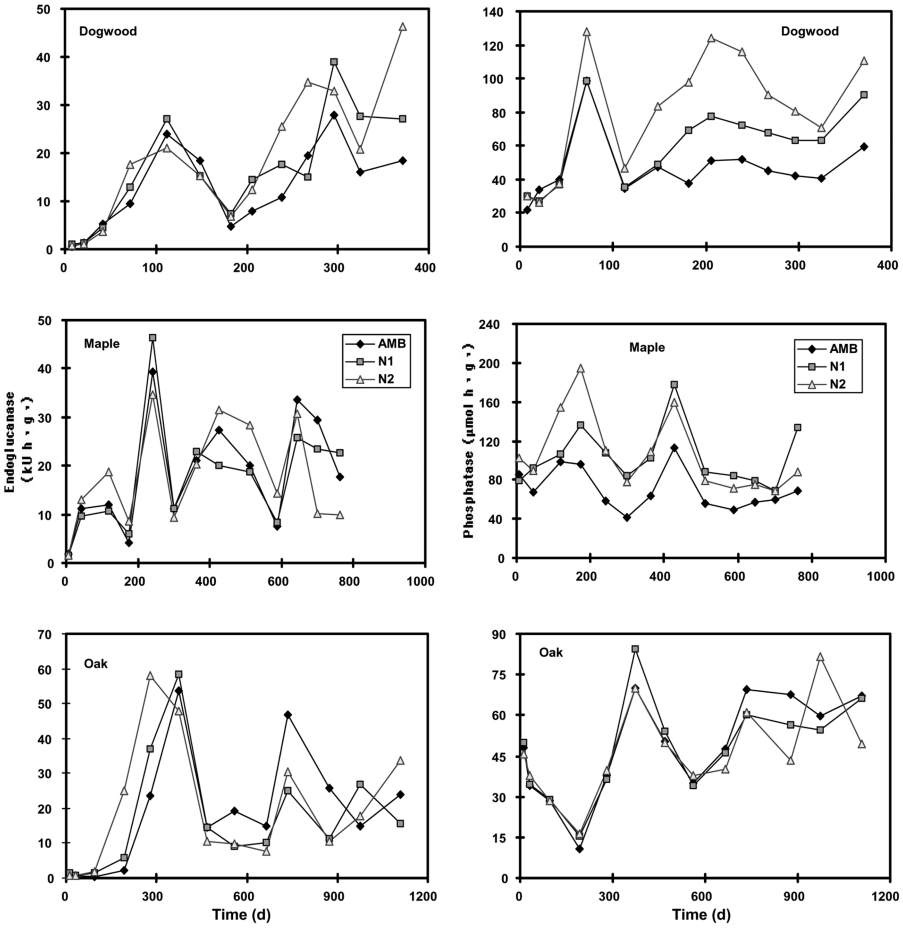

Figure 4. β-1,4-endoglucanase and acid phosphatase activities during the decomposition of dogwood, maple and oak leaf litter exposed to three levels of N deposition.

Acid phosphatase activity followed the same temporal trends as the lignocellulose-degrading enzymes (Figure 4). The most interesting difference was that the magnitude of phosphatase activity, like peroxidase, showed a marked variation among litter types. For both enzymes, peak activity was highest for maple litter and lowest for oak. Like the cellulases, phosphatase activity generally responded positively to N amendment: dogwood activity increased by 29% and 74% in the N1 and N2 treatments, maple activity increased by 49% and 54%, oak activity was little changed. Phosphatase activity was correlated with both cellulase (βG $r = 0.36$, CBH $r = 0.59$, EG $r = 0.25$) and N-acquiring enzyme activities (GAP $r = 0.39$, NAG $r = 0.28$), but not with fungal mass ($r = 0.06$).

β-N-acetylglucosaminidase and glycine aminopeptidase activities were strongly correlated ($r = 0.71$, $n = 117$) (Figure 6). Like phosphatase, there were litter spe-

