Title

Interactions among lignin, cellulose, and nitrogen drive litter chemistry–decay relationships

Journal

Ecology, 93(2)

ISSN

0012-9658

Authors

Talbot, Jennifer M

Treseder, Kathleen K

Publication Date

2012-02-01

DOI

10.1890/11-0843.1

Ecology, 93(2), 2012, pp. 345–354

Interactions among lignin, cellulose, and nitrogen drive litter chemistry–decay relationships

Jennifer M. Talbot[1] and Kathleen K. Treseder

Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92617-2525 USA

Abstract. Litter decay rates often correlate with the initial ratios of lignin : nitrogen (N) or lignin : cellulose in litter. However, the chemical and microbial mechanisms that give rise to these patterns are still unclear. To identify these mechanisms, we studied the decomposition of a model plant system, *Arabidopsis thaliana*, in which plants were manipulated to have low levels of lignin, cellulose, or litter N. Nitrogen fertilizer often increases the loss of cellulose, but it suppresses the breakdown of lignin in plant litter. To understand the mechanisms driving these patterns, we decomposed plants in litterbags for one year in control and N-fertilized plots in an Alaskan boreal forest. We found that litter N had a positive effect on total mass loss because it increased the loss of lignin, N, and soluble C. Lignin had a negative effect on rates of total litter mass loss due to decreases in the loss of cellulose and hemicellulose. Cellulose had a positive effect on lignin loss, supporting the concept of a "priming effect" for lignin breakdown. However, the low-cellulose plants also lost more of their original cellulose compared to the other plant types, indicating that decomposers mined the litter for cellulose despite the presence of lignin.

Low-lignin litter had higher fungal biomass and N-acetyl glucosaminidase (NAG, a chitinase) activity, suggesting that lignin restricted fungal growth and may have influenced competitive interactions between decomposers. Nitrogen fertilization increased NAG activity in the early stages of decay. In the later stages, N fertilization led to increased cellulase activity on the litters and tended to reduce lignin losses. The transition over time from competition among decomposers to high cellulase activity and suppressed lignin loss under N fertilization suggests that, in N-limited systems, N fertilization may alter decomposer community structure by favoring a shift toward cellulose- and mineral-N users.

Key words: Arabidopsis thaliana; *cellulose; decomposition; fungi; lignin; litter decay rates; nitrogen.*

Introduction

One of the most well-established patterns in ecosystem ecology is that litter decay rates are correlated with the initial ratios of C:N, lignin : N, or lignin : cellulose in litter (e.g., Melillo et al. 1982, Aerts 1997, Hobbie 2008). These chemical traits are strong predictors of litter decay, accounting for over 73% of the variation in litter decomposition rates worldwide (Zhang et al. 2008). However, the chemical and microbial mechanisms that give rise to these relationships are still unclear. Are these correlations a product of physical or chemical interactions among lignin, cellulose, and N-containing compounds; shifts in resource allocation by decomposers; changes in the community composition of decomposers; or a combination of these factors? It is rare that we identify factors that consistently predict ecological processes across a diversity of ecosystems; the C:N and lignin : N ratios are two of them. Identifying the mechanisms by which these factors control decay is a critical step towards explaining a large portion of variation in energy and nutrient flow—both within and among ecosystems—across the globe.

Many hypotheses have been invoked to explain lignin, cellulose, and N effects on litter decay rates. For example, litter N is hypothesized to control rates of decay by alleviating N limitation of litter C degradation (Berg and Staaf 1980). Lignin is hypothesized to protect labile litter components from microbial attack because lignin resists degradation and surrounds cellulose, hemicellulose, and protein in plant cell walls (Berg and McClaugherty 2003). While cellulose can be used as a sole C source by decomposers (primarily fungi; Waksman and Starkey 1924), lignin generally is not, because the C return on investing in lignin-degrading enzymes is hypothesized to be low or negative (Kirk and Farrell 1987). Therefore, the release of labile compounds from protection is often assumed to drive lignin breakdown by decomposers. This idea forms the basis for representing lignin dynamics in conceptual models (Berg and Matzner 1997) and predictive models of litter decay (Moorhead and Sinsabaugh 2006). It is challenging to test these hypotheses, because this requires that we isolate the effects of lignin, cellulose, and N on the decay of individual litter chemicals. As a result, our current conceptual model of litter chemistry controls over

Manuscript received 10 May 2011; revised 17 August 2011; accepted 23 August 2011. Corresponding Editor: S. D. Frey.
[1] E-mail: jmtalbot@umn.edu

decomposition (Berg and Staaf 1980) has remained unchallenged for over three decades.

Nitrogen fertilization has contrasting effects on cellulose and lignin loss in decomposing litter. Nitrogen fertilization often stimulates decomposition of "high quality" (i.e., low-lignin) litter (Hobbie 2000, Knorr et al. 2005) and increases cellulase activity by decomposer microbes (e.g., Fog 1988, Carreiro et al. 2000, Keeler et al. 2009), but tends to decrease the activity of lignolytic enzymes (e.g., Fog 1988, DeForest et al. 2004, Frey et al. 2004) and inhibit decomposition of "low quality" (i.e., high-lignin) litter (Knorr et al. 2005). It is unclear why N fertilization often slows lignin degradation during litter decay. One hypothesis is that N fertilization causes a shift in decomposer-community composition, where microbes with high N-assimilation efficiency and cellulolytic capability outcompete lignin decomposers (Couteaux et al. 1995). Another possibility is that lignin degradation is a mechanism of releasing N from the cell wall, so that microbes break down lignin to access protected N (i.e., "microbial N mining"; Craine et al. 2007). If N is readily available in mineral forms, there is less incentive for lignin degraders to invest resources in producing lignolytic enzymes. Finally, N fertilization may induce "browning" (i.e., production of melanoidins and other brown, recalcitrant compounds) of plant material, leading to the accumulation of compounds that are toxic to lignin-degrading microbes (Fog 1988).

Model plant systems provide the opportunity to identify the mechanistic effects of lignin, cellulose, and N on litter decomposition. In these systems, the concentrations of lignin, cellulose, or N in plant tissues can be altered without effects on other characteristics of the litter. Many laboratory studies have used genetically or chemically modified plants to examine lignin effects on litter degradation in the rumen (e.g., Grabber et al. 2009) or in soils (e.g., Hopkins et al. 2001, Webster et al. 2005, Yanni et al. 2011). These studies have found that plants with reduced levels of lignin consistently have faster degradation rates, and there is evidence that chemical protection of labile litter components is an important mechanism of lignin control over decomposition (Grabber et al. 2009, Talbot et al., *in press*). Understanding the importance of this control relative to others may provide insight into why lignin : N or lignin : cellulose ratios in litter consistently correlate with decomposition rates in ecosystems across the globe.

