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Contrasting Effects of Substrate and Fertilizer Nitrogen on the Early Stages of Litter Decomposition

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Abstract

Commonly observed positive correlations between litter nitrogen (N) concentrations and decomposition rates suggest that N frequently limits decomposition in its early stages. However, numerous studies have found little, if any, effect of N fertilization on decomposition. I directly compared internal substrate N and externally supplied inorganic N effects on decomposition in sites varying in soil N availability. I decomposed eight substrates (with initial %N from 0–2.5) in control and N-fertilized plots at eight grassland and forest sites in central Minnesota. N fertilization increased decomposition at only two of eight sites, even though decomposition was positively related to litter N at all sites and to soil N availability across sites. The effect of externally supplied N on decomposition was independent of litter N concentration, but was greater at sites with low N availability. The inconsistent effects of substrate and externally supplied N may have arisen because decomposers use organic N preferentially as an N source; because inorganic N availability across sites or with fertilization induced changes in microbial community attributes (for example, lower C:N or greater efficiency) that reduced the response of decomposition to increased inorganic N supply; or because the positive correlation between litter N or site N availability with decomposition was spurious, caused by tight correlations between litter or site N and some other factor that truly limited decomposition. These inconsistent effects of substrate N and external N supply on decomposition suggest that the oft-observed relationship between litter N and decomposition may not indicate N limitation of decomposition.

Key words: decomposition; fertilization; litter; Minnesota; nitrogen; nutrient limitation.

Introduction

Although nitrogen (N) has been shown to limit plant growth in a number of different ecosystems (Vitousek and Howarth 1991), the evidence for N limitation of decomposition is murky at best. Decomposers have lower carbon (C) to N ratios than does most fresh litter, suggesting that litter N content is frequently insufficient to support use of litter C, even given microbial inefficiency, and establishing the potential for N limitation of decomposition in its early stages. Indeed, many studies indicate that microbes import N into decomposing litter during initial decay (for example, Gosz and others 1973; Staaf and Berg 1981; Frey and others 2000). Furthermore, strong positive correlations that are sometimes observed between initial litter N concentrations and decomposition rates also suggest that N frequently limits decomposition (Melillo and others 1982; Enriquez and others 1993), particularly in the early stages (Berg and Matzner 1997).

Yet, despite compelling indirect evidence that N often limits decomposition, studies that have added N in the form of fertilizer show inconsistent effects

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on decomposition (Fog 1988). Some studies have found no effect of N fertilization on decomposition (for example, Van Vuuren and Van der Eerden 1992; Prescott 1995; Hobbie and Vitousek 2000; Johnson and others 2000), other studies have found positive effects of N fertilization on decomposition (for example, Hunt and others 1988; Conn and Day 1996; Hobbie 2000; Vestgarden 2001), and still others have found negative effects of N fertilization on decomposition (for example, Gill and Lavender 1983; Prescott 1995; Magill and Aber 1998).

Understanding the inconsistencies between the influence of litter N and fertilizer N on the early stages of decomposition is necessary for predicting how decomposition (and thus ecosystem carbon balance) responds to elevated N inputs resulting from human activities. Yet, few studies have directly compared the effects of substrate N with those of N added as fertilizer, and these studies have been relatively limited in scope. Some report similar positive effects (Reinertsen and others 1984; Vestgarden 2001), similar neutral effects (Prescott 1995), or contrasting effects of substrate and externally supplied N (Pastor and others 1987). Here I report the most comprehensive study to date that directly compares the effects of substrate N and exogenously supplied N on decomposition. Specifically, I test the following hypotheses:

Hypothesis 1. In N-poor ecosystems, both exogenous N and substrate N limit the rate of initial litter decomposition and N received from the environment and from decomposing substrates are equivalent N sources to decomposers.

Hypothesis 2. Exogenous N limits decomposition more strongly for low-N substrates than for high-N substrates, because of relatively greater N demand by decomposers breaking down low-N substrates compared to high-N substrates.

Hypothesis 3. Exogenous N limits decomposition more strongly in sites with low N availability, because of relatively greater N demand by decomposers breaking down litter in low-N compared to high-N sites.

To test these hypotheses, I established a decomposition experiment at eight different sites at the Cedar Creek Natural History Area (CCNHA) in Central Minnesota, a site with poorly developed soils that have low N availability. At each site, I established an N-fertilization experiment in which I decomposed an array of substrates that varied in their initial N concentration to directly compare the effects of exogenous N added as fertilizer with effects of variation in initial substrate N concentration on decomposition, testing Hypothesis 1. To test Hypothesis 2, I compared the magnitude of N fertilization effects on decomposition of substrates varying in their litter N concentration. And finally, to test Hypothesis 3, I examined relationships between the magnitude of N fertilization effects on decomposition and site N availability. In this study I focus exclusively on the first year of decomposition, because N limitation is expected to be strongest in the early stages of litter decomposition, when decomposing litter has its lowest N content. Interactions between lignin and N in the later stages of decomposition (Berg 1986; Fog 1988; Berg and Matzner 1997; Carreiro and others 2000) will be explored in a later paper.

Materials and Methods

Study Site

CCNHA is a Long Term Ecological Research (LTER) site 60 km north of Minneapolis, Minnesota and comprises a mosaic of wetlands, old fields, prairie and savanna remnants, and hardwood and pine stands on sandy, poorly developed soils (Grigal and Homann 1994). N limits net primary production (NPP) (Tilman 1984). I established an experiment to assess limitation by exogenous (soil) and endogenous (substrate) N of decomposition at eight upland sites within CCNHA, including two old fields (dominated by *Schizachyrium scoparium* and other C4 and C3 grasses), a mixed hardwood (maple-basswood) stand (dominated by *Acer saccharum*, *Tilia americana*, and *Quercus ellipsoidalis*), a bigtooth aspen stand (dominated by *Populus grandidentata*), two pin oak stands (dominated by *Quercus ellipsoidalis*), and two white pine stands (dominated by *Pinus strobus*). Sites are within 5 km of one another and have soils classified in the same Great Group (Udipsamments) (Grigal and others 1974).

