

## NITROGEN EFFECTS ON DECOMPOSITION: A FIVE-YEAR EXPERIMENT IN EIGHT TEMPERATE SITES

SARAH E. HOBBIE<sup>1</sup>

*Department of Ecology, Evolution and Behavior, University of Minnesota, Saint Paul, Minnesota 55108 USA*

**Abstract.** The influence of inorganic nitrogen (N) inputs on decomposition is poorly understood. Some prior studies suggest that N may reduce the decomposition of substrates with high concentrations of lignin via inhibitory effects on the activity of lignin-degrading enzymes, although such inhibition has not always been demonstrated. I studied the effects of N addition on decomposition of seven substrates ranging in initial lignin concentrations (from 7.4% to 25.6%) over five years in eight different grassland and forest sites in central Minnesota, USA. I predicted that N would stimulate the decomposition of lignin-poor substrates but retard the decomposition of lignin-rich substrates. Across these sites, N had neutral or negative effects on decomposition rates. However, in contrast to my hypothesis, effects of N on decomposition were independent of substrate initial lignin concentrations, and decomposition of the lignin fraction was unaffected by N fertilization. Rather, substrate–site combinations that exhibited more rapid decomposition rates in the control treatment were affected more negatively by addition of N fertilization. Taken together, these results suggest that decreased decomposition with added N did not result from inhibition of lignin-degrading enzyme activity, but may have resulted from abiotic interactions between N fertilizer and products of microbial degradation or synthesis or from N effects on the decomposer community. Low initial substrate N concentrations and N fertilization both stimulated N immobilization, but the differences among substrates were generally much larger than the effects of fertilization. This study suggests that atmospheric N addition could stimulate ecosystem carbon sequestration in some ecosystems as a result of reduced rates of forest floor decomposition.

*Key words:* decomposition; forest; grassland; immobilization; lignin; Minnesota, USA; nitrogen.

### INTRODUCTION

Production of nitrogenous fertilizers, cultivation of legume crops, and fossil fuel combustion all contribute to the transfer of nitrogen (N) from largely inert pools (atmospheric N<sub>2</sub>, fossil fuel reserves) to biologically reactive forms that can be transported from agricultural or industrial areas to ecosystems that may historically have experienced relatively low levels of N inputs, and in which N may limit rates of biological processes (Galloway and Cowling 2002, Galloway et al. 2003). Human activities have more than doubled the background rates of N fixation, and given likely trends in agricultural practices, population growth, and fossil fuel combustion, N deposition will likely continue to increase in the future (Galloway et al. 2004).

One of the major scientific challenges regarding the ecological effects of increased N deposition is to understand how it alters the cycling and storage of another biologically important element, carbon (C). Effects of N deposition on ecosystem C storage are best elucidated through increased understanding of the influence of N on ecosystem C inputs (i.e., productivity

and losses (i.e., decomposition). As N has historically limited plant growth in many temperate ecosystems where rates of N deposition are currently high (Vitousek and Howarth 1991), the potential for stimulation of net primary production (NPP) by increased N deposition is large (but see Nadelhoffer et al. 1999, Magill et al. 2000). Indeed, net ecosystem C uptake by temperate and boreal forests increases with rates of N deposition across the range of deposition rates measured (Magnani et al. 2007).

The role of chronic N addition in influencing C losses from ecosystems through decomposition is poorly understood. Indirect evidence suggests an important role for N limitation of decomposition, as litter often immobilizes N during the early stages of decomposition, suggesting that fresh litter contains insufficient N to meet the growth and maintenance requirements of decomposers (Gosz et al. 1973, Staaf and Berg 1981, Parton et al. 2007). Furthermore, litter decomposition rates often correlate positively with litter N concentrations (Melillo et al. 1982, Hobbie 2005) and is rapid in more fertile sites (Hobbie and Vitousek 2000).

Despite the indirect evidence for N limitation of decomposition, results of direct tests, in which N supply is experimentally increased through fertilization, are inconsistent. A recent meta-analysis of N fertilization effects on decomposition found that N can have stimulatory, neutral, or negative effects on decomposi-

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<sup>1</sup> E-mail: shobbie@umn.edu

tion, depending on substrate chemistry, ambient N deposition rates, and the amount of N fertilizer added (Knorr et al. 2005). Specifically, N generally reduced decomposition of substrates with high lignin concentrations but stimulated decomposition of substrates with low lignin concentrations. These results are consistent with those of other studies showing that substrate and/or exogenous N may reduce lignin decomposition (Berg and Matzner 1997), either by inhibiting synthesis of ligninolytic enzymes (Carreiro et al. 2000, Sinsabaugh et al. 2002) or by reacting with breakdown products of lignin degradation to form other recalcitrant compounds (Berg and Staaf 1981, Fog 1988, Dijkstra et al. 2004). The effects of N on decomposition may vary, increasing early stages of decomposition (when litter contains relatively high concentrations of labile C and low concentrations of N) but reducing decomposition in its later stages, when the concentrations of lignin in litter have increased (Berg and Matzner 1997).

At the Cedar Creek Ecosystem Science Reserve (formerly Cedar Creek Natural History Area) in central Minnesota, I conducted a five-year experiment addressing various specific questions regarding the effects of N on decomposition. I tested separate hypotheses regarding (1) the early stages of decomposition, when N is most likely to limit decomposition because of the large discrepancy between decomposer and litter C:N ratio (Hobbie 2005); and (2) the later stages of decomposition, when lignin degradation predominates and negative interactions between lignin decomposition and N addition are most likely to occur. In prior work, I found that after one year of decomposition, N fertilization increased decomposition at only two of eight grassland or forested sites, even though decomposition was positively related to litter N concentrations at all sites and to soil N availability across sites (Hobbie 2005). Here I report results after five years of decomposition and explicitly address the hypothesis that N will promote the decomposition of substrates with low lignin concentrations but will reduce that of substrates with high lignin concentrations, such that the effect of fertilizer N on decomposition will decrease with increasing initial substrate lignin concentration. I also hypothesized that N immobilization into decomposing litter would be enhanced by both N supply (i.e., fertilization) and demand for N by decomposers (i.e., low initial substrate N concentrations) (Parton et al. 2007). I tested these hypotheses by decomposing substrates varying in their initial lignin and N concentrations in control and N-fertilized plots at eight different sites comprising old fields and hardwood and conifer stands.