To identify the suite of mechanisms by which lignin, cellulose, and N control litter decay, we studied the decomposition of *Arabidopsis thaliana* mutants that were manipulated to vary in concentrations of lignin, cellulose, and N. Our study is the first to isolate the effects of each of these components, independently of other controls, on decomposition dynamics in a natural ecosystem. We used three *Arabidopsis* plant types: a low-lignin mutant, a low-cellulose mutant, and a wild-type plant. Each plant was grown to have high or low amounts of litter N. Plants were used in two different litterbag decomposition experiments, one designed to test the mechanisms of lignin, cellulose, and litter N effects on decomposition, and a second to test the effects of litter N compared to N fertilizer over time. We had several hypotheses of how the interactions between litter chemicals would control decay rates:

Hypothesis 1) Litter N should alleviate N limitation of decomposer activity. Therefore, we expected the low N plant to have low percentage mass loss due to lower losses of all litter C sources.

Hypothesis 2) Cellulose should alleviate C limitation of decomposer activity. We predicted that the low-cellulose plant would have lower total percentage mass loss due to lower losses of lignin, cellulose, and N during decay.

Hypothesis 3) Plants with low lignin would offer less protection of cell-wall polysaccharides and protein. Therefore, we expected that the low-lignin plant would have higher percentage mass loss during decomposition due to greater losses of cellulose, hemicellulose, and N.

Hypothesis 4) Adding fertilizer N should increase cellulose losses by alleviating any N limitation of decomposition and should reduce lignin losses by eliminating the need for microbes to access litter N. We also expected an interaction between N fertilization and *Arabidopsis* plant type, such that the low-cellulose plant decomposed slower under N fertilization, but the low-lignin plant decomposed faster due to more easily available C sources.

Methods

Study system: Arabidopsis thaliana

We examined the role of lignin, cellulose, and N in controlling litter decay rates using *Arabidopsis thaliana* mutants that were manipulated to vary in the concentration of each of these chemical components. To isolate lignin and cellulose effects on decay, we used two mutant *Arabidopsis* plants that had low levels of either cellulose or lignin in their tissue. The low-lignin plant had a single nucleotide polymorphism in the gene AT1G15950.1, which codes for cinnamoyl-CoA reductase (CCR) (Jones et al. 2001), and the low-cellulose plant was deficient in *AtCesA7*, which codes for cellulose synthase catalytic subunit 7 (Turner and Somerville 1997). Both plants were derived from the Landsberg erectica ecotype. To examine the effects of litter N on decomposition, we grew *Arabidopsis* plants to have one of two N levels in stem tissue by watering the plants with either high-N (15 mmol KNO_3/L) fertilizer or low-N (3 mmol/L KNO_3) fertilizer.

Arabidopsis plants were germinated from seed according to the procedure in Talbot et al. (*in press*). Every seven days, pots were watered with a solution containing calcium chloride (0.5 mmol/L), magnesium sulfate (0.25 mmol/L), sodium phosphate (0.25 mmol/L), and potassium nitrate (15 mmol/L). Low-N plants were grown with 3 mmol/L potassium nitrate. At the seven-week

harvest date, stem tissue was isolated from each plant and dried at 60°C for 48 h. All stems of a plant type were pooled and subsamples were taken for litterbag construction and initial chemical analyses.

Study site

Litterbag decomposition experiments using the *Arabidopsis* plants were conducted in a mature black spruce (*Picea mariana* (P. Mill.) B.S.P) forest in an upland boreal ecosystem in central Alaska, USA (Treseder et al. 2007). This site was chosen because of the critical need to understand chemical controls over decomposition rates in boreal systems (Talbot et al., *in press*). Soils at this site are gelisols and have an O-horizon thickness of 9.8 cm with a soil pH of 4.9. The vegetation understory is dominated by mosses, lichens, ericoid shrubs, and ectomycorrhizal shrubs. The growing season begins with bud break in mid-May and extends until leaf senescence in mid-September. The local climate is cold and dry, with a mean annual temperature of 2°C and a precipitation rate of 303 mm/yr (Treseder et al. 2007).

Lignin, cellulose, and litter-N effects on decomposition: Litterbag experiment 1

To test for the direct effects of lignin, cellulose, and litter N on decay rates, we studied the decomposition of four plant types: the wild-type *Arabidopsis* (Columbia ecotype), the low-lignin mutant, the low-cellulose mutant, and the wild-type *Arabidopsis* with low N content. Plants were grown from February to April 2008. Approximately 1000 plants were cultivated to maturity (~250 individuals/plant type) and stem tissue was isolated and dried as described above.

Whole stems from each plant type were placed in 10 × 10 cm double-layered 1 mm mesh litterbags constructed from a layer of nylon mesh inside a layer of fiberglass mesh. One gram of stem tissue from a given *Arabidopsis* plant type was placed into a litterbag. Litterbags were sterilized overnight via gamma-irradiation (Talbot et al., *in press*). Replicate bags of each plant type were affixed to the top of the forest floor in each of four plots, spaced 10 cm apart, resulting in a total of 16 litterbags deployed. All litterbags were deployed at the site in July 2008 and retrieved in July 2009. The retrieved litterbags were placed on ice and transported to the University of California at Irvine campus, where a portion of each bag was either frozen at −80°C or freeze-dried within 48 h.

Litter-N and fertilizer-N effects over time: Litterbag experiment 2

To test for effects of litter N and N fertilization on decay, we conducted a second experiment in which we manipulated the litter N content of each *Arabidopsis* mutant, as well as the mineral-N content of soil. To manipulate litter N, we grew two batches of *Arabidopsis* plants; one with low-N fertilizer to produce low-N litter, and another with high-N fertilizer to produce high-N litter. Each batch included the wild-type *Arabidopsis* (L. erectica ecotype), the low-cellulose mutant, and the low-lignin mutant, for a total of six different litter types. Plants were grown from January to June 2009 in seven-week batches. In total, ~3000 plants were cultivated to maturity as described above (~500 individuals/plant type). In this experiment, we focused on the decomposition of cell-wall components by pre-extracting stems with warm deionized water prior to placing into litterbags. Litterbags were sterilized by gamma irradiation and placed in either N-fertilized or unaltered (control) plots at the site.