Decomposition Experiment

Within each site, I established twelve 2.5 m × 2.5 m plots. Six plots were left as control plots, and six were randomly chosen to receive 10 g N m^{-2} y^{-1} as NH_4NO_3 in solution, beginning in October 1999. I applied fertilizer by spraying 1 L of NH_4NO_3 solution (or water, for control plots) over each plot in three applications each year (May, July, October).

To examine effects of endogenous (substrate) N on decomposition, as well as interactions between exogenous and endogenous N, I measured decomposition rates of eight substrates that vary in their initial N concentrations (and C chemistry, see below) within each plot. Substrates included leaf litter of *Schizachyrium scoparium*, *Acer saccharum*,

Quercus ellipsoidalis, and *Pinus strobus*, green leaves of *Acer saccharum* and *Quercus ellipsoidalis*, cellulose filter paper (Whatman number one), and commercially available untreated birch wood applicators (14.5 × 0.2 cm) as a wood substrate (Cotton Tail Medical Products quality wood applicators, CITMED, Citronelle AL). Pure leaf litter of *Schizachyrium* was obtained from Prairie Restorations, Inc. (Princeton MN). Leaf litter of *Acer*, *Quercus*, and *Pinus* was collected in autumn 1998 by picking up freshly fallen litter from the ground in several stands. Litter collected from different stands was thoroughly mixed for each species and air-dried at 22°C. Green leaves of *Acer* and *Quercus* were collected in mid-July 1999 by pruning branches from understory individuals of *Acer* in a sugar-maple basswood stand and from short-statured canopy individuals of *Quercus* in a savanna site and stripping branches of all green leaves. Green leaves were also air-dried.

I constructed 400 cm^2 litter bags using fine-mesh polyester (200 μm) bottoms and small-mesh (0.3 mm) tops. Fine-mesh bottoms were used to minimize contamination of the litter bags by sand, but still allow fungal colonization of the litter (fungal hyphal diameter = 3–8 μm, Richards 1987); hyphal connections between the underlying soil and the litter bags were visible with the naked eye upon collection. The mesh size of the tops (0.3 mm) did not exclude most soil fauna (for example, earthworms and insect larvae were found inside bags upon harvest). Approximately 6 g (air-dry mass) of each substrate were placed in each bag. Exceptions were filter paper and wooden dowels: three pieces of filter paper (~3.8 g) and 3 wooden dowels (~1 g) were used per bag. Subsamples of each substrate were used to develop air-dry to oven-dry (65°C) conversions and were analyzed for initial concentrations of C and N ($n = 5$) by combustion on a Carlo-Erba CHN Analyzer (Carlo Erba Instruments, Milan Italy) and for phosphorus (P) and base cations ($n = 3$) by inductively coupled argon plasma emissions spectometry (ICP, Applied Research Laboratory 3560) following digestion in 10% HCl (Munter and Grande 1981) at the University of Minnesota's Research Analytical Laboratory. A single subsample of each substrate was analyzed for C fractions using Forest-Products techniques (Ryan and others 1989) at the Center for Water and the Environment (Natural Resources Research Institute, University of Minnesota, Duluth). Fractions determined included nonpolar extractives (NPE: fats, oils, waxes), water-solubles (WS: amino acids, simple sugars, soluble phenolics), acid-solubles (AS: cellulose, hemicellulose, starch, polypeptides, nucleic acids), and acid-insolubles (hereafter, lignin). Carbon fractions are presented on an ash-free dry mass basis.

In early December 1999, six strings with each of the eight substrates (in random positions along the string) were placed on the soil surface in each of the twelve plots (6 control and 6 N-fertilized) within each site, with enough strings for harvests in spring and fall 2000, and in fall of 2001–2004 (4608 total bags). Here I present results from the first year's harvests (17 April and 8 October, 2000). Harvested substrates were cleaned of soil, plant roots, invertebrates, and so on, dried (65°C) and weighed. Decomposition was calculated as the proportion initial mass remaining.

Substrate Nitrogen Dynamics

I determined substrate N concentration on harvested litter using near-infrared reflectance spectroscopy (NIRS) (Gillon and others 1999). For each of the two harvests, one of the eight sites was randomly chosen to generate separate calibrations for each harvest. Harvested litter from these sites was analyzed for N by combustion on an ECS 4010 element analyzer (Costech Analytical, Valencia CA) at the University of Nebraska, Lincoln. I generated calibration equations using replicates 1–4 ($n = 64$) and validated these equations using replicates 5–6 ($n = 32$). All replicates were used to generate the final equations that were used to predict litter N at the remaining seven sites. Cross-validation was used to estimate the optimal number of terms to include in the calibration. Equations developed using all substrates sometimes yielded negative values for low-N substrates (filter, wood), so calibration equations developed excluding the high-N substrates (*Acer* and *Quercus* leaves, $n = 72$) were used to predict the remaining six substrate N concentrations. I used equations developed using all eight substrates ($n = 96$) to predict the N concentrations of *Acer* and *Quercus* leaves.

Dried and ground (Wiley Mill, 20-mesh) samples were scanned on an NIR 5000 near-infrared reflectance spectrophotometer (Foss NIRSystems/Tecator, Silver Springs, MD) at 2 nm intervals from 1100–2500 nm. Calibrations and predictions were done using WinISI II (v. 1.02A, Infrasoft International, LLC., Silver Spring, MD) using the modified partial least-squares regression (PLS) method. Different mathematical treatments (first and second derivative, gap, and smooth) were tried, and the treatment yielding the greatest ratio of the standard deviation (SD) to the standard error of cross-validation (SECV), along with a high coefficient of

determination (r^2), was used to generate predicted values (Williams 1987; Gillon and others 1999). Final calibration equations used to predict unknown N concentrations had SD/SECV values ranging from 4.62–7.74 and r^2 values of 0.96–0.98. I determined the proportion of initial N at each harvest by multiplying litter N concentration by litter mass for the beginning of the experiment and for each harvest, and dividing the final N pool by the initial N pool. The proportion of initial N was compared between treatments and among substrates for each harvest separately using two-way ANOVA.