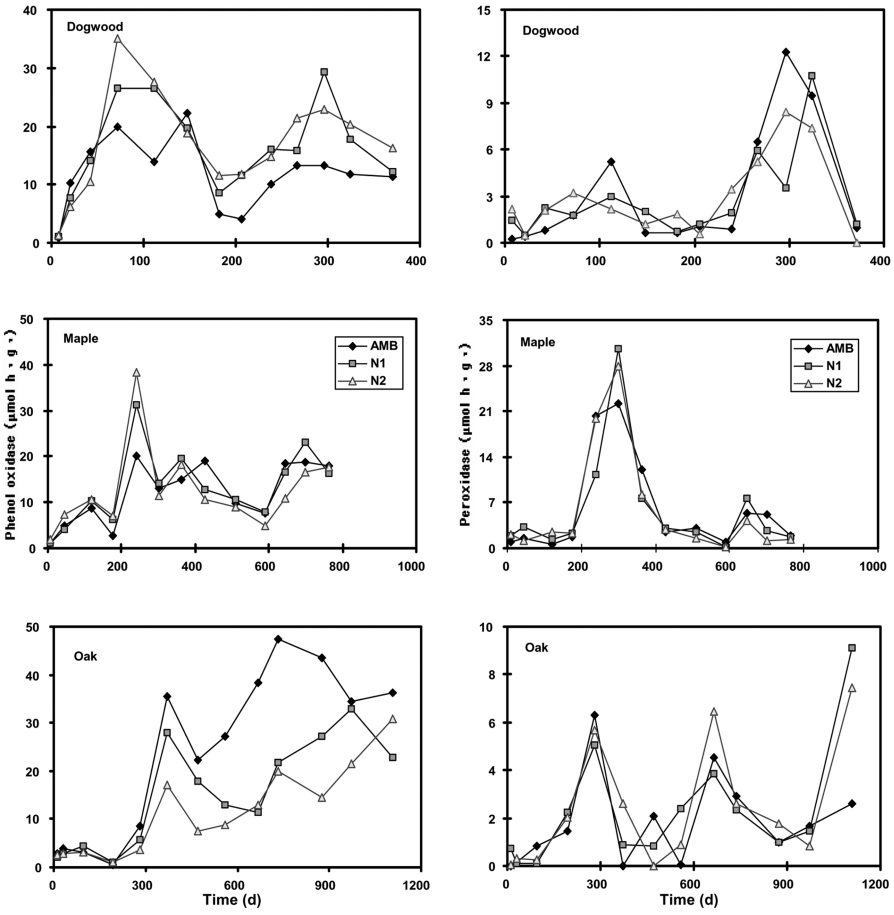

Figure 5. Phenol oxidase and peroxidase activities during the decomposition of dogwood, maple and oak leaf litter exposed to three levels of N deposition.

cific differences in the magnitude of activity, but N-acquisition activities were highest for dogwood litter rather than maple. Within litter type, N amendment had little effect on activities (Table 2). NAG and GAP activities were correlated with β-glucosidase (NAG $r = 0.39$, GAP $r = 0.29$), cellobiohydrolase (NAG $r = 0.57$, GAP $r = 0.56$, and phosphatase activities (NAG $r = 0.28$, GAP $r = 0.54$), as well as fungal mass (NAG $r = 0.39$, GAP $r = 0.26$).

PCA results

Principal components analysis (PCA) using varimax rotation highlighted the emergent relationships among enzymes (Figure 7). The first factor, which accounted for 31% of total variance, was associated with labile nutrient acquisition; it grouped