Nitrogen-fertilized plots were established at the site in May of 2009. N fertilizer was applied as ammonium nitrate to replicate plots in May 2009 at a rate of 200 kg N/ha, followed by an application in May 2010 at a rate of 100 kg N/ha. Each N-fertilized plot was paired with a control plot. Plots were 1.5 × 1.5 m, and paired plots were separated by at least 8 m to avoid leaching of fertilizer N into control plots. Litterbags of each plant type and litter N level were placed in each plot. To examine treatment effects at different stages of decay, sets of litterbags were harvested at two different time points, after two months of decay (to represent an early stage of decay) or after one year of decay (to represent a mid-to-late stage of decay). After two months, sets of the low-lignin and wild-type *Arabidopsis* litterbags were collected. After one year, litterbags of all six plant types were collected. The design was replicated 3 times, resulting in a total of 58 litterbags placed into the field in July 2009.

Chemical analyses of initial and decomposed litters

Initial and decomposed stem tissue samples from Experiments 1 and 2 were processed for total C and N, soluble C, hemicellulose, cellulose, and lignin content. Freeze-dried samples were ground on a Spex SamplePrep 8000D mixer/mill (Spex SamplePrep, Metuchin, New Jersey, USA) using stainless steel vials and grinding media. Total C and N were determined by combustion on an elemental analyzer (Flash EA 1112; Thermo Scientific, Waltham, Massachusetts, USA). Samples were then fractionated following the International Association of Analytical Communities (AOAC) official Uppsala method (Theander et al. 1995). Concentrations of lignin, cellulose, and hemicelluloses in the cell walls were analyzed by standard spectrophotometric methods. Total lignin content was determined by the acetyl bromide method (Iiyama and Wallis 1990). Total cellulose and hemicellulose content of cell walls was determined by the Updegraf method (Updegraf 1969). Soluble C was calculated as total C content of stem tissue minus C content of the cell-wall fraction. For initial samples, results are the means of three subsamples of stems from each plant type. For final samples, results are the means of 3–4 replicate litterbags for each plant type.

Extracellular enzymes

We assayed the potential activities of six extracellular enzymes involved in litter C and nutrient cycling:

TABLE 1. Initial litter-chemistry traits of *Arabidopsis* plants used in Experiment 1.

Plant type	Cellulose (μg/mg stem)	Lignin (μg/mg stem)	Hemicellulose (μg/mg stem)	Soluble C (μg/mg stem)	N (μg/mg stem)
Wild-type control	242.40[a] (12.8)	79.72[a] (6.54)	83.24[a] (1.05)	170.24[b] (12.51)	29.27[a] (1.40)
Low cellulose	79.09[b] (6.93)	84.37[a] (3.77)	98.66[a] (6.93)	253.20[a] (6.89)	27.63[a] (0.52)
Low lignin	193.89[a] (16.12)	54.60[b] (1.38)	94.36[a] (4.36)	205.89[b] (7.94)	31.52[a] (0.87)
Low N	241.72[a] (19.89)	93.46[a] (5.24)	96.73[a] (5.52)	208.25[b] (12.51)	14.04[b] (0.58)

Notes: Data are means with SE in parentheses. Significant differences ($P < 0.05$) among plant types are designated by different lowercase letters superscripted on the means (from Tukey's multiple comparisons test; $n = 3$ replicate litterbags for each plant type.

cellobiohydrolase (CBH, an exocellulase), β-glucosidase (BG, which hydrolyzes cellobiose into glucose), β-xylosidase (BX, which degrades the xylose component of hemicellulose), polyphenol oxidase (PPO, which oxidizes phenols) and peroxidase (PER, including oxidases that degrade lignin), N-acetyl-glucosaminidase (NAG, which breaks down chitin), and leucine-amino-peptidase (LAP, which breaks down polypeptides). Potential enzyme activities were assayed on a subsample of each litterbag that was stored frozen at −80°C after litterbag harvest. Samples were processed within six months using either a fluorometric or colorimetric procedure on a microplate reader following Allison et al. (2008).

Hyphal length

Lengths of fungal hyphae were determined on stained litter samples using the gridline intersect method following McGonigle et al. (1990). Whole litter samples were thawed and cleared with 10% potassium hydroxide for 48 h and 1% HCl for 24 h. Samples were then stained with acid fuchsin (0.1% in lactoglycerol) for 24 h and stored in destain (14:1:1 lactic acid : glycerol : water). Approximately 20 pieces of litter (each 1–2 cm in length) in each sample were mounted on glass slides with polyvinyl lactic acid (PVLG) and covered with a glass coverslip. Litter was examined for the presence of fungal hyphae at 200× magnification using a Nikon phase-contrast microscope (Nikon Eclipse e400; AG Heinze, Lake Forest, California, USA).

Data analysis

In Experiment 1, we tested for differences in initial litter chemistry among plant types by conducting ANOVA with plant type as a fixed effect (wild-type plant, low- lignin plant, low-cellulose plant, and low-N wild-type plant) and the chemical components of stem tissue as dependent variables (concentrations of lignin, cellulose, hemicellulose, soluble C, and total N). To test for plant-type effects on decay dynamics, we conducted one-way ANOVA with plant type as main effect and percentage total litter mass loss, percentage loss of litter chemicals, hyphal length, and extracellular enzyme activities as the dependent variables.

Data analysis for Experiment 2 was similar to that for Experiment 1. To test for differences in initial litter chemistry among plants, we conducted two-way ANOVA with plant type (low-lignin plant, low-cellulose plant, wild-type plant) and litter N level (high litter N, low litter N) as fixed effects and the chemical components of stem tissue as dependent variables. To test for litter-chemistry and N-fertilization effects on decay dynamics, we conducted three-way ANOVA with plant type, litter N level, and N-fertilization treatment (control vs. N fertilized) and their interactions as main effects and percentage total litter mass loss, percentage loss of litter chemicals, hyphal length, and extracellular enzyme activities as the dependent variables. All percentage mass-loss and hyphal-length data met assumptions of normality and homogeneity of variance. In cases where enzyme-activity data did not meet these assumptions, data were log-transformed prior to analysis. Comparisons among means were analyzed by Tukey HSD post hoc contrasts. All data were analyzed using JMP statistical software (JMP version 8.0; SAS Institute 2009).