Site Characterization

In 2001, I characterized sites for N availability, litter moisture content, and litter layer and soil pH. I assumed that relative differences among site were similar in 2000 and 2001. I assessed inorganic N availability using ion-exchange resin (IER) bags in the litter layer and the surface soil. 15 ml of resins (Dowex Marathon MR-3 mixed bed resins [R-100835], Supelco Parke, Bellefonte, PA) were placed in nylon stocking bags, acid-washed in 10% HCl for two hours, and rinsed. On 7 May, 2001, I placed one IER bag into the soil (5–10 cm depth) and one bag in the litter layer in control plots at each site. Bags were collected and replaced on 2 July and 30 August, with a final collection on 30 October. After collection, resins were rinsed with deionized water and allowed to air dry in weighing tins for one week and weighed. Resins were extracted in pre-leached 30-ml syringes with GF/A filter paper in the bottom using 100 ml of acidified 2 M NaCl (in 0.1 M HCl) (Giblin and others 1994). Extracts were analyzed for inorganic N on an Alpkem autoanalyzer (OI Analytical, College Station, TX).

Gravimetric moisture (65°C) was measured in the litter layer at seven times between 15 June and 6 September, 2001 by collecting the entire litter layer (O horizon) from an approximately 10-cm diameter circle at each of 6 points spaced 2 m apart along a transect that was close to the actual treatment plots. The pH of the litter layer and the surface soil was determined on 15 June, 2001. Litter samples were collected as described for litter moisture. In addition, at each point along the transect, 3 soil cores (2.54-cm diameter, 5 cm depth) were collected and composited. Subsamples (2 g litter, 10 g soil) were placed in 20 ml of 0.01 M $CaCl_2$, shaken for 30 min, and allowed to settle for 30 min. pH was measured using an Orion pH meter.

Statistical Analyses

Effects of Nitrogen on Decomposition within Sites. To determine the effects of fertilizer N on decomposition *within a site*, I used 2-way ANOVA with substrate and fertilizer treatment as main effects for each site separately. To determine the effect of substrate N and C chemistry *within a site*, I used analysis of covariance (ANCOVA). Fertilization treatment was included as the main effect and initial concentrations of N, WS, lignin, and NPE were included as covariates. Percent AS was excluded from the model because of its tight negative correlation with both WS and NPE (see *Results*). The mean decomposition in either the control or N-fertilized treatment was used as the dependent variable, as I did not have independent measures of initial litter chemistry for each plot. Initial models included all interactions between covariates and fertilization to test the assumption of homogeneity of slopes required by ANCOVA. Interaction terms were not significant in all cases; so I present results from ANCOVA without interaction terms.

To further assess whether the influence of added N *within a site* depended on substrate chemistry, I calculated the ''effect of added N'' on decomposition as the ratio of the proportion of initial mass remaining in control plots to that in N-fertilized plots. Equal mass remaining in control and N-fertilized plots yields a ratio of one. Greater mass loss in N-fertilized plots yields a ratio greater than one (that is, less of the initial mass would be remaining in the N-fertilized plots relative to the control plots). The ratio, rather than the absolute difference, was used because I expected negative exponential decay. I calculated the index using the proportion mass remaining, rather than mass lost, because some litter types exhibited mass loss very close to zero and dividing by those values resulted in large outliers. I regressed this ratio against initial substrate nutrient and C fraction concentrations in separate regressions.

Effects of Nitrogen on Decomposition among Sites. I determined the influence of site characteristics on decomposition by regressing decomposition in control plots against litter and soil pH, litter and soil IER-N, and litter moisture in separate bivariate regressions. In addition, to determine whether the influence of added N depended on site N availability (or some other site characteristic), I regressed the site means of the ''effect of added N'' (see previous section) for all substrates at a site against the site means of IER-N, litter pH, and litter moisture. Litter pH, soil pH and litter moisture were also compared among sites using one-way ANOVA.

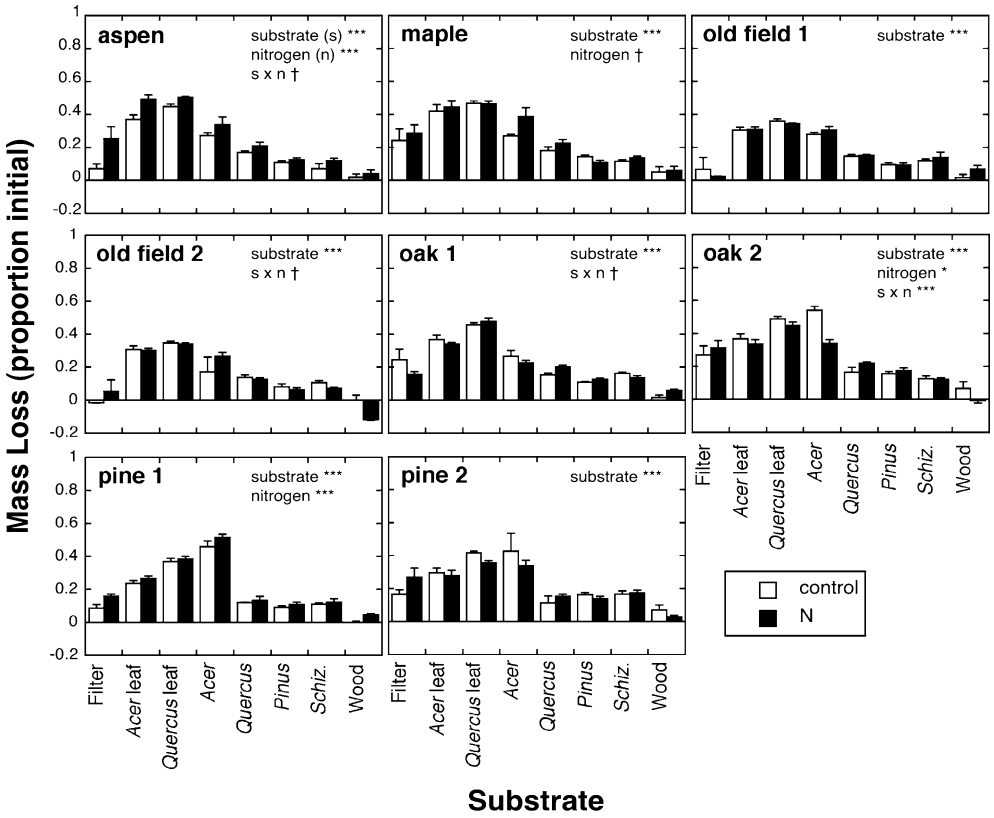

Figure 1. Mass loss of eight substrates decomposed for ten months in control and N-fertilized plots ($n = 6$) at all sites. For two-way ANOVA comparing substrates and N fertilization within each site: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, $^{\dagger}P < 0.10$.