## METHODS

### *Study site*

The experiment was established at Cedar Creek Ecosystem Science Reserve (CCESR), a Long Term Ecological Research (LTER) site in central Minnesota (45°40' N, 93°20' W, elevation 270 m) that 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). The average ambient wet N deposition rate is 5.6 kg N·ha<sup>-1</sup>·yr<sup>-1</sup> (1997–2006; measured at the Cedar Creek National Atmospheric Deposition Program site). The climate is temperate, with a mean annual temperature of 6.7°C and mean annual precipitation of 801 mm. In 1999 I established an experiment to explore interactions between exogenous (fertilizer) N supply and substrate initial lignin and N concentrations on decomposition at eight upland sites within CCESR (Hobbie 2005), including two old fields (dominated by *Schizachyrium scoparium* and other C<sub>4</sub> and C<sub>3</sub> 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, all on Udipsamments (Grigal et al. 1974).

### *Decomposition experiment*

The details of the decomposition experiment have been described previously along with initial substrate chemistry and the first year of decomposition and litter N dynamics (Hobbie 2005). Briefly, within each site I established 12 plots (six control, six N-fertilized). The N-fertilized plots were sprayed with 10 g N·m<sup>-2</sup>·yr<sup>-1</sup> as aqueous NH<sub>4</sub>NO<sub>3</sub>, beginning in October 1999, in three applications per year. Control plots received the equivalent volume as water. In each plot, I used litter bags to decompose seven substrates with varying initial N and lignin concentrations: leaf litter of *Schizachyrium scoparium*, *Acer saccharum*, *Quercus ellipsoidalis*, and *Pinus strobus*; green leaves of *Acer saccharum* and *Quercus ellipsoidalis*; and commercially available untreated birch wood applicators (“wood” hereafter). I exclude data from cellulose filter paper (presented in Hobbie [2005]) because its rapid decomposition made it an outlier in most analyses. Initial substrate C, N, P, K, Ca, Mg, nonpolar extractive (NPE), water-soluble (WS), acid-hydrolyzable (AH), and nonhydrolyzable (hereafter lignin) fractions are presented in Hobbie (2005). Initial lignin concentrations ranged from 7.4% to 25.6%, and initial N concentrations ranged from 0.45% to 2.53%. Litter bags were deployed in December 1999 and harvested on 17 March and 9 October 2000 and in October 2001–2004. Harvested substrates were cleaned of soil, plant roots, invertebrates, and other debris, dried (65°C), and weighed.

### *Substrate nitrogen dynamics*

I determined substrate N concentration on harvested litter using near-infrared reflectance spectroscopy (NIRS; Gillon et al. 1999), calibrated using a subset of samples analyzed for N by combustion on an ECS 4010 element analyzer (Costech Analytical, Valencia,

California, USA) at the University of Nebraska, Lincoln, Nebraska, USA (see Hobbie 2005 for details). 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 by dividing the final N pool by the initial N pool.

#### *Substrate lignin dynamics*

I analyzed all substrates in all plots of one randomly selected site (Pine 2) for substrate lignin concentrations using forage-fiber techniques (Van Soest 1967) on an ANKOM Fiber Analyzer (Ankom Technology, Macedon, New York, USA). These analyses were done at the four-year (October 2003) harvest in order to determine N fertilization effects on lignin decomposition in the later stages of decomposition, while allowing sufficient mass for lignin determination. I determined the proportion of initial lignin after four years by multiplying substrate lignin concentrations by substrate mass and dividing by the initial lignin pool. For this purpose, initial lignin concentrations were also determined using forage-fiber techniques on subsamples of undecomposed substrates used to construct litter bags. Values obtained from these analyses were significantly correlated with values of initial lignin obtained using forest-products techniques, although the values obtained using forage-fiber techniques were about 70% of those using forest-products techniques (Hobbie 2005) ( $y = 0.76 + 0.67x$ ,  $r = 0.80$ ,  $P < 0.05$  including *Schizachyrium*;  $y = 0.91 + 0.74x$ ,  $r = 0.97$ ,  $P < 0.05$  excluding *Schizachyrium*;  $y =$  forage fiber value and  $x =$  forest-products value).

#### *Site characterization*

In 2001 sites were characterized for N availability, litter moisture content, and litter layer and soil pH (Hobbie 2005). Additionally, I assessed inorganic N availability using ion-exchange resin (IER) bags (Giblin et al. 1994) in the litter layer and the surface soil in each plot in control and N-fertilized treatments. (In 2001 only control plots were characterized.) Nylon stocking bags containing 15 ml of resins (Dowex Marathon MR-3 mixed bed resins, Supelco Parke, Bellefonte, Pennsylvania, USA) were acid-washed in 10% HCl for two hours and rinsed. Bags were placed in the soil or in the litter layer from May–June, July–August, and September–October 2003. Upon collection, resins were rinsed with deionized water, air-dried, weighed, and extracted with acidified 2 mol/L NaCl. Extracts were analyzed for inorganic N on an Alpkem autoanalyzer (OI Analytical, College Station, Texas, USA). In July 2005 I measured pH in the litter layer of all plots. Within each plot, litter (entire O horizon) was collected from four separate locations using a 20 × 20 cm sampling frame and combined in the field into one composite litter sample per plot. In the lab the litter was mixed, homogenized by hand, and dried (65°C) before pH determination. Subsamples (2 g) were placed in 20 mL of water, shaken

for 30 min, and allowed to settle for 30 min. I measured pH using an Orion pH meter (Thermo Scientific, Waltham, Massachusetts, USA).

#### *Statistical analyses*

To determine site and treatment effects on N availability and pH, the total quantity of inorganic N accumulated over the season on IER bags was compared among sites, between treatments (control and N-fertilized), and between positions (litter layer vs. soil) using three-way ANOVA. Values were ln-transformed to homogenize variances. O horizon pH (water) was compared between treatments and among sites using two-way ANOVA.

The proportion of initial substrate mass remaining against time was fit with three alternative models (sensu Weider and Lang 1982): a single-exponential decomposition model,  $X = e^{-kt}$ ; a double-exponential decomposition model,  $X = Ae^{k_1t} + (1 - A)e^{k_2t}$ ; and an asymptotic model,  $X = C + (1 - C)e^{-k_Ct}$ , where  $X$  is the proportion of initial mass remaining at time  $t$ . In the single-exponential decomposition model,  $k$  is the decomposition constant. In the double-exponential decomposition model,  $A$  is the fraction of the initial mass that decomposes with decomposition rate  $k_1$ , while the remaining fraction  $(1 - A)$  decomposes with decomposition rate  $k_2$ . In the asymptotic model,  $C$  is the fraction of the initial mass with a decomposition rate of zero (i.e., the asymptote), while the remaining fraction  $(1 - C)$  decomposes with decomposition rate  $k_C$ . Note that all models constrain the proportion of initial mass remaining at time zero to be 1. All models were fit using SAS version 9.1 or JMP version 6.0.3 (SAS Institute, Cary, North Carolina, USA), and biologically unreasonable parameters (e.g., negative parameter values) were not allowed. Of the models that achieved convergence in the curve-fitting process, the final model was selected using Akaike's Information Criterion (AIC), which rewards good fit and penalizes for more model terms (Burnham and Anderson 1998). If the difference between the lowest AIC in any case and the AIC from any other candidate model was less than three, I concluded that the model with the lowest AIC and the other model(s) were indistinguishable in their abilities to describe the data.