RESULTS

Experiment 1: Lignin, cellulose, and N controls

In Experiment 1, modified *Arabidopsis thaliana* plants differed from the wild type in concentrations of lignin, cellulose, or N, with little variation in other traits. There was a significant effect of plant type on total N content ($P < 0.0001$), such that the low-N plant had 52% less N than the high-N wild type. There was also a significant effect of plant type on cellulose concentration ($P = 0.0001$), in which the low-cellulose plant had 67% less cellulose compared to the wild type. Finally, there was a significant effect of plant type on lignin concentration ($P = 0.0020$), such that the low-lignin plant had 32% less lignin relative to the wild-type plant (Table 1). These chemical differences resulted in plant type effects on initial C:N, lignin : N, and LCI of stem tissue (Table 1). Plant types did not differ in hemicellulose content ($P = 0.2037$), but the low-cellulose plant had significantly higher levels of soluble C in stem tissue compared to the other plant types ($P = 0.0021$).

After a year of decomposition at the field site, there was an average of 57% total litter-mass loss across all the *Arabidopsis* plants. Percentage mass loss was significantly different among the plant types ($P = 0.0005$), due to differences in percentage loss of lignin

TABLE 1. Extended.

C:N	Lignin : N	Lignocellulose index, LCI (lignin/[lignin + cellulose])
12.47[b] (0.79)	2.76[b] (0.36)	0.21[bc] (0.015)
13.87[b] (0.32)	3.06[b] (0.17)	0.37[a] (0.007)
11.65[b] (0.32)	1.73[c] (0.04)	0.17[c] (0.007)
29.64[a] (1.29)	6.65[a] (0.21)	0.24[b] (0.018)

($P < 0.0001$), cellulose ($P < 0.0001$), hemicellulose ($P = 0.0040$), soluble C ($P = 0.0013$), and total N ($P < 0.0001$). The low-N plant retained more mass after one year of decay than the wild type with higher N due to lower percentage loss of lignin, soluble C, and N (Fig. 1). However, litter N did not have a significant effect on percentage hemicellulose loss or percentage cellulose loss. The low-cellulose plant had similar percentage total mass loss compared to the wild type, but differed from the wild type in litter chemistry dynamics during decay. The low-cellulose plant lost no lignin and less soluble C relative to the wild type, but lost a significantly higher percentage of its original cellulose (Fig. 1). Like the low-cellulose plant, the low-lignin plant had a percentage total mass loss that was similar to the wild type. However, the low-lignin plant lost significantly more mass than the low-N plant and the low-cellulose plant (Fig. 1) and lost more cellulose and hemicellulose relative to all other plant types. This plant also had higher fungal activity relative to the other plant types; fungal biomass was significantly greater in the low-lignin plant ($P = 0.0022$), as was N-acetyl-glucosaminidase (NAG) activity ($P < 0.0001$) (Fig. 2). The low-lignin plant also had significantly higher activity of CBH (cellohydrolase) and BG (β-glucosidse) relative to the low-N plant, and lower activity of PPO+PER (polyphenoloxidase and peroxidase) compared to other plant types (Appendix A).

Experiment 2: Litter-N and fertilizer-N effects over time

Plants used in Experiment 2 had differences in litter chemistry that were similar to differences between plants used in Experiment 1 (Appendix B). However, there was a significant effect of *Arabidopsis* plant type on litter N content ($P < 0.0001$), such that the low-lignin and low-cellulose plants accumulated more N in their tissues relative to the wild-type plants (Appendix B).

After two months of decay, there was an average of 9% total litter-mass loss across all plant types. During this early stage of decomposition, percentage mass loss was controlled by N fertilizer; litter decomposed

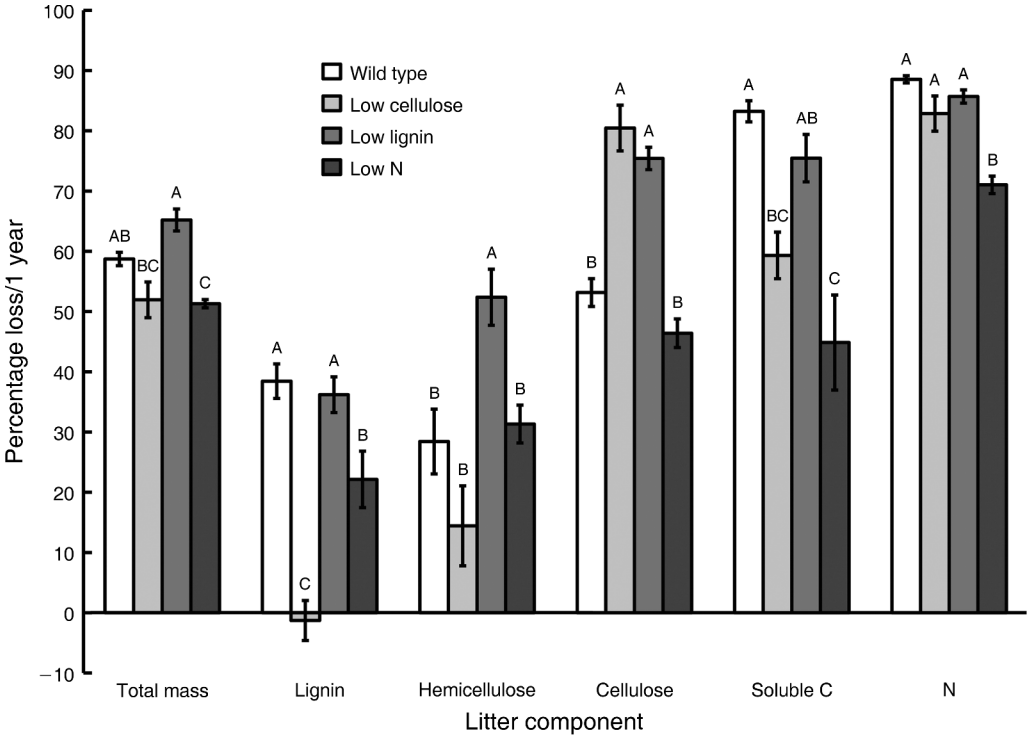

FIG. 1. Percentage loss of total mass, lignin, hemicelluloses, cellulose, soluble C, and N in stem tissue from different *Arabidopsis thaliana* plant types in Experiment 1. Plants were decomposed in litterbags for one year in an Alaskan boreal forest. A negative value indicates accumulation of decomposer metabolites during decay. Data are means ± SE, with $n = 3$–4 replicate litterbags for each plant type. Different uppercase letters above the bars represent significant differences from Tukey's multiple comparisons test among plant types ($P < 0.05$).