Inorganic N accumulated on IERs was compared among sites, between positions (litter layer versus soil) and among seasons (spring, summer, fall) using repeated-measures ANOVA.

Results

Effects of Nitrogen on Decomposition within a Site

N fertilization increased decomposition only at the Aspen and Pine 1 sites (Figure 1). At the Oak 2 site, there was a significant interaction between substrate and N fertilization with N increasing decomposition of some substrates, but decreasing or having no effect on decomposition of others. At the other five sites, the effect of N fertilizer was not significant, indicating a lack of limitation by exogenously supplied N. First-year mass loss differed significantly among substrates at all sites, with *Quercus* and *Acer* leaves and *Acer* litter decomposing most quickly and wood decomposing most slowly (Figure 1).

As expected, N and C fraction concentrations varied widely among substrates, with N ranging from 0–2.53% (Table 1). N was not correlated with any aspect of litter C chemistry (Table 2), allowing separate determination of the effects of N and C chemistry on decomposition. However, N was tightly and positively correlated with P and K concentrations. Also, both lignin:N and C:N were positively correlated with each other and with % acid soluble (AS), and negatively correlated with % water-soluble (WS). Litter N was positively and significantly related to decomposition at all sites except the two Pine sites (Figure 2), and if *Acer* litter was excluded from the analyses, mass loss was positively related to litter N at these sites as well. *Acer* litter likely had more rapid decomposition than predicted by its N content because of its high concentrations of water-soluble C and its low lignin content (Table 1). The positive relationship between decomposition and litter N may reflect significant positive effects of litter P or K, rather than N, on decomposition, as litter N was tightly correlated with both P and K (Table 2). In fact, separate ANCOVAs at each site with N fertilization as main effect and either P or K as a covariate indicated significant effects of both P and K on decomposition (analyses not shown). First-year mass loss was also

Table 1. Initial Substrate Chemistry of Eight Common Substrates Decomposed at Each Site.

	Litter Nutrients (mg/g)						Ash (mg/g)	Litter Carbon Fractions (mg/g)			
Substrate	N	P	K	Ca	Mg	C		NPE	WS	AS	Lignin
Acer leaves	18.2 (0.4)	2.19 (0.15)	7.35 (0.37)	15.34 (0.43)	2.50 (0.12)	441.5 (0.9)	78	97	312	452	139
Quercus leaves	25.3 (0.7)	1.80 (0.03)	8.49 (0.15)	7.56 (0.39)	2.50 (0.20)	474.6 (1.8)	43	103	284	394	219
Acer litter	4.5 (0.1)	1.65 (0.28)	2.41 (0.30)	25.99 (1.68)	3.05 (0.30)	412.4 (2.9)	130	158	339	429	74
Quercus litter	9.9 (0.7)	0.87 (0.05)	3.79 (0.60)	7.63 (0.28)	1.69 (0.12)	480.3 (2.4)	41	74	259	412	256
Pinus litter	4.6 (0.3)	0.47 (0.07)	0.75 (0.04)	8.24 (0.17)	1.42 (0.02)	500.6 (1.6)	37	250	182	367	202
Schizachyrium litter	3.3 (0.2)	0.28 (0.02)	0.57 (0.01)	1.49 (0.22)	0.49 (0.03)	463.2 (1.1)	13	37	63	666	234
Filter paper	0.0 (0.0)	0.00 (0.00)	0.01 (0.01)	0.04 (0.00)	0.00 (0.00)	423.9 (0.2)	1	1	17	982	0
Wooden applicators	0.9 (0.0)	0.08 (0.01)	0.31 (0.01)	0.56 (0.02)	0.13 (0.00)	462.2 (0.4)	4	8	35	811	146

Values are means (SE). No standard errors are presented for C fractions, which were measured on single bulk samples.
NPE = nonpolar extractives, WS = water soluble, AS = acid soluble, lignin = acid insoluble. Carbon fractions are expressed per g ash-free dry mass.

Table 2. Correlation Matrix of Various Aspects of Initial Substrate Chemistry.

	N	C:N	Lignin	Lignin:N	WS	AS	NPE	P	K	Ca
C:N	−0.62									
lignin	0.40	−0.23								
lignin:N	−0.65	**0.98*** **	−0.04							
WS	0.68	**−0.76***	0.21	**−0.88****						
AS	−0.61	**0.87****	−0.58	**0.91****	**−0.86****					
NPE	0.20	−0.47	0.22	−0.52	0.59	**−0.78***				
P	**0.81***	−0.67	0.09	−0.79	**0.91****	−0.69	0.37			
K	**0.98*** **	−0.61	0.30	−0.67	**0.75***	−0.60	0.16	**0.88****		
Ca	0.27	−0.48	−0.16	−0.63	**0.85****	−0.64	0.59	**0.76***	0.38	
Mg	0.67	−0.74	0.14	**−0.86***	**0.99*** **	**−0.84****	0.60	**0.93*** **	0.73	**0.89****

Values are Pearson correlation coefficients. Significant correlations (P<0.05) are indicated in **bold.**
****P < 0.001, **P < 0.01, *P < 0.05*
NPE = nonpolar extractives, WS = water soluble, AS = acid soluble, lignin = acid insoluble.

positively related to the concentration of water-soluble C at the four of eight sites, suggesting limitation by labile C and/or the contribution of leaching to mass loss in the first year. Decomposition was also negatively related to initial lignin concentrations at five of the sites, indicating a negative effect of lignin even in the early stages of decay. The ANCOVA analyses done for each site also indicated a significant N fertilization effect at the Aspen site, but not at any other sites (Figure 2). The discrepancy between the results of the ANCOVAs and the two-way ANOVAs with substrate and treatment as main effects likely arise from lower power associated with the ANCOVAs, which were done using mean decomposition of all substrates for each treatment.