Because a single negative exponential decomposition model described decomposition across most site, treatment, and substrate combinations better than double negative exponential or asymptotic decomposition models (see *Results: Decomposition models*), I used that model to estimate  $k$  for the following analyses: (1) To investigate site and N fertilization effects on decomposition, I compared  $k$  (determined separately for all replicates of each site–treatment–substrate combination) among substrates and between treatments using two-way analysis of variance (ANOVA) for each site. (2) To determine whether the effect of fertilizer N depended on initial substrate chemistry, I calculated the “effect of added N” ( $k_N - k_C$ ) on decomposition as the difference

TABLE 1. Summary of results of model fitting.

Model	Total fits when particular model was best fit (%)	
	Each replicate fit separately	All replicates pooled
None	2 (0.3)	0
Single	556 (82.7)	0
Double	23 (3.4)	12 (10.7)
Asymptotic	0	0
Single, double	16 (2.4)	0
Single, asymptotic	0	57 (50.9)
Double, asymptotic	26 (3.9)	26 (23.2)
Single, double, asymptotic	49 (7.3)	17 (15.2)

Notes: Replicates were fit within a site–treatment–substrate combination;  $n = 672$  fits for each replicate separately, and  $n = 112$  fits for all replicates pooled. Where more than one model is indicated, they were equivalent fits for the data using the criterion that the difference between the minimum Akaike's Information Criterion (AIC) and the AIC of other candidate model(s) was less than three.

between  $k$  of substrates in control ( $k_C$ ) vs. N-fertilized plots ( $k_N$ ) (determined for all replicates combined of each site–treatment–substrate combination). I then analyzed the relationship between  $k_N - k_C$  and initial substrate chemistry using analysis of covariance (ANCOVA) with site as a main effect and initial substrate chemical constituents as covariates, including initial WS, AH, NPE, lignin, N, P, Ca, Mg, and K (separate ANCOVA for each covariate). Initial models that included interactions between covariates and site tested the assumption of homogeneity of slopes required by ANCOVA. (3) To determine the relationship between  $k$  (determined for all replicates combined of each site–treatment–substrate combination) and substrate initial chemistry, I used ANCOVA with site, fertilization treatment, and their interaction as main effects, and initial substrate chemical constituents as covariates (separate analyses for each covariate). Initial models included all interactions between covariates and main effects to test the assumption of homogeneity of slopes required by ANCOVA. (4) To determine whether environmental differences among sites could explain site differences in average  $k$  (in control plots) or the effect of added N on  $k$ , I used backward stepwise regression of  $k_C$  and  $k_N - k_C$  (determined for all replicates combined of each site–treatment–substrate combination and averaged across substrates within each site and treatment) against site-averaged soil and litter IER-N (mean of 2001 and 2003 control plot measurements), litter layer gravimetric moisture (Hobbie 2005), and soil and litter pH (Hobbie 2005). I used AIC to select predictors to include in the model in a stepwise manner, eliminating predictors one at a time in the order of which one produced the greatest reduction in AIC until elimination no longer reduced AIC (Weisberg 2005).

To determine the effects of N fertilization on substrate N dynamics, I used ANCOVA to analyze the proportion of initial N of each substrate in each site (all harvests

combined), with N fertilization as a main effect and the proportion of initial mass remaining as a covariate. I also analyzed the effects of site, N fertilization, and substrate chemistry on maximum N immobilization (mg N/g initial substrate) over the course of the five-year experiment using ANCOVA with site, N-fertilization treatment, and their interaction as main effects, and substrate initial C and nutrient constituents as covariates (each covariate was analyzed in a separate ANCOVA).

Finally, to determine the effects of N fertilization on the dynamics of the lignin fraction (all substrates combined), I analyzed the proportion of initial lignin remaining at the fourth harvest in the Pine 1 site using ANCOVA with N fertilization as a main effect and the proportion of initial mass remaining as a covariate.

## RESULTS

### Decomposition models

Substrates lost between 20% and 99% of their initial mass (mean 70%; median 72%) by the end of five years. Using Akaike's Information Criterion (AIC), the single-exponential model was either the best fit or was equally as good as the other two models in 92% of all cases (mean  $R^2 = 0.90$ , median  $R^2 = 0.93$ , range: 0.05–1.00; Table 1). In the remaining cases, a double-exponential model was the best fit or was equally as good as an asymptotic model. The majority of cases where a double-exponential fit was best was for *Acer* litter or *Quercus* leaves. However, even for these substrates, a single-exponential model was the best fit or was equally as good as the other two models in 89/96 or 78/96 cases, respectively.

When fitting the decomposition models to site, treatment, and substrate combinations (six replicates combined), the models were less distinct in their ability to describe the data (Table 1). A single-exponential model was equally as good a fit as an asymptotic model, or both an asymptotic or double-exponential model in 66% of all cases. A double-exponential decomposition model was the best fit (10.7%) or was equally as good as an asymptotic model (23.2%) in the remainder. In no case was a single or asymptotic model the sole best fit.

### Effects of N fertilization on decomposition

Fertilization effectively increased N availability as assessed by ion-exchange resins (Table 2). Fertilization effects on ion-exchange resin N (IER-N) differed among sites but not between positions (three-way ANOVA: site  $\times$  treatment  $\times$  placement,  $P < 0.0001$ ; treatment  $\times$  site,  $P = 0.0003$ ; placement  $\times$  treatment,  $P = 0.43$ ; placement  $\times$  site,  $P = 0.007$ ; placement  $\times$  site  $\times$  treatment,  $P = 0.17$ ). In the litter layer in the N-fertilized plots, IER-N ranged from 13 times higher in the Pine 1 site to 35 times higher in the Aspen site than in control plots. In contrast, fertilization had no effects on the pH of the litter layer (two-way ANOVA: site,  $P < 0.0001$ ; treatment,  $P = 0.18$ ; site  $\times$  treatment,  $P = 0.43$ ; Table 2).