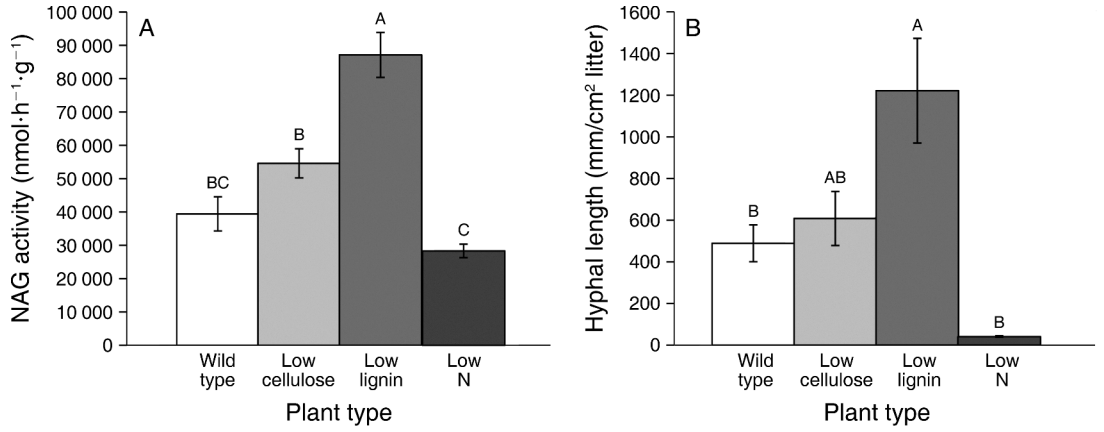

FIG. 2. (A) N-acetyl glucosaminidase (NAG) activity and (B) fungal biomass (i.e., hyphal length) in *Arabidopsis* plants decomposed for one year in Experiment 1. Data are means ± SE, with $n = 3$–4 replicate litterbags for each plant type. Different uppercase letters above the bars represent significant differences from Tukey's multiple comparisons test among plant types ($P < 0.05$).

significantly faster in N-fertilized plots than in control plots ($P = 0.0244$; Fig. 3). Higher rates of litter decay under N fertilization were due to increased losses of cellulose compared to litter decomposed in control plots ($P = 0.0153$). Plants decomposed in N-fertilized plots also had higher NAG activity compared to plants decomposed in control plots ($P = 0.0307$) but did not have higher fungal biomass (data not shown; $P = 0.8004$). At this stage of decay, plant type, litter N level, N fertilization, and their interactions did not have significant effects on fungal biomass, any other extracellular enzymes, or the loss of any other litter chemical.

After one year of decay, there was an average of 43% total litter-mass loss across plant types. At this stage, controls over decay rate switched from N fertilizer to litter N and lignin content. Similar to Experiment 1, plant types differed significantly in percentage mass loss ($P < 0.0001$), and the percentage loss of cellulose ($P < 0.0001$), hemicellulose ($P = 0.0077$), lignin ($P = 0.0005$), soluble C ($P < 0.0001$), and N ($P = 0.0064$). The low-lignin plants with higher litter N decomposed faster than the other plant types due to larger losses of hemicellulose, cellulose, soluble C, and N (Appendix C). These plants also had significantly higher fungal biomass, activities of NAG, BG, CBH, and BX, but lower levels of PPO+PER, relative to the wild type (Appendix D). The low-cellulose plant lost more of its original cellulose but less lignin than the wild type (Appendix C).

After one year, there was no effect of N fertilization on percentage mass loss or the loss of cellulose, hemicellulose, soluble C, or N. However, at this stage of decay, N fertilization increased the activity of the polysaccharide-degrading enzymes CBH ($P = 0.0006$) and BX ($P = 0.0004$), as well as the activity of BG ($P = 0.0002$), without any affect on NAG activity or fungal biomass (Appendix D). Nitrogen fertilization also tended to reduce % lignin loss across all plant types ($P = 0.0528$).

DISCUSSION

Lignin, cellulose, and N are frequently associated with rates of litter decay in ecosystems around the world (Zhang et al. 2008, Prescott 2010). To identify the mechanisms that give rise to these associations, we directly manipulated lignin, cellulose, and N concentrations in litter using the model system *Arabidopsis thaliana*. In some cases, our original hypotheses about these mechanisms were supported. Litter N increased total mass loss by accelerating the loss of lignin and soluble C (Fig. 1), supporting the hypothesis that litter N induces C limitation of decomposer activity (Hypothesis 1). In addition, cellulose increased lignin loss (Fig. 1), which supports the hypothesis that cellulose alleviates C limitation of lignin degradation (Hypothesis 2). Lignin reduced total litter-mass loss during the later stages of decay by slowing the loss of hemicellulose and cellulose (Fig. 1). This supports the hypothesis that lignin protects cell-wall polysaccharides from degradation (Hypothesis 3).

Nevertheless, some aspects of our original hypotheses were not supported. Lignin did not reduce N loss from decomposing litter as expected (Fig. 1), implying that lignin did not protect N-rich litter compounds in the cell wall from degradation. In addition, the low-cellulose plant lost N at a rate similar to the wild-type plant, suggesting that cellulose did not induce decomposers to immobilize litter N during decay. These results have led us to develop a new model of lignin, cellulose, and N controls over decomposition for our system, which may explain patterns of litter decay observed in other systems. We developed this model by considering the evidence in support or in contrast to each prediction generated from our original hypotheses, as described below.

Hypothesis 1: Litter N controls over decay

Lower losses of soluble C in low-N plants support the hypothesis that litter N alleviates N limitation of labile-

C degradation by decomposer microbes (Berg and Staaf 1980). This mechanism may explain why rates of litter-mass loss often correlate positively with absolute N concentrations in the litter during the early stages of decay (Enriquez et al. 1993). However, we found that microbial degradation of structural carbohydrates was insensitive to initial litter N; low-N plants lost cellulose and hemicellulose at a rate similar to the high-N wild type (Fig. 1). This was surprising, given that hemicellulose and cellulose decomposition require degradation by extracellular enzymes prior to uptake, and so likely have higher N requirements compared to soluble-C metabolism. The insensitivity of cellulose and hemicellulose breakdown to litter N suggests that either (1) decomposers did not use litter N to break down structural polysaccharides, (2) the decomposer community shifted in response to litter N level (e.g., fungal : bacterial ratio decreased), or (3) decomposers adjusted the efficiency with which they invested litter N in these processes (N-use efficiency). By contrast, litter N had a positive effect on lignin degradation (Fig. 1). Lignin is depolymerized by nonspecific oxidative mechanisms (Baldrian 2006), which makes lignin breakdown less efficient than carbohydrate decomposition. Therefore, one possibility is that decomposers invested N resources more conservatively in lignin degradation than in carbohydrate degradation.