My hypothesis that fertilizer N would have larger effects on decomposition of low-N substrates was not supported. As mentioned previously, there were no significant interactions between N fertilization and litter N in the ANCOVA analyses. Furthermore, linear regressions of the ''effect of added N'' on decomposition against initial litter N concentration were not significant at any site (Figure 3, P > 0.30 in all cases); significant negative relationships would have supported my hypothesis. The ''effect of added N'' was also unrelated to other aspects of initial litter chemistry, including % WS (data not shown).

Effects of Nitrogen on Decomposition among Sites

Inorganic N accumulated on IERs was highest in the Oak 2 and Pine sites and lowest in the Aspen site (Table 3). Differences among sites changed through the season (data not shown, Repeated-Measures ANOVA, Date*Site: $F_{14,154} = 1.93$, $P = 0.03$) and

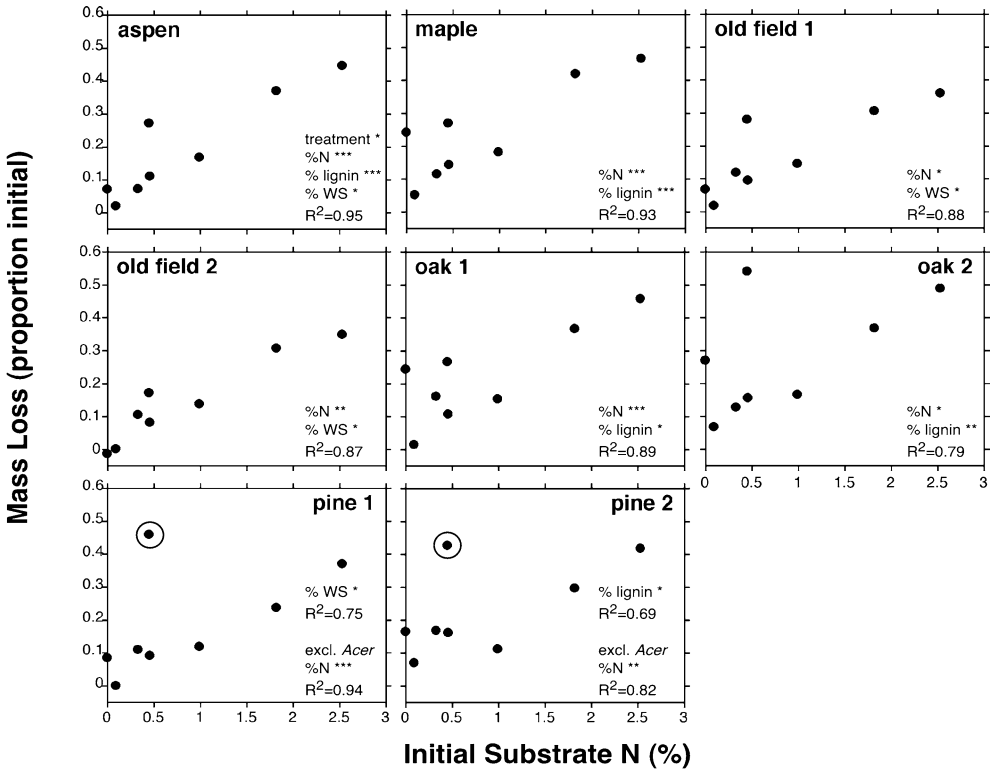

Figure 2. Relationships between mass loss after ten months and initial substrate % N for eight substrates decomposed in control plots at each site. Statistics are from ANCOVAs of mass loss with fertilization treatment as the main effect and substrate N, lignin, WS, and NPE as covariates. ANCOVAs were run with and without *Acer* litter (circled) at both Pine sites. Significant relationships with N or WS were positive and those with lignin were negative. Percent AS was excluded from the analysis because of tight negative correlations with percent NPE and WS (Table 2). Statistical significance indicated as in Figure 1.

IER-N was higher in the soil than in the litter layer (Repeated-Measures ANOVA, Date: $F_{2,77}$ = 122.81, Position: $F_{1,78}$=109.83, Date*Position: $F_{2,77}$ = 52.74, $P < 0.0001$ in all cases). Litter pH was greatest in the Aspen site, whereas soil pH was highest in the Pine 2 and Old Field sites and lowest in the Maple and two Oak sites. Litter moisture was lower in the Old Field sites than in the forested sites, although a significant Site*Date interaction arose because site differences were greater on dates with higher litter moisture contents (data not shown, two-way ANOVA, Site: $F_{7,280}$ = 32.08, Date: $F_{6,280}$ = 298.37, Site*Date: $F_{42,280}$ = 4.68, $P < 0.0001$ in all cases). Across sites, decomposition in the control plots was positively related to IER-N in soils ($r = -0.74$, $P = 0.04$) and to litter moisture content ($r = -0.82$, $P = 0.01$), but was unrelated to IER-N in the litter layer alone ($P = 0.42$) or to litter ($P = 0.99$) or soil ($P = 0.12$) pH. Negative correlation coefficients indicate positive relationships with decomposition, as the dependent variable was proportion initial mass remaining.

My hypothesis that N fertilization would have a greater effect on litter decomposition in sites with lower inorganic N availability was supported by a significant negative relationship between the site mean ''effect of added N'' and site mean seasonal total litter IER N (Figure 4). In other words, fertilizer N had a larger effect on decomposition in sites with lower N availability in the litter layer. The relationship between the ''effect of added N'' and soil IER N was also negative, but not significant ($P = 0.15$), and the relationship with litter+soil IER N was significant ($r = -0.80$, $P = 0.02$). There was no significant relationship between the ''effect of added N'' and either litter ($P = 0.23$) or soil ($P = 0.85$) pH or litter moisture content ($P = 0.84$). I confirmed these results by analyzing proportion mass remaining in an ANCOVA with litter moisture and litter+soil IER N included as covariates, and N fertilization as a main effect, finding a significant interaction between N fertilization and litter+soil IER N (IER N was negatively related to proportion mass remaining in the control treatment, and

Table 3. Environmental Characteristics of All Sites.