TABLE 2. Litter and soil ion-exchange resin N (IER-N) and pH in control and fertilized plots at eight sites.

Site	Growing season ion exchange resin N ( $\mu\text{g N/g resin}$ )				pH (water) of litter			
	Soil		Litter		Control		N-fertilized	
	Control	N-fertilized	Control	N-fertilized	Control	N-fertilized	Control	N-fertilized
Aspen	323.7 (76.3)	10 611.9 (2322.0)	88.8 (8.8)	3123.8 (560.4)	5.83 (0.07)	5.75 <sup>a</sup> (0.05)		
Maple	957.8 (165.8)	13 730.0 (2652.9)	136.9 (32.4)	2193.6 (498.2)	5.51 (0.07)	5.40 <sup>b</sup> (0.06)		
Old Field 1	410.3 (41.8)	8982.1 (1192.1)	197.8 (9.6)	3426.5 (642.4)	5.71 (0.10)	5.70 <sup>a</sup> (0.10)		
Old Field 2	410.2 (116.6)	16 788.5 (5162.9)	193.7 (9.6)	4139.7 (482.0)	5.44 (0.03)	5.52 <sup>b</sup> (0.06)		
Oak 1	1034.5 (261.2)	16 388.6 (2335.7)	199.0 (28.4)	4880.6 (1434.4)	5.23 (0.10)	5.11 <sup>cd</sup> (0.08)		
Oak 2	1460.3 (103.6)	15 975.1 (4652.7)	279.2 (21.0)	5596.9 (914.6)	5.44 (0.06)	5.28 <sup>bc</sup> (0.06)		
Pine 1	1168.6 (186.7)	18 751.7 (3337.7)	397.9 (92.0)	8019.4 (1481.1)	5.41 (0.05)	5.51 <sup>b</sup> (0.08)		
Pine 2	2656.8 (145.8)	19 417.7 (4916.2)	417.3 (56.2)	5613.1 (1776.4)	5.19 (0.04)	5.11 <sup>d</sup> (0.05)		

Notes: Values are means (with SE in parentheses). For pH, different letters within a column indicate significant differences among sites (Tukey's hsd;  $P < 0.05$ ). See *Results: effects of N fertilization on decomposition* for other statistical analyses.

For all substrates in all sites taken together, decomposition rates in the control treatment were significantly correlated with decomposition rates in the N-fertilized treatment. However, the slope of this relationship was significantly less than 1 (Fig. 1), such that site–substrate combinations (as well as site averages of all substrates) that exhibited more rapid decomposition rates in the control treatments were more negatively affected by addition of N fertilizer.

In one Oak (Oak 1) and one Pine (Pine 2) site, N fertilization significantly slowed decomposition rates (Fig. 2; Appendix A: Table A1). In the other Oak and Pine sites, the effect of N fertilization was also negative but varied among substrates, giving rise to significant substrate  $\times$  N interactions. In the two Old Fields, N fertilization effects were more varied, ranging from positive to neutral to negative, again giving rise to significant substrate  $\times$  N interactions. N fertilization had no effect on decomposition in the Aspen or Maple sites.

In contrast to my hypothesis that N would stimulate the decomposition of low-lignin substrates but reduce the decomposition of high-lignin substrates, the effect of fertilizer N on  $k$  was independent of substrate initial lignin concentration (Table 3). However,  $k_N - k_C$  was negatively related to initial nutrient, water-soluble (WS), and nonpolar extractive (NPE) concentrations and positively related to initial acid-hydrolyzable (AH) concentrations (ANCOVAs of  $k_N - k_C$  with site as a main effect and initial substrate chemical constituents as covariates; Table 3). Teasing apart the relative importance of different aspects of initial substrate chemistry was impossible because many components of initial substrate were highly correlated with one another (Table 4). For example, initial WS was correlated tightly with initial AH, P, Ca, and Mg concentrations, as were N with P and K; P with K, Mg, and WS; and AH with NPE and Mg.

After four years, in the Pine 1 site, decomposition of the lignin fraction was related positively to overall decomposition ( $P < 0.001$ ). However, there was no difference between the control and N-fertilized treatments in the proportion of initial lignin, adjusted for variation in mass remaining (overall  $R^2 = 0.25$ ;  $P = 0.54$ ).

#### Effects of substrate chemistry on decomposition

Decomposition was negatively related to initial concentrations of lignin and AH and positively related to initial concentrations of WS and all nutrients (ANCOVAs of  $k$  of each substrate, all replicates combined within each site–treatment combination, with site, N fertilization, and their interaction as main effects and substrate initial chemical constituents as covariates; Table 5). Teasing apart the relative importance of different substrate constituents was impeded by tight correlations among them. Neither the main effect of N fertilization nor its interaction with site was significant. Although the lack of a fertilization or site  $\times$  fertilization

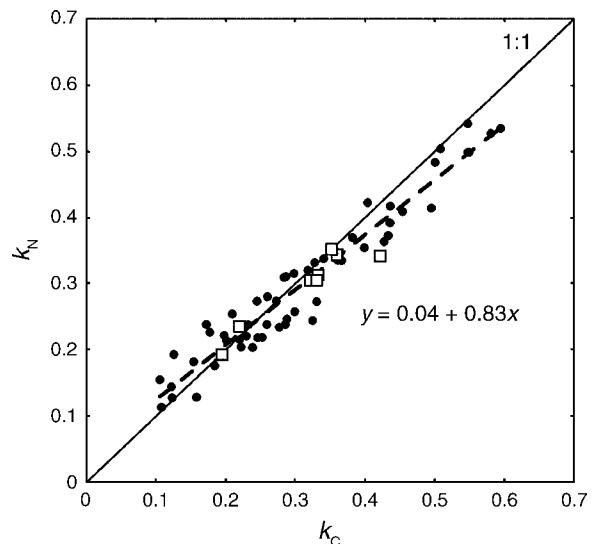


FIG. 1. The decomposition rate in N-fertilized plots ( $k_N$ ) vs. the decomposition rate in control plots ( $k_C$ ) for each substrate in all sites (solid circles) and averaged across all substrates in a site for all sites (open squares). The 1:1 line is shown with a solid line. The dashed line and equation represent the fitted line for the solid circles. This line has a slope significantly less than 1.0 ( $t_{53} = -5.73$ ,  $P < 0.001$ ,  $R^2 = 0.94$ ). One observation (not shown) was excluded as an outlier (Cook's  $D > 1.0$ ). A line fit through the open squares ( $y = 0.07 + 0.71x$ ; line not shown) also has a slope significantly less than 1.0 ( $t_6 = -2.82$ ,  $P < 0.05$ ,  $R^2 = 0.89$ ).