Litter N also significantly affected rates of N loss from litter; low-N plants lost less N compared to high-N plants (Fig. 1). This phenomenon is hypothesized to occur as a consequence of high microbial N demands during initial stages of litter decomposition (Berg 1986) when microbes must invest in N-rich proteins like extracellular enzymes and uptake transporters. High-N substrates meet these demands more sufficiently than low-N substrates, causing a net release of N as decomposition progresses. Our study is the first to provide evidence of this mechanism by isolating the effect of litter N on N loss independently of other controls.

Hypothesis 2: Cellulose controls over decay

Cellulose was not a major control over total litter decay rates. The low-cellulose plant had 67% less cellulose compared to the wild-type plant, but had rates of total mass loss that were similar to the wild type (Fig. 1). However, cellulose was an important control over litter chemistry dynamics. In both experiments, there was little to no lignin degradation in the low-cellulose plant, supporting the concept that cellulose "primes" lignin decomposition due to low C return on producing lignolytic enzymes (Kuzyakov et al. 2000). These results also support the hypothesis that lignin is not used as a C source by decomposers. Nevertheless, the low-cellulose plants lost 80–90% of their original cellulose after one year of decay, compared to 50–60% in the wild type (Fig. 1). This response occurred despite the presence of other C forms in litter (hemicellulose and soluble C),

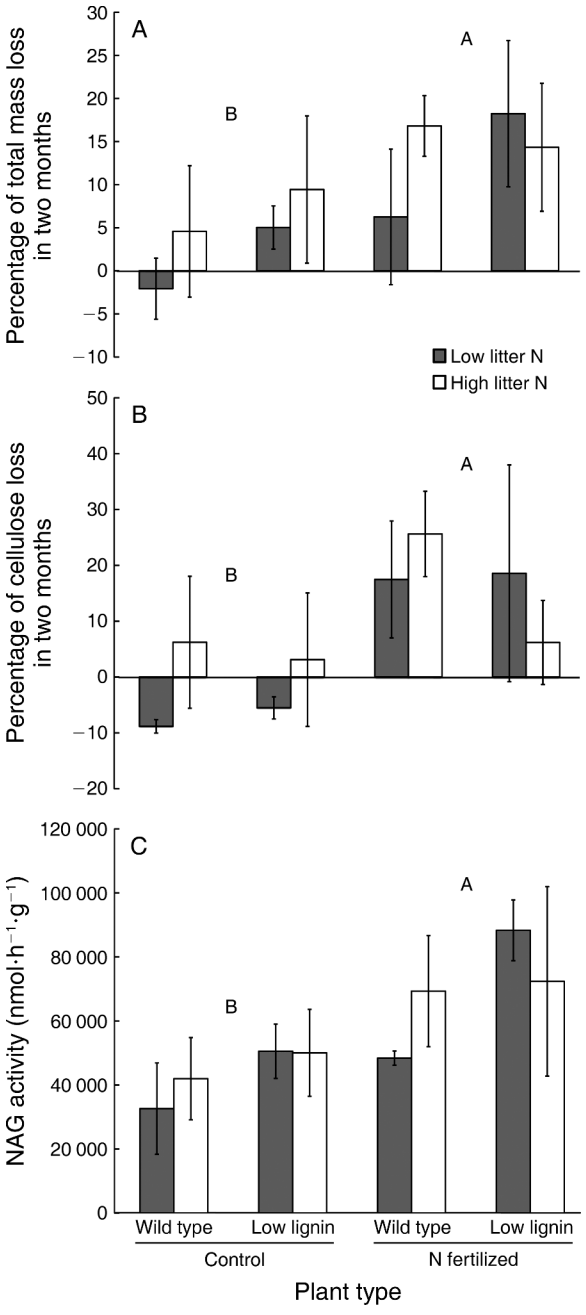

FIG. 3. (A) Percentage total mass loss, (B) percentage cellulose loss, and (C) N-acetyl glucosaminidase (NAG) activity in stem tissue from wild-type and low-lignin *Arabidopsis thaliana* plant types decomposed for two months in control and N-fertilized plots (Experiment 2). Negative values indicate accumulation of decomposer biomass and metabolites during decay. Data are means ± SE among litterbags, $n = 3$ replicate litterbags for each plant type. Different uppercase letters above the bars represent significant differences from Tukey's multiple comparisons test between control and N-fertilized plots ($P < 0.05$).

which were not degraded to the same level as cellulose during either experiment. These results suggest that low cellulose levels in litter induced C limitation and caused decomposers to mine the litter for cellulose despite the presence of lignin.

Hypothesis 3: Lignin controls over decay

Large losses of cellulose and hemicellulose in the low-lignin plant (Fig. 1) and high activity of cellulose- and hemicellulose-degrading enzymes (Appendices A and D) support the long-standing hypothesis that lignin protects cell-wall polysaccharides from degradation by decomposers. By contrast, protection of N-rich compounds was not a mechanism of lignin control over litter decay; both the wild-type and low-lignin plants lost over 85% of their original N during one year of decay (Fig. 1), despite ~50% of initial litter N being bound in the cell wall. Rather than breaking down lignin to access N (Hammel 1997), decomposers may have decomposed lignin to access more C substrates for growth. We found that low-lignin plants had significantly higher fungal biomass and activity of N-acetyl-glucosaminidase (NAG) (Fig. 2), which breaks down chitin in fungal cell walls. NAG is produced by fungi during cellular growth (Haran et al. 1995), so could reflect increased growth of fungal biomass in the presence of high cellulose availability on low-lignin litters.