Site	Litter pH	Soil pH	Litter moisture (%)	Soil inorganic N (μg N/g resin)	Litter inorganic N (μg N/g resin)	Total inorganic N (μg N/g resin)
Aspen	5.1 ± 0.1^{a}	4.0 ± 0.1^{ab}	89.1	508.5 ± 77.2	167.9 ± 55.3	676.4 ± 130.8
Maple	4.3 ± 0.1^{b}	3.8 ± 0.2^{a}	93.2	724.6 ± 62.0	137.4 ± 19.5	862.0 ± 60.4
Old Field 1	4.7 ± 0.1^{b}	4.4 ± 0.0^{bc}	56.8	528.7 ± 124.2	237.6 ± 35.0	766.3 ± 148.7
Old Field 2	4.2 ± 0.1^{b}	4.6 ± 0.1^{bc}	36.2	598.7 ± 130.8	216.0 ± 37.5	814.7 ± 142.8
Oak 1	4.2 ± 0.1^{b}	3.6 ± 0.2^{a}	96.5	659.8 ± 117.6	328.7 ± 55.1	988.6 ± 157.5
Oak 2	4.5 ± 0.2^{b}	3.7 ± 0.2^{a}	95.4	758.3 ± 106.1	282.1 ± 38.3	1040.4 ± 95.0
Pine 1	4.4 ± 0.2^{b}	4.1 ± 0.1^{ab}	85.2	674.3 ± 95.7	184.7 ± 21.7	859.0 ± 93.7
Pine 2	4.7 ± 0.1^{b}	4.7 ± 0.1^{c}	88.9	725.3 ± 61.2	367.2 ± 43.4	1092.5 ± 98.4

Values are means ± SE. Sample sizes are n = 6 for litter and soil pH; n = 6 at each of seven dates for litter moisture; n = 6 at each of three dates for litter and soil inorganic N. Litter moisture is the mean of all dates measured. Inorganic N is the sum of NH_4^+-N and NO_3^--N that accumulated on ion-exchange resins over three different two-month time periods. Different letters within a column indicate significant differences between sites for pH (Tukey's HSD, $p < 0.05$). See text for statistical analyses of other environmental factors.

unrelated to mass remaining in the N-fertilized treatment). Litter moisture was significantly negatively related to mass remaining, but there was no interaction with N fertilization.

Litter Nitrogen Dynamics

All substrates, except *Quercus* leaves, immobilized N at most sites over the first winter of decomposition (Figure 5A). Proportion initial N was negatively correlated with initial N concentration at only two of the eight sites (Aspen and Maple, $P < 0.05$). N fertilization significantly increased N immobilization at only one site, Oak 2. In contrast, after the summer (that is, after ~one year of decomposition), all substrates exhibited N immobilization. Significant differences among substrates were still apparent, with wood consistently immobilizing relatively more N than other substrates. Nitrogen fertilization led to significantly greater immobilization at three sites, Old Field 1, Pine 1, and Pine 2, and marginally greater N immobilization at Aspen. Thus, there was a lack of perfect correspondence between sites where N stimulated decomposition and sites where N stimulated immobilization. Nitrogen stimulated decomposition at one site where it had only marginal effects on N immobilization (Aspen) and stimulated immobilization at two sites where it did not affect decomposition (Old Field 1 and Pine 2).

Discussion

Substrate versus Fertilization Effects on Decomposition

My hypothesis that both exogenous and endogenous N availability would limit decomposition in these N-poor ecosystems where N limits net primary production was only partially supported. Substrate N was positively correlated with decomposition at all sites, but aspects of C chemistry (water-soluble C, lignin) also explained variation in decomposition among substrates, suggesting that C and N chemistry are both important aspects of litter quality to decomposers in the early stages of decomposition for these substrates in these sites. Although substrate N concentrations were strongly and positively related to decomposition at all sites, increasing the supply of exogenous N through addition of inorganic fertilizer increased decomposition at only two of the eight sites. Other studies have also found positive relationships between litter N concentrations and decomposition while finding no effect of added N (Prescott 1995).

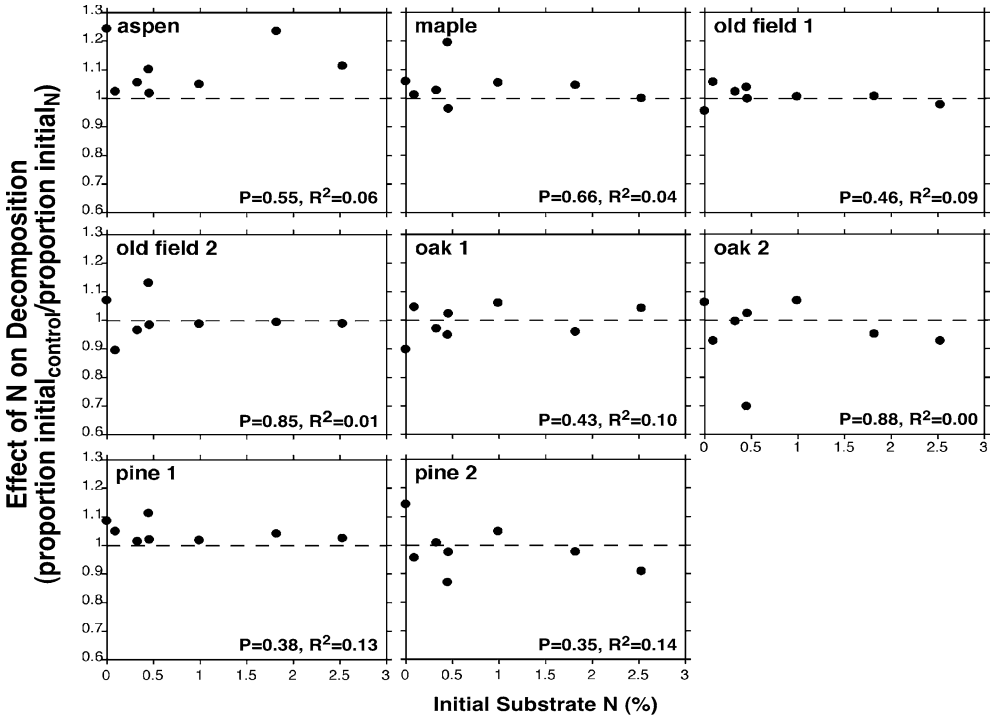

Figure 3. Linear regressions of the effect of fertilizer N (see text) on decomposition and initial substrate N concentration at each of the eight sites. A value of one (no difference in decomposition between control and N-fertilized plots) is indicated by a dashed line.