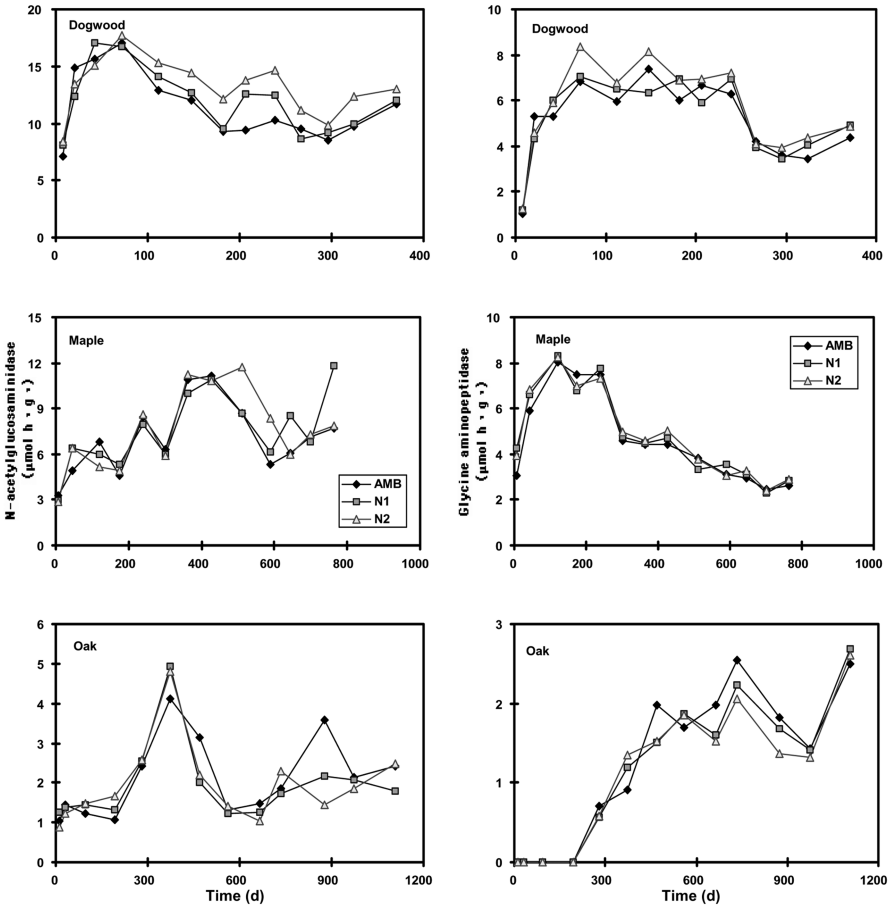

Figure 6. β-1,4-N-acetylglucosaminidase and glycine aminopeptidase activities during the decomposition of dogwood, maple and oak leaf litter exposed to three levels of N deposition.

β-glucosidase and cellobiohydrolase, enzymes that generate assimilable saccharides, with β-N-acetylglucosaminidase, glycine aminopeptidase, and acid phosphatases, enzymes that generate assimilable forms of N and P from organic substrates. Factor 2, which accounted for 20% of the total variance, was associated with lignocellulose degradation; it grouped β-1,4-endoglucanase, an enzyme that attacks the interior of cellulose molecules, with phenol oxidase, an enzyme involved in the degradation of lignin and other polyphenolic molecules. Fungal mass was also weighted toward factor 2. Factor 3, which accounted for 15% of the total variance, was highly correlated with peroxidase, an enzyme involved in the degradation of lignin and humic molecules. Because the production of lignin peroxidases

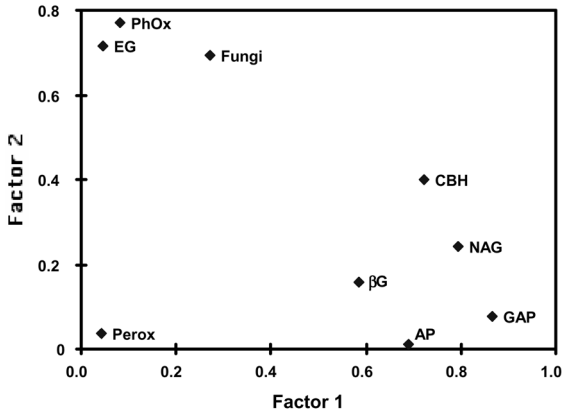

Figure 7. Factor plot from a principal components analysis of enzyme activity and fungal mass data for dogwood, maple and oak litter exposed to three levels of N deposition (117 cases). Using varimax rotation, the first three factors accounted for 31%, 20% and 15% of the variance, respectively. Factor 1 was weighted toward labile nutrient acquisition (βG, CBH, GAP, NAG, AP). Factor 2 was weighted toward lignocellulose degradation (EG, PhOx) and fungal mass (Fungi). Factor 3 was weighted toward peroxidase activity (Perox). βG: β-1,4-glucosidase, CBH: cellobiohydrolase, EG: β- 1,4-endoglucanase, PhOx: phenol oxidase, Perox: peroxidase, NAG: β-1,4-N-acetylglucosaminidase, GAP: glycine aminopeptidase, AP: acid phosphatase.

by decomposer fungi is repressed by high N availability, much of this activity could be of prokaryotic origin.

Turnover activities: comparisons among litter types

Turnover activities, calculated from regressions of LN (% mass remaining) vs. cumulative EEA provide a basis for comparing the allocation of EEA and the efficiency of microbial decomposition among litter types and across treatments (Table 3). Dogwood, maple and oak litters, which contrast in chemical composition (Carreiro et al. 2000) and mass loss rate, also show marked differences in the amount and allocation of EEA required for decomposition (Figure 8). In terms of lignocellulolytic activity, dogwood decomposition was considerably more efficient than either maple or oak. But, maple litter decomposition required 1.6X more peroxidase and 1.4X more cellobiohydrolase activity than oak litter decomposition, while oak litter decomposition required 3.7X more phenol oxidase, 2.0X more endoglucanase and 1.2X more β-glucosidase activity than maple. There were also large differences in EEA allocation to N and P acquisition. Again, dogwood decomposition appeared to be the most efficient, or least nutrient limited, followed by oak then maple. Oak litter had the lowest turnover activities for the N-acquiring enzymes GAP and NAG, but the highest turnover activity for phosphatase. Maple decomposition required about twice as much GAP and NAG activity as dogwood or oak, and nearly as much phosphatase activity as oak.