An alternative motivation for lignin degradation may have been to reduce competition between decomposers for space and labile-C substrates. Increased NAG activity has been associated with antagonistic interactions between species of pathogenic fungi (Brunner et al. 2003) and wood-decay fungi (Baldrian 2008), as well as between fungi and bacteria (De Boer et al. 1998). Decomposers often display antagonistic behavior when colonizing wood (reviewed in Boddy [2000]) or decomposing leaves (Romani et al. 2006). Recently, it has been shown that the successful establishment of decomposer fungi on wood is dependent on community assembly history, where the first species to colonize a substrate is more successful at establishing than when it arrives on a substrate previously colonized by another species (Fukami et al. 2010). Priority effects on microbial establishment have also been observed in bacteria (Fukami et al. 2007) and in ectomycorrhizal fungi colonizing plant roots (Kennedy et al. 2009). Only a subset of decomposer microbes are capable of modifying lignin (Osono 2007). Lignin degradation by these taxa would allow them to colonize substrates before other decomposers that lack this capability, reducing competition and potentially accelerating rates of litter decay.

Hypothesis 4: Nitrogen fertilization effects on decay

Nitrogen fertilization increased mass loss of litter after two months of decay due to increased loss of cellulose (Fig. 3). In addition, nitrogen fertilization increased the activity of CBH, BG, and BX at the later stage of decay (Appendix D). These observations are consistent with our original hypothesis that mineral N alleviates N limitation of cellulose degradation (Hypothesis 4).

Nitrogen fertilization tended to increase the retention of lignin in litter, yet we did not find evidence that this effect was due to a reduced need for microbes to access litter N protected by lignin. After a year of decomposition, percentage litter N loss was similar in control and N fertilized plots and there was no interaction between litter N and N fertilization, which is consistent with other studies (Hobbie 2005). Instead, nitrogen fertilizer effects may have resulted from interactions between lignin and cellulose decomposers. Indeed, it is possible that competition between decomposers occurred during the early stage of decay; nitrogen fertilization increased NAG activity (Fig. 3) without increasing fungal biomass on the litters, suggesting that NAG activity may represent antagonism towards fungi, rather than mechanisms of fungal growth. This mechanism is supported by numerous studies demonstrating shifts in microbial community composition with N fertilization, particularly in N-poor systems (e.g., Treseder 2004, Allison et al. 2007, Papanikolaou et al. 2010). Increases in cellulases and hemicellulases and the suppression of lignin degradation (Appendices C and D) under N fertilization during later stages of decay suggest that cellulose degraders were competitively dominant under N fertilization.

Conclusion

Interactions among lignin, cellulose, and N were major controls over litter decomposition in our system. We found support for several long-standing hypotheses about how these controls operate: lignin protects cell-wall polysaccharides from decay, cellulose is a labile-C co-substrate for lignin degradation, and litter N alleviates N limitation of C use by decomposers. However, our results also provided evidence that these chemicals can affect decay by alternative mechanisms. In particular, lignin controlled fungal activity without affecting microbial access to litter N. Furthermore, we hypothesize that lignin and N fertilizer play a role in structuring competitive interactions between decomposers. Levels of lignin, cellulose, and N in the *Arabidopsis* litters were within the range of these chemical components in natural litter (Zhang et al. 2008). Therefore, our results may explain patterns of litter decay in other systems where decay rates vary by plant species, across systems that vary in soil N availability, or during shifts in plant and microbial communities as a result of changing environmental conditions.

Acknowledgments

We thank Steven Allison, Diane Pataki, and the Treseder laboratory for their review and support of this work. Clint Chapple generously supplied seeds of *Arabidopsis* plants used in this experiment. NSF-EAR-044548, a UCI Environment Institute Research Grant, a Lewis and Clark scholarship from the American Philosophical Society, a Graduate Research Fellowship from NSF, and a NSF Doctoral Dissertation Improvement Grant supported this project.

Literature Cited

Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos 79:439–449.

Allison, S. D., C. I. Czimczik, and K. K. Treseder. 2008. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Global Change Biology 14:1156–1168.

Allison, S. D., C. A. Hanson, and K. K. Treseder. 2007. Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. Soil Biology and Biochemistry 39:1878–1887.

Baldrian, P. 2006. Fungal laccases – occurrence and properties. FEMS Microbiology Reviews 30:215–242.

Baldrian, P. 2008. Enzymes of saprotrophic basidiomycetes. Chapter 2, pages 19–41 *in* British Mycological Society Symposia Series. Volume 28. Elsevier, Amsterdam, The Netherlands.

Berg, B. 1986. Nutrient release from litter and humus in coniferous forest soils—a mini review. Scandinavian Journal of Forest Research 1:359–370.

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

Berg, B., and C. McClaugherty. 2003. Plant litter: decomposition, humus formation, carbon sequestration. Springer-Verlag, Berlin, Germany.

Berg, B., and H. Staaf. 1980. Decomposition rate and chemical changes of Scots pine needle litter. II. Influence of chemical composition. Ecological Bulletins (Stockholm) 32:373–390.

Boddy, L. 2000. Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Microbiology Ecology 31:185–194.

Brunner, K., C. K. Peterbauer, R. L. Mach, M. Lorito, S. Zeilinger, and C. P. Kubicek. 2003. The Nag1 N-acetylglucosaminidase of *Trichoderma atroviride* is essential for chitinase induction by chitin and of major relevance to biocontrol. Current Genetics 43:289–295.

Carreiro, M. M., R. L. Sinsabaugh, D. A. Repert, and D. F. Parkhurst. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81:2359–2365.

Couteaux, M. M., P. Bottner, and B. Berg. 1995. Litter decomposition, climate, and litter quality. Trends in Ecology and Evolution 10:63–66.

Craine, J. M., C. Morrow, and N. Fierer. 2007. Microbial nitrogen limitation increases decomposition. Ecology 88:2105–2113.

De Boer, W., P. Gunnewiek, P. Lafeber, J. D. Janse, B. E. Spit, and J. W. Woldendorp. 1998. Anti-fungal properties of chitinolytic dune soil bacteria. Soil Biology and Biochemistry 30:193–203.

DeForest, J. L., D. R. Zak, K. S. Pregitzer, and A. J. Burton. 2004. Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. Soil Biology and Biochemistry 36:965–971.

Enriquez, S., C. M. Duarte, and K. Sand-Jensen. 1993. Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94:457–471.

Fog, K. 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Reviews of the Cambridge Philosophical Society 63:433–462.

Frey, S. D., M. Knorr, J. L. Parrent, and R. T. Simpson. 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management 196:159–171.

Fukami, T., H. J. E. Beaumont, X. X. Zhang, and P. B. Rainey. 2007. Immigration history controls diversification in experimental adaptive radiation. Nature 446:436–439.

Fukami, T., I. A. Dickie, J. Paula Wilkie, B. C. Paulus, D. Park, A. Roberts, P. K. Buchanan, and R. B. Allen. 2010. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. Ecology Letters 13:675–684.