These seemingly contradictory responses of decomposition to substrate versus externally supplied N could arise for several reasons that I consider in turn. First, the positive correlation between substrate N and decomposition might arise for reasons other than N limitation of decomposition. Second, N supplied in fertilizer and substrate might not be equivalent in quantity or quality. Third, inorganic N fertilizer might alter aspects of the microbial community that make it less responsive to addition of that fertilizer.

Spurious correlation with substrate N. A correlation between substrate N concentration and decomposition might arise in the absence of N limitation of decomposition if (1) substantial substrate N is soluble and readily lost through leaching, or (2) substrate N is correlated with another aspect of substrate chemistry that influences decomposition. Previous studies have suggested that litter N might correlate with mass loss because much of the litter N is soluble and is lost through leaching in the very early stages of decomposition rather than because of N limitation to decomposition (Pastor and others 1987). However, given that the difference in N content between high-N and low-N substrates was about 2% units (for example, *Quercus* leaves versus *Pinus* litter) and that proteins are about 17% N by mass (Sterner and Elser 2002), even if all of the additional N in high-N litter types was soluble

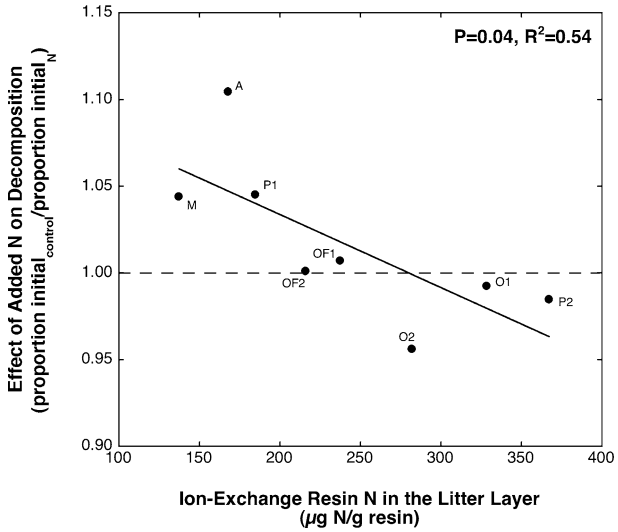

Figure 4. The relationship between the effect of fertilizer N (see text), averaged for all substrates, on decomposition and the availability of inorganic N in the litter layer (the sum of IER-N across the season) across the eight sites. A value of one is indicated by a dashed line. A, Aspen; M, Maple; O1, Oak 1; O2, Oak 2; OF1, Old Field 1; OF2, Old Field 2; P1, Pine 1; P2 Pine 2.

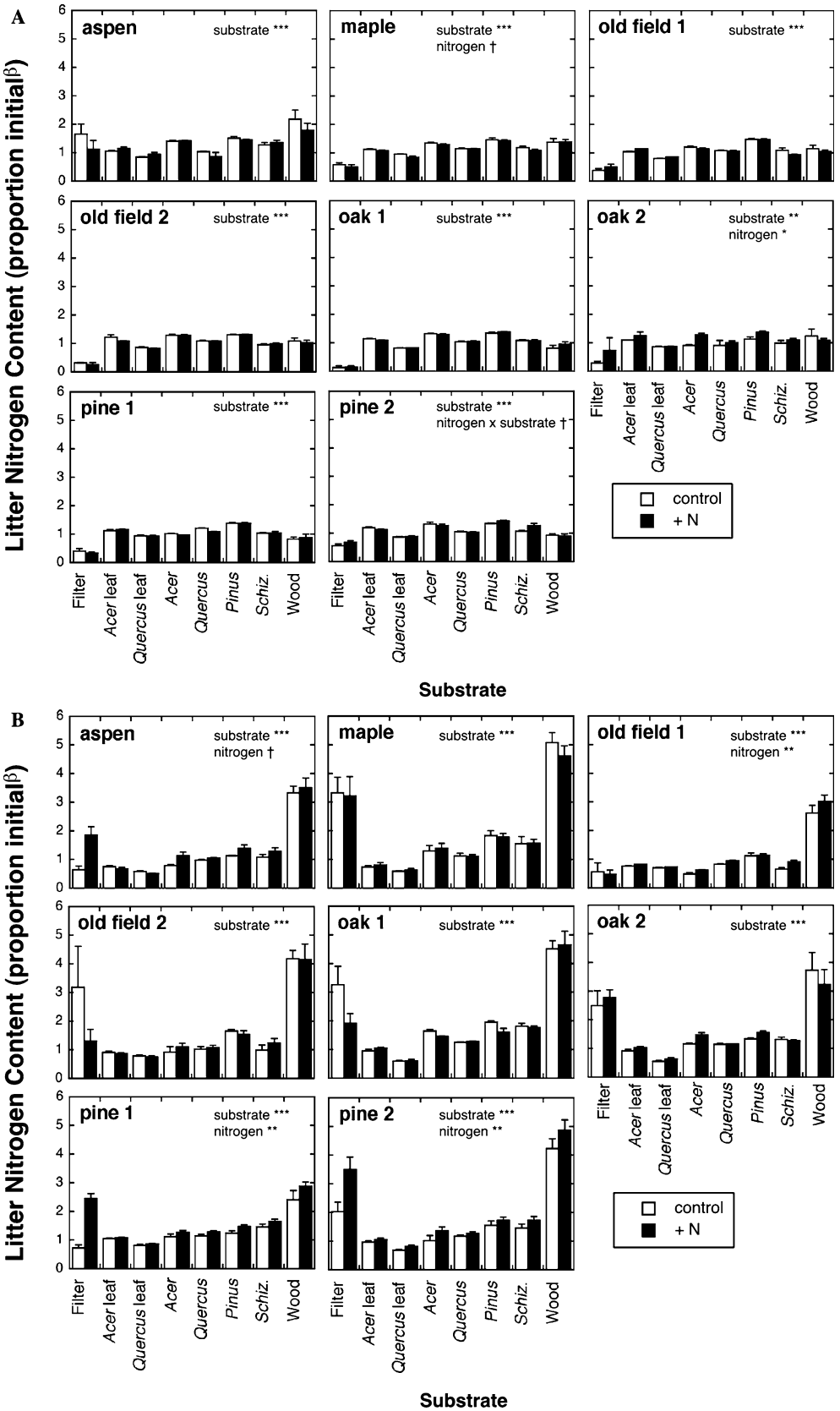

Figure 5. Nitrogen content (proportion initial) of eight substrates decomposed four months over winter (A) and ten months (B) in control and N-fertilized plots ($n = 6$) at each site. Statistical significance is indicated for two-way ANOVA comparing substrates (litter type) and N fertilization within each site: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, †$P < 0.10$. β Note that units for filter substrate, which was excluded from ANOVA, are mg N/g initial litter.