Table 3. Regression statistics for LN (%mass remaining) as a function of cumulative enzyme activity for decomposing dogwood, maple and oak leaf litter subjected to three levels of N deposition. k_a is the first-order rate constant (slope) for each regression in units of g mol^{-1}, except for endoglucanase where k_a has units of g/GU (GU = 10^9 units). T_a ($1/k_a$) is the turnover activity, i.e. the amount of accumulated enzyme activity, expressed as mol g^{-1} (except for endoglucanase where T_a has units of GU g^{-1}) estimated for the complete decomposition of the litter

Enzyme	Statistic	Dogwood			Maple			Oak		
		AMB	N1	N2	AMB	N1	N2	AMB	N1	N2
β-1,4-glucosidase										
	k_a	−3.03	−3.08	−2.57	−1.28	−1.05	−0.92	−1.06	−0.74	−0.66
	T_a	0.330	0.324	0.389	0.783	0.955	1.085	0.943	1.346	1.508
	r^2	0.95	0.93	0.89	0.92	0.92	0.93	0.98	0.96	0.97
Cellobiohydrolase										
	k_a	−11.33	−10.49	−8.14	−3.40	−2.67	−2.23	−4.89	−3.13	−2.65
	T_a	0.088	0.095	0.123	0.294	0.375	0.439	0.205	0.319	0.378
	r^2	0.96	0.94	0.91	0.90	0.90	0.89	0.98	0.94	0.94
β-1,4-endoglucanase										
	k_a	−7.44	−8.84	−8.60	−2.56	−2.64	−1.82	−1.32	−1.21	−0.97
	T_a	0.134	0.113	0.116	0.390	0.379	0.549	0.759	0.827	1.026
	r^2	0.95	0.94	0.96	0.94	0.92	0.88	0.95	0.93	0.94
Phenol oxidase										
	k_a	7.88	−8.24	−8.09	−3.90	−3.48	−2.88	−1.05	−1.34	−1.65
	T_a	0.127	0.121	0.124	0.257	0.287	0.347	0.953	0.747	0.606
	r^2	0.88	0.88	0.85	0.93	0.92	0.91	0.88	0.78	0.73
Peroxidase										
	k_a	−31.5	−55.1	−55.2	−6.21	−6.50	−4.85	−15.05	−10.67	−8.69
	T_a	0.032	0.018	0.018	0.161	0.154	0.206	0.066	0.094	0.115
	r^2	0.98	0.96	0.98	0.83	0.84	0.83	0.98	0.88	0.90
β- 1,4-NAGase*										
	k_a	−8.73	−11.8	−11.2	−6.49	−6.35	−4.55	−13.4	−11.7	−10.4
	T_a	0.115	0.085	0.089	0.154	0.157	0.220	0.075	0.085	0.096
	r^2	0.88	0.84	0.84	0.93	0.92	0.88	0.97	0.94	0.94
Gly-aminopeptidase										
	k_a	−17.4	−25.0	−23.7	−10.04	−9.95	−7.82	−20.4	−16.7	−15.6
	T_a	0.057	0.040	0.040	0.100	0.101	0.128	0.049	0.060	0.064
	r^2	0.86	0.84	0.82	0.91	0.92	0.96	0.88	0.77	0.77
Acid phosphatase										
	k_a	−2.09	−2.43	−1.81	−0.727	−0.468	−0.360	−0.617	−0.493	−0.454
	T_a	0.479	0.411	0.552	1.38	2.14	2.78	1.62	2.03	2.20
	r^2	0.90	0.91	0.90	0.94	0.93	0.94	0.94	0.89	0.86

*NAGase = N-acetylglucosaminidase.

Across litter types turnover activities were more closely related to fungal mass than to turnover times. Maple litter had the lowest standing stock of fungi and required the most enzymatic work to decompose, even though the turnover time for