Grabber, J. H., D. R. Mertens, H. Kim, C. Funk, F. C. Lu, and J. Ralph. 2009. Cell wall fermentation kinetics are impacted more by lignin content and ferulate cross-linking than by lignin composition. Journal of the Science of Food and Agriculture 89:122–129.

Hammel, K. E. 1997. Fungal degradation of lignin. Pages 33–46 *in* G. Cadisch and K. E. Giller, editors. Driven by nature: plant litter quality and decomposition. CAB International, Wallingford, UK.

Haran, S., H. Schickler, A. Oppenheim, and I. Chet. 1995. New components of the chitinolytic system of *Trichoderma harzianum*. Mycological Research 99:441–446.

Hobbie, S. E. 2000. Interactions between litter lignin and soil nitrogen availability during leaf litter decomposition in a Hawaiian Montane forest. Ecosystems 3:484–494.

Hobbie, S. E. 2005. Contrasting effects of substrate and fertilizer nitrogen on the early stages of litter decomposition. Ecosystems 8:644–656.

Hobbie, S. E. 2008. Nitrogen effects on decomposition: a five-year experiment in eight temperate sites. Ecology 89:2633–2644.

Hopkins, D., E. Webster, J. Chudek, and C. Halpin. 2001. Decomposition in soil of tobacco plants with genetic modifications to lignin biosynthesis. Soil Biology and Biochemistry 33:1455–1462.

Iiyama, K., and A. F. A. Wallis. 1990. Determination of lignin in herbaceous plants by an improved acetyl bromide procedure. Journal of the Science of Food and Agriculture 51:145–161.

Jones, L., A. R. Ennos, and S. R. Turner. 2001. Cloning and characterization of irregular xylem4 (irx4): a severely lignin-deficient mutant of *Arabidopsis*. The Plant Journal 26:205–216.

Keeler, B. L., S. E. Hobbie, and L. E. Kellogg. 2009. Effects of long-term nitrogen addition on microbial enzyme activity in eight forested and grassland sites: implications for litter and soil organic matter decomposition. Ecosystems 12:1–15.

Kennedy, P. G., K. G. Peay, and T. D. Bruns. 2009. Root tip competition among ectomycorrhizal fungi: Are priority effects a rule or an exception? Ecology 90:2098–2107.

Kirk, T. K., and R. L. Farrell. 1987. Enzymatic combustion: the microbial degradation of lignin. Annual Review of Microbiology 41:465–505.

Knorr, M., S. D. Frey, and P. S. Curtis. 2005. Nitrogen additions and litter decomposition: A meta-analysis. Ecology 86:3252–3257.

Kuzyakov, Y., J. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32:1485–1498.

McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115:495–501.

Melillo, J. M., J. D. Aber, and J. M. Muratore. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63:621–626.

Moorhead, D. L., and R. L. Sinsabaugh. 2006. A theoretical model of litter decay and microbial interaction. Ecological Monographs 76:151–174.

Osono, T. 2007. Ecology of ligninolytic fungi associated with leaf litter decomposition. Ecological Research 22:955–974.

Papanikolaou, N., A. J. Britton, R. C. Helliwell, and D. Johnson. 2010. Nitrogen deposition, vegetation burning and climate warming act independently on microbial community structure and enzyme activity associated with decomposing

litter in low-alpine heath. Global Change Biology 16:3120–3132.
Prescott, C. 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 101:133–149.
Romani, A. M., H. Fischer, C. Mille-Lindblom, and L. J. Tranvik. 2006. Interactions of bacteria and fungi on decomposing litter: Differential extracellular enzyme activities. Ecology 87:2559–2569.
Talbot, J., D. Yelle, J. Nowick, and K. Treseder. In press. Lignin chemistry controls litter decomposition rates. Biogeochemistry. [doi:10.1007/s10533-011-9599-60]
Theander, O., P. Aman, E. Westerlund, R. Andersson, and D. Petersson. 1995. Total dietary fiber determined as neutral sugar residues, uronic acid residues, and Klason lignin (the Uppsala method): collaborative study. Journal of AOAC International 78:1030–1044.
Treseder, K. K. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytologist 164:347–355.
Treseder, K. K., K. M. Turner, and M. C. Mack. 2007. Mycorrhizal responses to nitrogen fertilization in boreal ecosystems: potential consequences for soil carbon storage. Global Change Biology 13:78–88.
Turner, S. R., and C. R. Somerville. 1997. Collapsed xylem phenotype of *Arabidopsis* identifies mutants deficient in cellulose deposition in the secondary cell wall. Plant Cell. 9:689–701.
Updegraf, D. 1969. Semimicro determination of cellulose in biological materials. Analytical Biochemistry 32:420–425.
Waksman, S. A., and R. L. Starkey. 1924. Influence of organic matter upon the development of fungi, actinomycetes and bacteria in the soil. Soil Science 17:373–378.
Webster, E. A., C. Halpin, J. A. Chudek, E. L. Tilston, and D. W. Hopkins. 2005. Decomposition in soil of soluble, insoluble and lignin-rich fractions of plant material from tobacco with genetic modifications to lignin biosynthesis. Soil Biology and Biochemistry 37:751–760.
Yanni, S. F., J. K. Whalen, M. J. Simpson, and H. H. Janzen. 2011. Plant lignin and nitrogen contents control carbon dioxide production and nitrogen mineralization in soils incubated with Bt and non-Bt corn residues. Soil Biology and Biochemistry 43:63–69.
Zhang, D., D. Hui, Y. Luo, and G. Zhou. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. Journal of Plant Ecology 1:85.

Supplemental Material

Appendix A

A table presenting potential extracellular enzyme activities for different *Arabidopsis plants* decomposed for one year in Experiment 1 (*Ecological Archives* E093-033-A1).

Appendix B

A table of initial litter chemistry traits of *Arabidopsis* plants used in Experiment 2 (*Ecological Archives* E093-033-A2).

Appendix C

A figure presenting percentage loss of total mass, soluble C, cellulose, lignin, hemicelluloses, and N in all *Arabidopsis* plants decomposed for one year in control and N-fertilized plots, Experiment 2 (*Ecological Archives* E093-033-A3).

Appendix D

A figure presenting activities of extracellular enzymes and fungal biomass on *Arabidopsis* plants decomposed for one year in control and N-fertilized plots, Experiment 2 (*Ecological Archives* E093-033-A4).