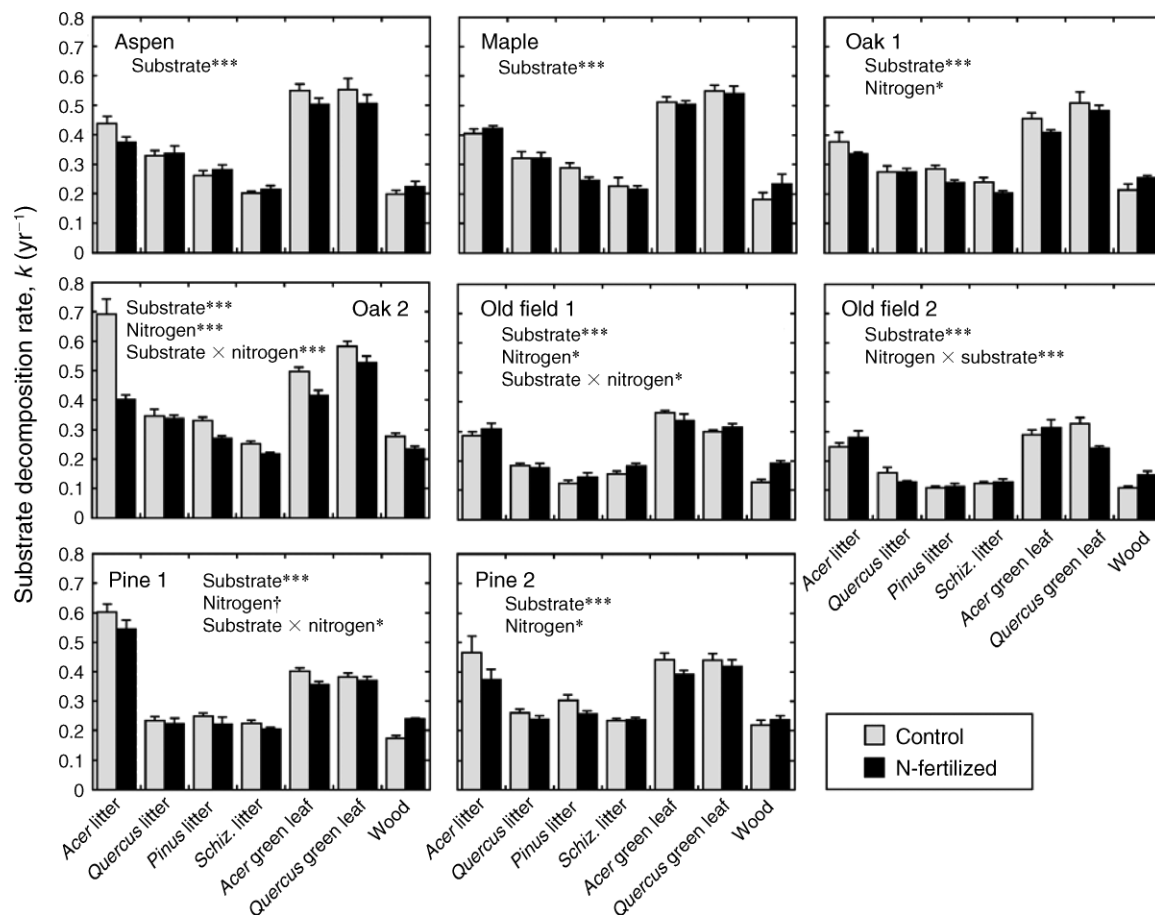


FIG. 2. Decomposition rates ( $k$ ) of substrates decomposed in control and N-fertilized sites in eight grassland and forested sites. Results of two-way ANOVAs (with site and N fertilization as main effects) are shown for each site. Values are means + SE. *Schiz.* = *Schizachyrium scoparium*, a grass.

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ; †  $P < 0.1$ .

effect is inconsistent with the results of the two-way ANOVA (Fig. 1), in which some sites showed N fertilization effects, the discrepancy likely arises from lower power associated with the ANCOVAs, which were done using a single decomposition rate for each substrate in each treatment.

#### *Effects of site on decomposition and its response to N fertilization*

Sites varied in ambient N availability, with the Old Fields, Maple, and Aspen sites having the lowest and the Oak and Pine sites the highest IER-N (Table 2), ranking similarly to measurements of IER-N made in 2001 and reported previously (Hobbie 2005; Spearman rank correlation,  $P = 0.03$  and  $P = 0.05$  for soil and litter layer, respectively). Sites also differed in litter layer pH, with the Aspen and Old Field 1 sites having the highest pH and the Pine 2 and Oak sites having the lowest pH (total range of pH among sites less than one pH unit; Table 2). Sites did not rank similarly in litter layer pH (water) measured in 2005 and litter layer pH (CaCl<sub>2</sub>)

measured in 2001 (reported in Hobbie 2005; Spearman rank correlation,  $P = 0.23$ ). Sites also varied in the moisture content of the litter layer; old field sites were drier than forest sites (Hobbie 2005).

Decomposition in control plots (averaged across substrates) was positively related to litter layer moisture ( $P < 0.001$ ,  $R^2 = 0.88$ ) but was unrelated to any other site characteristic. In large part, this relationship was driven by slow decomposition in the two old fields, which had the lowest litter layer moisture. Decomposition was unrelated to litter layer moisture among the forested sites alone ( $P > 0.10$ ). Averaged across substrates within each site,  $k_N - k_C$  was unrelated to any site characteristic (litter moisture, soil or litter pH, or soil or litter IER-N;  $P > 0.10$ ).

#### *Litter nitrogen dynamics*

For a number of site-substrate combinations, N fertilization increased the proportion of initial N throughout decomposition when adjusted for variation in mass remaining, although the effects were generally

TABLE 3. The significance of ANCOVAs of the effect of added N on decomposition ( $k_N - k_C$ ) with site and measures of substrate initial C and nutrient chemistry as covariates.

Covariate in analysis	Significance of covariate	Significance of site	$R^2$
WS	*** (-)	**	0.48
NPE	** (-)	*	0.38
AH	*** (+)	**	0.51
Lignin	ns	ns	...
N	** (-)	*	0.38
P	*** (-)	*	0.46
K	** (-)	*	0.38
Ca	** (-)	*	0.39
Mg	*** (-)	**	0.49

Notes: The effect of added N is the difference between decomposition rate  $k$  of substrates in control ( $k_C$ ) vs. N-fertilized plots ( $k_N$ ). Each covariate was analyzed in a separate ANCOVA. The sign of the relationship between the covariate and  $k_N - k_C$  is indicated in parentheses. Site  $\times$  covariate interactions were not significant in preliminary analyses (not shown). Key to abbreviations: WS, water-soluble fraction; NPE, nonpolar extractive fraction; AH, acid-hydrolyzable fraction.