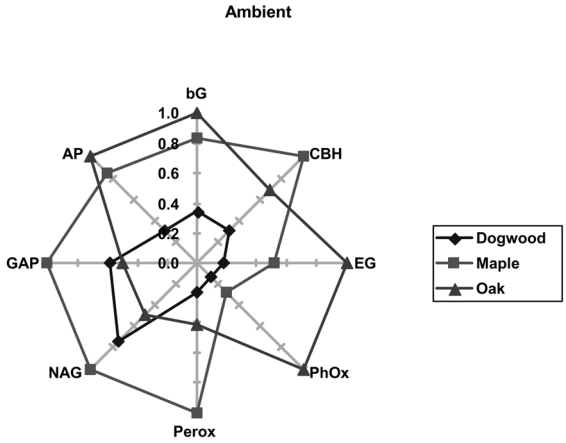

Figure 8. Relative turnover activities for three types of deciduous leaf litter under ambient conditions. The comparisons are based on the turnover activities listed in Table 3. βG: β-1,4-glucosidase, CBH: cellobiohydrolase, EG: β- 1,4-endoglucanase, PhOx: phenol oxidase, Perox: peroxidase, NAG: β- 1,4-N-acetylglucosaminidase, GAP: glycine aminopeptidase, AP: acid phosphatase.

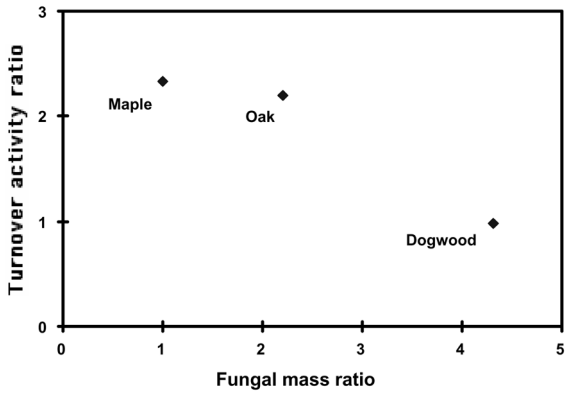

Figure 9. Comparison of the total enzymatic resources needed to decompose litter and the mean fungal mass associated with the litter. The ratios of enzymatic effort were calculated by summing the relative turnover activities of the eight enzymes we monitored (shown in Figure 8). Ratios of mean fungal mass for each litter were calculated over 13 sampling dates (shown in Figure 2).

maple was half that of oak. Overall, there appeared to be an inverse relationship between total enzymatic effort (measured by summing the relative turnover activities of the eight enzymes measured) and mean fungal mass (Figure 9). The significance of this relationship is difficult to assess because we do not know the turnover rates for either the biomass or enzyme stocks; if they are correlated it appears that there is a trade-off between resources expended to acquire nutrients from the environment and resources directed toward biomass development.

Turnover activities: N deposition responses

N amendment altered turnover activities for all litter types (Figure 10). For dogwood, the N1 treatment increased the efficiency and rate of decomposition, in part by reducing expression of the N-acquiring enzymes, GAP and NAG, as well as peroxidase. In the N2 treatment, there was a marginal increment in mass loss rate but the efficiency of decomposition was lower relative to the N1 treatment because of 'over expression' of phosphatase and the cellulolytic enzymes βG and CBH. For maple litter, N amendment reduced the efficiency of enzymatic decomposition with little effect on allocation among enzymes. Responses were modest in the N1 treatment: AP, CBH and βG turnover activities increased, as observed in the dogwood N2 treatment, but no change in mass loss rate was observed. In the N2 treatment, mass loss rate declined in parallel with losses in apparent efficiency for all enzymes measured. For oak litter, the suppression of phenol oxidase activity in response to N addition reduced decomposition rates and increased the turnover activities of the other enzymes.

The general effect of N deposition was to shift EEA away from N acquisition and toward P acquisition and away from polyphenol oxidation and toward polysaccharide hydrolysis (Figure 11). The strength of each effect varied with litter composition. For oak, the most P-limited litter (highest mean C:P, highest AP turnover activity, lowest GAP and NAG turnover activities, lowest E_P: E_N), N amendment had little effect on mean E_P: E_N, increasing it from 2.6 to 2.8 (8%) in the N2 plots. For maple, E_P: E_N increased 38% (0.80 to 1.07 to 1.10) from the ambient to N2 treatment. For dogwood, the most N-limited litter (highest C:N, lowest mean E_C: E_N, lowest E_P: E_N), E_P: E_N increased 51% (0.39 to 0.50 to 0.59) from the ambient to N2 treatment.

The effect of added N on the distribution of lignocellulolytic activity was most pronounced during the latter stages of decomposition when oxidative activities became more prominent. For the final seven sampling dates, the mean E_{cel}: E_{ox} ratio for oak was 0.91 in the ambient treatment, 1.49 (64% increase) in the N1 plots, and 2.23 (145% increase) in the N2 plots. For maple, the E_{cel}: E_{ox} ratio for the final seven samples, increased from 3.00 to 3.64 (+21%) to 4.56 (+52%) from ambient to N1 to N2 plots. Dogwood, which had the lowest LCI, showed the weakest response; the ratio increased from 4.02 to 4.07 (+1%) to 4.51 (+12%) from ambient to N1 to N2 plots.