protein (which is unlikely), leaching of that protein could only account for a 12% difference in mass loss of the high-N relative to the low-N substrates, about half of the observed difference. Furthermore, most substrates, including the high-N *Acer* leaves, exhibited net N immobilization, rather than N release, over the first winter of decomposition when leaching most likely would have occurred.

Decomposition would also be positively correlated with substrate N concentration if substrate N was negatively correlated with some factor that inhibited decomposition or positively correlated with a labile C fraction or with another nutrient that was truly limiting decomposition. However, although substrate lignin and water-soluble C concentrations were negatively and positively correlated with decomposition, respectively, substrate N was unrelated to concentrations of both of these C fractions. Litter N concentration was positively correlated with both litter P and litter K concentrations, as is often observed (Garten 1976; Cornelissen and others 1997; Hobbie and Gough 2002), so tightly that I could not determine the independent influence of each of these three nutrients on decay. The discrepancy between decomposer and litter N:P ratios, along with the immobility of P in soils, makes P-limitation of decomposition plausible (Paul and Clark 1996). Indeed, some studies have found positive correlations between litter P concentration and decomposition rate (Berg and others 1987; Vitousek and others 1994; Hobbie and Vitousek 2000; Ostertag 2001). However, in two Cedar Creek old fields (similar to those studied here), decomposition rates of *Schizachyrium* litter in plots fertilized with both P and K as well as NH_4NO_3 did not differ from rates in unfertilized control plots (Pastor and others 1987), suggesting that P or K limitation of decomposition is an unlikely explanation for the positive relationship between decomposition and substrate N observed in this study.

Quantity and quality of N supplied in fertilizer versus substrate. The contradictory responses of decomposition to substrate versus externally supplied N cannot easily be explained by differences in the quantity or availability of N supplied in litter versus fertilizer. The quantities of N added in litter and fertilizer were similar (6 g of *Quercus* leaves in a 0.0225 m^2 litter bag = 6.7 g N/m^2 compared with 10 g N/m^2 added as NH_4NO_3). Furthermore, the stimulation of N immobilization by N fertilization indicates that decomposers were accessing N added as fertilizer at some sites. In addition, N fertilization stimulated immobilization in some sites where it did not stimulate decomposition, suggesting that lack of accessibility to fertilizer N alone cannot explain the lack of N effects on decomposition, although the stimulation of immobilization was relatively small.

However, even though similar amounts of N were added in litter and fertilizer, N added as NH_4NO_3 fertilizer may not be equivalent to organic N contained within litter or leaves as an N source for decomposing microbes. For example, different soil microbes use different forms of N, with some unable to grow on inorganic forms alone, requiring organic N forms such as amino acids (Lochhead and Chase 1943). The complete absence of decomposing microorganisms that can assimilate NH_4^+ or NO_3^- seems unlikely (Brown 1980), and ^{15}N tracer studies have demonstrated immobilization of inorganic N (both NH_4^+ and NO_3^-) into decomposing litter (for example, Van Vuuren and Van der Eerden 1992; Downs and others 1996; Frey and others 2000). In this study, higher N content in litter decomposing in N-fertilized plots also suggests that decomposers were accessing inorganic N. However, organic N may be a preferred N source to NH_4^+ or NO_3^- because it is does not require reduction (as does NO_3^-) or assimilation (for example, Brown 1980). Given the lower energetic costs of using organic N, those decomposers relying on organic N may have competitively excluded those relying on inorganic N, minimizing the response of decay to inorganic N addition.

Effects of N on the decomposer community. The discrepancy between the influence of inorganic N supply and substrate N might also have occurred because inorganic N influenced characteristics of the decomposer community. For example, previous studies have shown that fertilization with N reduces microbial C:N ratio (Knapp and others 1983), consistent with studies showing greater N immobilization without concomitant increased mass loss in response to N fertilization (for example, Pastor and others 1987; Fog 1988; Hunt and others 1988; Downs and others 1996; Hobbie and Vitousek 2000). Reductions in decomposer C:N ratio might have resulted in a population response to added N that did not translate into greater C use and thus mass loss. Inorganic N enrichment might also increase the efficiency of microbial decomposers (Ågren and others 2001), which would also be consistent with little effect of added N on decay. However, why supplementation of inorganic N supply would influence microbial C:N ratio or efficiency, while increased substrate N would not, is unclear.

Site Variation in Decomposition and its Response to Fertilization

The positive relationship between site N availability and decomposition as well as the greater response of decomposition to added N at N-poor sites are seemingly inconsistent with the lack of N fertilization effects on decomposition. What could explain this inconsistency? Possibly, site N availability covaries with the real factor that explains site variation in decomposition, such as the availability of some other nutrient or an aspect of the composition or physiology of the microbial community that influences decomposition. For example, if high-N sites are characterized by more efficient decomposers or by decomposers with a lower C:N ratio, decomposition in those sites might respond less to N addition than in low-N sites, consistent with my results. However, additional research is required to determine whether such site-to-site differences in decomposer attributes actually exist.

Conclusion

The inconsistent effects of substrate N and external N supply on decomposition observed here suggest that the oft-observed relationship between litter N and decomposition cannot be interpreted as straightforward N limitation. My results suggest that more careful studies of the influence of various resources on decomposition (for example, organic versus inorganic N; N versus other elements besides N), along with characterization of physiological attributes of the decomposer community, are necessary before the influence of N on the early stages of decomposition can be fully understood.

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