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ; ns, not significant ( $P > 0.05$ ).

small (Table 1). However, N fertilization had no significant effects on N immobilization by wood, and few significant effects on N immobilization by green leaves. Substrates generally showed distinctive patterns of N dynamics (Appendix B: Table B1; Fig. 3). For example, for *Acer* and *Quercus* green leaves and *Acer*, *Quercus*, and *Pinus* litter, the proportion of initial N correlated positively with the proportion of initial mass remaining such that differences within and among substrates in the proportion of initial N were driven largely by differences in how far along decomposition had progressed (Fig. 3). In addition, these substrates (excepting *Pinus*) exhibited little or no N immobilization. By contrast, *Schizachyrium* litter and the wooden applicators, the substrates with the lowest initial N (and all other nutrient) concentrations (Hobbie 2005), exhibited either no relationship between mass remaining and N immobilization (proportion of initial N) or exhibited a negative correlation between them (opposite of the other substrates). These relationships (or lack thereof)

arose because these substrates exhibited N immobilization during most of their decomposition, and at any level of mass remaining, these substrates varied considerably in the proportion of initial N (Fig. 3), both within and among sites. The relationship between mass remaining and the proportion of initial N remaining broke down in the Old Field sites, where it was positive only for *Acer* and *Quercus* leaves (Appendix B: Table B1).

Because of the close correspondence between mass remaining and the proportion of initial N, maximum N immobilization was correlated with many of the same substrate characteristics as decomposition. For example, maximum N immobilization was correlated negatively with initial substrate N, P, K, Mg, WS, and lignin concentrations and correlated positively with initial substrate AH (Appendix C: Table C1). The relationships with initial N and K were the strongest.

Across sites, maximum N immobilization (averaged across substrates) in control plots was significantly negatively related to average 2001 litter pH in control plots (backward stepwise regression;  $P = 0.05$ ) but was unrelated to 2005 litter pH or to any other site characteristic.

## DISCUSSION

### *Decomposition within and among sites*

For a wide variety of substrates and sites, a single-exponential decomposition model best described decomposition. These results suggest that even after five years of decomposition, most substrates were still decomposing as though comprising a single substrate pool throughout decomposition rather than two substrate pools with distinctive decomposition rates. In the relatively small number of cases when a double-exponential model best described the data (e.g., some cases of *Acer* litter and green leaves of *Quercus*), initial decomposition was quite rapid, perhaps driven by relatively high N concentrations in leaves (Hobbie 2005). Interestingly, an asymptotic model was never the sole best predictor of decomposition and could only be fit to a small number of cases (11% of cases when

TABLE 4. Correlation coefficients from pairwise correlations among concentrations of initial substrate chemical constituents, from Hobbie (2005).

Substrate chemical constituent	WS	NPE	AH	Lignin	N	P	K	Ca	Mg
WS	1.0								
NPE	0.47	1.0							
AH	-0.83*	-0.76*	1.0						
Lignin	-0.30	-0.16	-0.09	1.0					
N	0.61	0.04	-0.53	0.19	1.0				
P	0.90**	0.22	-0.62	-0.36	0.77*	1.0			
K	0.70	0.00	-0.53	0.05	0.98***	0.86*	1.0		
Ca	0.83*	0.50	-0.57	-0.70	0.15	0.71	0.28	1.0	
Mg	0.98***	0.49	-0.79*	-0.39	0.60	0.91**	0.68	0.87*	1.0

Notes: Abbreviations are as in Table 3. Significant coefficients are in bold type.

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ .

TABLE 5. The significance of ANCOVAs of decomposition ( $k$ ) with site, N-fertilization treatment, and their interaction as main effects, and aspects of substrate initial C and nutrient chemistry as covariates.

Covariate in analysis	Effect				$R^2$
	Covariate	Site (S)	N-fertilization (N)	S $\times$ N	
WS	*** (+)	***	ns	ns	0.71
NPE	ns	***	ns	ns	0.27
AH	*** (-)	***	ns	ns	0.45
Lignin	*** (-)	***	ns	ns	0.36
N	*** (+)	***	ns	ns	0.34
P	*** (+)	***	ns	ns	0.84
K	*** (+)	***	ns	ns	0.69
Ca	** (+)	***	ns	ns	0.32
Mg	*** (+)	***	ns	ns	0.77

Notes: Each covariate was analyzed in a separate ANCOVA. The sign of the relationship between the covariate and the decomposition rate is indicated in parentheses. There were no interactions between any covariate and site, fertilization, or their interaction in preliminary analyses (not shown). Abbreviations are as in Table 3.

\*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ; ns, not significant.

decomposition constants were determined for each replicate individually). Thus I found no evidence that decomposition approached a "limit value," or asymptote, in contrast to studies of decomposition of *Pinus sylvestris* and *Picea abies* (Berg and Ekbohm 1991, Berg 2000).

Across substrates, decomposition rates generally were related positively to initial concentrations of water-soluble (WS) C and nutrients and negatively to initial concentrations of lignin and acid-hydrolyzable (AH) C. These results indicate that over five years, both C and nutrient quality were likely important in influencing decomposition. However, the results also highlight the difficulties associated with linking decomposition rates to any single component of substrate chemistry, since many aspects of leaf and litter chemistry and structure tend to be closely related (Wright et al. 2004; W. K. Cornwell et al., *unpublished manuscript*). Across plant taxa, concentrations of different foliar and litter nutrients are tightly correlated because of their associations in biochemical functioning in plants. For example, the nutrient pairs N–P and Ca–Mg are often positively related in leaves (Garten 1976, Reich et al. 1998, Wright et al. 2004). In this study, N additionally was correlated with K, as was P with K and Mg. Furthermore, foliar C constituents also are related closely to one another, to foliar nutrient concentrations, and to decomposition rates (W. K. Cornwell et al., *unpublished manuscript*). For example, here WS was negatively correlated with AH and positively correlated with P, Ca, and Mg.

Among sites, litter moisture was the best predictor of decomposition, as found in other studies (Bryant et al. 1998, Gholz et al. 2000). Decomposition was slowest in the two Old Field sites where the litter layer is exposed and dries out relatively quickly after precipitation events. This lack of moisture is likely exacerbated in the old fields because of poor contact between litter and

the soil surface caused by the presence of standing dead grasses (Dukes and Field 2000).