Discussion

The magnitude of the EEA responses, and their subsequent impact on decomposition rates, depended on the N availability and the lignin and polyphenol content of each litter. For dogwood litter, N addition mitigated a strong N limitation which led to increased cellulolytic activity and faster mass loss. In the N1 plots, cellulase activity increased by 40% and mass loss by 45%; for the N2 plots the corresponding

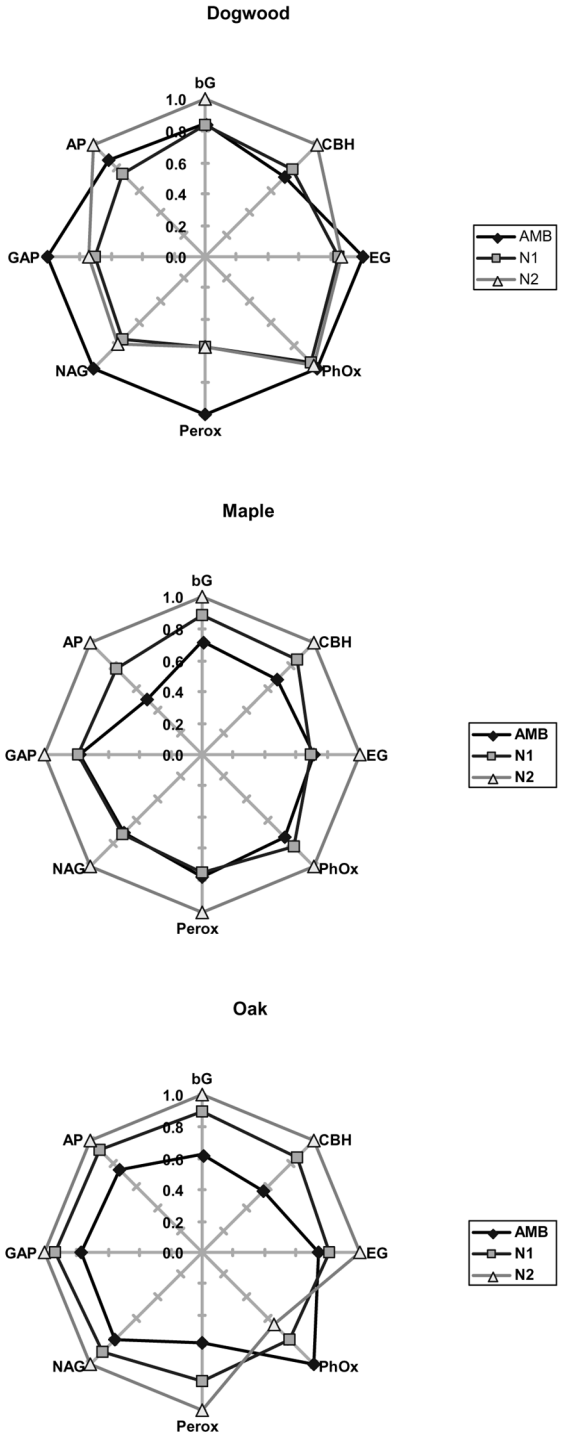

Figure 10. Relative turnover activities for enzymes associated with leaf litter decomposing under varying levels of N deposition. The values used for the comparisons are taken from Table 3.

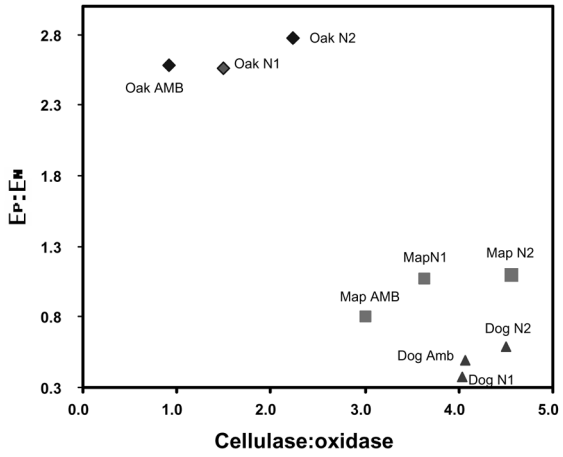

Figure 11. Effects of N amendment on the distribution of EEA. N addition shifts EEA toward P acquisition relative to N (E_P: E_N) and toward cellulolytic activity relative to oxidative activity (cellulase:oxidase).

responses were 70% and 53%. Because dogwood litter had a low LCI, the oxidation of lignin and polyphenols did not limit decomposition.

Microbial activity on oak litter was principally P – limited, so N amendment had minimal effects on the distribution of N and P – acquiring enzymes. But with a high initial LCI, mass loss rate was directly linked to oxidative activity. For the last seven samples, oxidative activity and mass loss rate declined by 36% and 42%, respectively, in the N1 plots and 48% and 44%, respectively, in the N2 plots.

Maple litter, intermediate to oak and dogwood in N availability and LCI, responded to N addition with moderate shifts in both nutrient and carbon acquisition activities. These shifts increased decomposition during the early stages (up to about 40% mass loss) and retarded it during the later stages. For the N1 treatment, there was no net change in mass loss rate. For the N2 treatment, the 52% decrease in oxidative activity during the last half of the exposure period was large enough to reduce k_t by 22% (Table 1).

That N amendment tends to increase the mass loss rate of cellulosic litter and retard the decomposition of lignified or humified litter is well known (Fog 1988; Berg and Matzner 1997; Carreiro et al. 2000) and the ecosystem-scale implications of this differential mass loss response for carbon and nitrogen storage in forest soils has been discussed (Berendse et al. 1987; Berg and Ekbohm 1991; Berg and Matzner 1997). The leading hypothesis for the N inhibition effect is repression of ligninase expression by white rot basidiomycetes (Söderström et al. 1983; Berg 1986; Fog 1988). The hypothesis is supported by culture studies (Kirk and Farrell 1987; Eriksson et al. 1990; Blanchette 1991; Hammel 1997) and by observations that fungal abundance, particularly basidiomycete, is depressed in N fertilized systems (Miller and Lodge 1997). Further support comes from the demonstration that oxidative activities directed toward phenols are reduced by high levels of N appli-

cation and that the magnitude of this effect is directly related to changes in mass loss rates (Carreiro et al. 2000). However, so little known is known about the processing of lignin and other polyphenols, e.g. the identity of significant enzymes, number of potential pathways, consortial interactions (Kirk and Farrell 1987; Eriksson et al. 1990; Hammel 1997), that it is not possible to conclude that repression of white rot fungi is the principal mechanism for reductions in oxidative activity. A more general hypothesis is that high N availability affects all three elements of the successional loop (substrate, biota, extracellular enzyme), interfering with the establishment and function of the oligotrophic microbiota typically associated with the degradation of humified organic matter (Dix and Webster 1995; Miller and Lodge 1997).

N deposition alters the composition of plant litter through increased humification reactions between oxidative free radicals and nitrogen compounds. This effect contributes to lower mass loss rates in the latter stages of litter decomposition (Stevenson 1994; Berg and Matzner 1997; Magill and Aber 1998). Assuming a C:N ratio of 5, microbial mass (data for fungi in Figure 2, bacterial data not shown), on average, accounted for 42% of oak litter N, 18% of dogwood, and 10% of maple. These fractions did not increase with N amendment suggesting that the faster rates of N immobilization observed in the N1 and N2 treatments (Figure 1) was a humification effect. Humification, like loss of oxidative activity, would tend to reduce the apparent efficiency of enzymatic decomposition, an effect we observed across all litter types, by increasing the diversity and recalcitrance of linkages. Several studies (reviewed by Berg and Matzner (1997)) have shown that community respiration declines with the endogenous C:N ratio of humus, as well as in response to exogenous N amendments.

The MARCIE model provides a framework for representing the functional organization of microdecomposer communities in relation to decomposition. To capture N deposition effects, it is necessary to consider four axes of activity: N acquisition, P acquisition, polysaccharide hydrolysis and polyphenol oxidation. Within the context of the model, it is possible to make qualitative predictions about microbial community response to alterations in nutrient availability or carbon quality, but empirical relationships can only be constructed a posteriori (e.g. Sinsabaugh et al. (1994b); Jackson et al. (1995)). Even then, high substrate heterogeneity, high microbial diversity and the myriad interactions between them, severely limit the range of predictive application. Apparent enzymatic efficiency is an expression of this complexity (Table 3).