#### *Nitrogen effects on decomposition within and among sites*

In contrast to the initial year of this experiment when they were largely neutral or slightly positive (Hobbie 2005), N fertilization effects on decomposition were largely neutral or negative after five years of decomposition, reducing decomposition by up to nearly 20% (average of all substrates in Oak 2 site). However, I have no evidence to suggest that inhibitory effects of N addition on lignin-degrading enzyme activity explain the negative effects of N observed here, in contrast to another study in which both phenol oxidase activity and decomposition rates of high-lignin oak litter responded negatively to N addition (Carreiro et al. 2000, Sinsabaugh et al. 2002). First, the effects of N fertilization on decomposition after five years were independent of substrate initial lignin concentrations, a result that was robust across the eight sites. Second, at least in one site, the proportion of initial lignin remaining after four years was unaffected by N fertilization when adjusted for variation in mass remaining. Third, a separate study in the same fertilization experiment studied here found no significant effects of N addition on phenol oxidase or peroxidase activity in the litter layer or soil (B. L. Keeler et al., *unpublished manuscript*). Interestingly, in the aforementioned study of oak decomposition, N inhibition of phenol oxidase activity was not observed for two other litter types (Carreiro et al. 2000, Sinsabaugh et al. 2002). Another study similarly found no significant effect of N addition on phenol oxidase activity in the litter layer of a sugar maple forest (Saiya-Cork et al. 2002). Taken together, these results suggest that reduction of decomposition by N can occur even when lignin degradation per se is unaffected.

So, if N inhibition of ligninolytic enzyme activities cannot explain the negative effects of decomposition in this study, then what mechanisms might be responsible



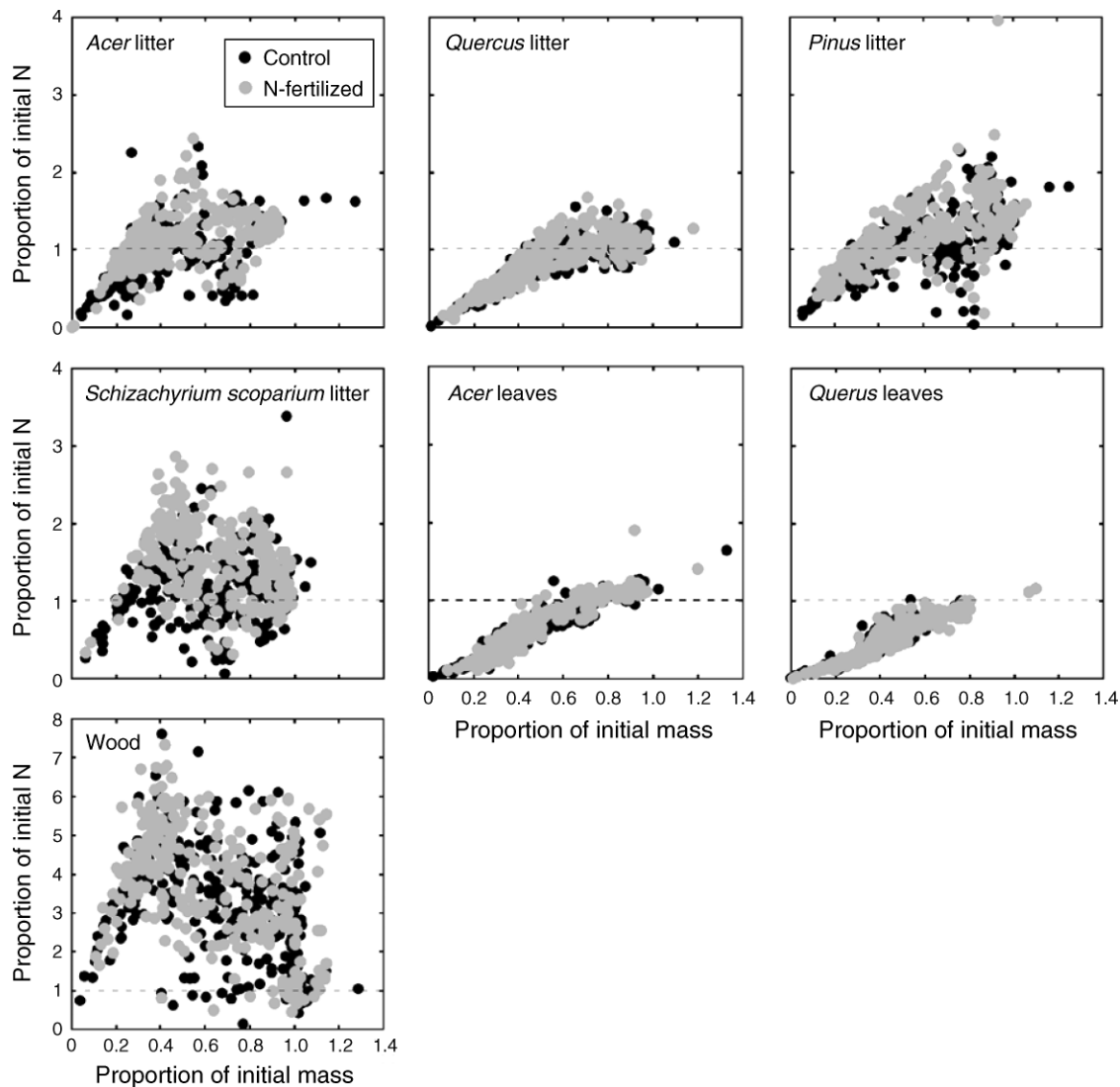


FIG. 3. The proportion of initial N vs. the proportion of initial mass for all replicates, harvests, and sites for each substrate in control and N-fertilized plots. A dashed line indicates the proportion of initial N value of 1.0. Analyses for individual substrate-site combinations are shown in Appendix B: Table B1.

and why do sites vary in the degree to which N reduces decomposition? Across substrates and sites, N effects were more negative for those site-substrate combinations that decomposed more rapidly in unfertilized control plots (i.e., the slope of  $k_N$  vs.  $k_C$  was less than one). In other words, litter that decomposed more rapidly, was in the later stages of decomposition, and had been subject to greater microbial processing exhibited more negative effects of added N. Nitrogen fertilizer effects on decomposition were also related to a number of aspects of substrate chemistry besides lignin. However, interpretation of these relationships is impeded by the tight correlations among different initial litter constituents.