Relative to the conditions under which our assays were conducted, the enzymatic efficiency of decomposition was low. Consider the following example: the turnover activity for βG ranged from 0.33–1.51 mol g^{-1} (Table 3). One gram of pure cellulose contains 0.0056 mole of glucose. Assuming for simplicity that βG was the only enzyme needed to hydrolyze this cellulose, the relative efficiency would be 0.4–1.7%. Applying a couple of corrections – that one gram of litter is 20% cellulose and that all the cellulose is converted to cellobiose before βG acts on it – changes the relative efficiency of βG to 0.15–0.68%. There are lots of reasons for this low value: cellulolysis requires the activity of enzymes besides βG,

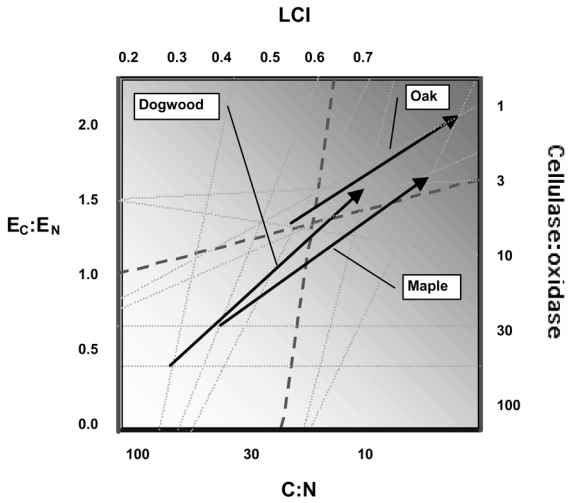

Figure 12. A fuzzy synthesis of relationships among litter composition and microbial activity that determine decomposition responses to N deposition. Conditions that lead to N inhibition of decomposition include high lignin/humus content, low C:N ratio, a high ratio of lignocellulase activity to hydrolytic N-acquisition activities, and a low ratio of cellulase to polyphenol oxidase activities. Approximate threshold values for these measures are shown by dashed lines. Approximate initial and final values for dogwood, maple and oak litter decomposition are indicated by the arrows.

βG acts on other substrates in addition to cellulose, environmental conditions of temperature, water activity, pH and substrate availability are far from optimal, βG may be immobilized or sequestered at sites where no substrate is available, cellulose may in inaccessible to enzymes, etc. (see Burns (1983) for review of enzyme-substrate interactions). The aggregate effect of these factors is to weaken the connection between enzyme expression and product generation. Similar environmental interactions affect all extracellular enzymes and apparent enzymatic efficiencies (AEEs) decline as litter is transformed from a structured polymer matrix to humified soil organic matter (Sinsabaugh et al. 1994b; Jackson et al. 1995). N deposition introduces additional elements into this complexity, the magnitude of which can be inferred from the declines in AEEs relative to those for litter decomposing in the ambient plots (Table 3).

N clearly has complex effects on microbial activity and community organization and we are far from a predictive understanding of the phenomenon. If N inhibition of decomposition is principally a fungal effect, it may have limited impact on storage of soil organic matter (SOM). Basically, the residual 'work' of decomposition will be passed from litter fungi to soil bacteria, which are enzymatically capable though less efficient at the task. If N selects against humus-degrading biota in general, then the inhibition effect could include SOM as well as litter, which could significantly impact SOM accumulation. Driscoll (2001) provides some evidence for a wider effect. She sampled litter and soil from a sugar maple (*Acer saccha-*

rum) stand containing plots that had been N-amended for several years. Oxidative activity was 20% and 40% lower, respectively, than that of the control plots.

The subtlety of these effects merits emphasis. Microbial responses to N addition can easily be dismissed as statistically insignificant when compared to the background 'noise' of spatiotemporal variation. A 'signal' change of 10–50% in the activity of one or two critical enzymes, measured against a background 'noise' an order of magnitude larger, can lead to proportional increases or decreases in mass loss rates, even if other measures of activity appear unresponsive. At the plot scale, even respiration rates may be misleading because the decomposition of some litter fractions may increase while others are slowed.

Extrapolating these complexities to the ecosystem scale is fuzzy (Figure 12). The biggest impacts of N on EEA were release of N limitation, repression of oxidative activity, and losses of AEE. The transition from stimulation to inhibition of decomposition occurred when the E_C: E_n ratio exceeded 1.0 and E_C: E_{ox} ratio decreased below 3.0. At the substrate level, N addition accelerated the decline in C:N ratio, probably through humification reactions. A C:N ratio of about 40 was the threshold for potential inhibition effects. Dose-response relationships were linked to the fiber composition of the litter; the critical LCI value for the transition from enhanced to reduced decomposition was about 0.5.

It is difficult to speculate on the generality of these findings; at present it is not even clear whether N inhibits decomposition in bacterial dominated systems as well as fungal. But there is ample evidence that large-scale models that predict decomposition and carbon storage from stoichiometric relationships may not accurately describe the effects of atmospheric N deposition on these processes.

Acknowledgements

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