One explanation for N fertilization having more negative effects on decomposition of more decomposed

litter types is that during decomposition, microbial decomposers synthesize phenolic compounds and/or break down litter lignin and other polyphenolic compounds into compounds that react with inorganic N to form additional compounds that are resistant to decomposition (Fog 1988, Davidson et al. 2003). The formation of these resistant compounds would be stimulated by  $\text{NH}_4\text{NO}_3$  addition, and their greater abundance could slow decomposition in N-fertilized plots compared to control plots (Nommik and Vahtras 1982, Stevenson 1994, Berg and Matzner 1997). This interpretation is consistent with previous findings that the rate of decomposition of litter with higher N concentrations or in N-fertilized plots sometimes asymptotes at some lower level of accumulated mass loss ("limit value") than does low-N litter or litter in

unfertilized plots (e.g., Berg and Ekbohm 1991, Berg 2000), even though decomposition in this study generally did not fit an asymptotic model, even in N-fertilized plots.

An alternative explanation for the negative effects of N on decomposition is that N altered the microbial community in ways that impeded its overall degradation ability. Nitrogen addition has been shown to change microbial community composition (Bardgett et al. 1999, Compton et al. 2004, Frey et al. 2004). Indeed, Fog (1988) hypothesized that one explanation for negative effects of N addition on decomposition might result from competitive exclusion of white-rot basidiomycetes by ascomycete cellulose degraders under condition of high N.

My results are largely consistent with those of a meta-analysis of N effects on decomposition (Knorr et al. 2005). The switch from neutral or positive effects to neutral or negative effects of N on decomposition is consistent with negative effects (an 18% reduction) of N in longer studies (>24 months) and positive effects of N in shorter studies (Knorr et al. 2005). However, in contrast to this study, Knorr et al. (2005) found that negative effects of N fertilization on decomposition were prevalent for substrates with relatively high initial lignin concentrations, even though the range of initial lignin concentrations was similar between this study and the meta-analysis. However, the average reduction in decomposition rate for high-lignin litter caused by added N (5%) was small relative to the reduction in decomposition caused by N in long-term (>24 months) studies. Assessing whether N effects on decomposition generally become independent of initial lignin concentrations in long-term studies is difficult because only two studies reviewed by Knorr et al. (2005) lasted as long as the present study. One study lasted six years (Berg and Tamm 1991) but measured only one species. Another (Magill and Aber 1998) found negative effects of N fertilization after six years that, as in this study, were independent of initial litter lignin concentrations (regression of effect of added N vs. initial litter lignin, using the high N and control treatments only;  $P = 0.25$ ).

#### *Litter nitrogen dynamics*

Nitrogen immobilization likely was enhanced by both N supply and demand for N, as N fertilization generally stimulated N immobilization (when adjusted for variation in mass remaining), and substrates with the lowest concentrations of N (as well as other nutrients) immobilized the most N, both because these substrates decomposed most slowly and because they exhibited greater immobilization at any given level of mass loss. Numerous other studies have also shown that added N stimulates N immobilization (e.g., Hunt et al. 1988, Berg and Tamm 1994, Hobbie and Vitousek 2000). Given the evidence that N may be reacting with decomposition breakdown products via abiotic reactions, it is unclear

how much of N immobilization is occurring through microbial uptake vs. abiotic reactions.

Although N fertilization stimulated N immobilization by some substrates, the effects were small relative to differences among substrates. Greater N immobilization by substrates with lower initial percent N in this study is consistent with results of a 10-yr cross-site study (Parton et al. 2007). Interestingly, there were large differences among substrates, not just in the magnitude of immobilization, but in variation among substrates, apparently driven by site-substrate interactions. For example, substrates exhibiting substantial N immobilization also exhibited large variation in N immobilization at any given level of mass remaining. As in the Parton et al. (2007) study, within sites, immobilizing substrates exhibited considerable variation in immobilization, particularly at higher values of mass remaining. In addition, sites exhibited different relationships between mass N remaining: in particular, the old fields often exhibited negative relationships in contrast to the positive relationships exhibited in other sites. These results could arise from variation in the degree, timing, or effectiveness of fungal connections between decomposing litter and soil among substrates and sites, with particularly slow colonization and lengthy periods of immobilization for low-N substrates in the drier old fields.

#### *Conclusions*

The results presented here show that for litter approaching the later stages of decomposition, high inputs of N had mainly neutral or negative effects on decomposition, slowing decomposition for site-substrate combinations with the most rapid decomposition, regardless of initial litter lignin concentrations. The contrast between results of this and prior studies (Carreiro et al. 2000) suggests that multiple potential mechanisms of negative effects of N on decomposition (biotic and abiotic) may operate among different ecosystems, and further research is necessary to elucidate those mechanisms and to predict when and where particular mechanisms are important. The negative effects of N on decomposition were not apparent in the first year (Hobbie 2005) or at all of the sites studied, underscoring the importance of long-term studies in multiple sites for understanding N effects on decomposition.

These results suggest that effects of atmospheric N deposition on decomposition likely will contribute to neutral or positive effects of N deposition on ecosystem C sequestration. If atmospheric N deposition (rates of which are lower than rates of fertilizer added here) slow forest floor decomposition rates, forest floor accumulation and net ecosystem production should be stimulated, given no change in litter inputs. In sites where N stimulates litter production, C storage would be further stimulated, unless higher litter N concentrations in plants subject to relatively high N deposition have

offsetting stimulatory effects on decomposition (Berg and Matzner 1997). However, synthesis of data from the northeastern United States indicates no relationship between N deposition and foliar (and presumably litter) N concentrations (Aber et al. 2003). Thus such offsetting effects on long-term decomposition may not occur. While effects of N deposition on productivity have been considered in ecosystem models (e.g., Townsend et al. 1996), to my knowledge, its effects on decomposition have not. However, the results presented here suggest that such effects should also be considered when making predictions about effects of N deposition on ecosystem C dynamics. Indeed, when variation in disturbance is accounted for, net ecosystem production is strongly positively correlated with wet N deposition in temperate and boreal forests (Magnani et al. 2007), but whether slower decomposition is associated with greater N deposition and contributes to that pattern is unknown.

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#### APPENDIX A

A table showing decomposition constants from single-exponential decomposition models fit for all replicates of each site-treatment-substrate combination (*Ecological Archives* E089-148-A1).

#### APPENDIX B

A table showing results of ANCOVAs for each site-substrate combination of the proportion of initial N at all harvests with N fertilization as a main effect and the proportion of initial mass remaining as a covariate (*Ecological Archives* E089-148-A2).

#### APPENDIX C

A table showing the significance of ANCOVAs of maximum N immobilization by decomposing substrates with site, N-fertilization treatment, and their interaction as main effects and measures of substrate initial carbon and nutrient chemistry as covariates (*Ecological Archives* E089-148-A3).