

# Chronic nitrogen deposition influences the chemical dynamics of leaf litter and fine roots during decomposition



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## ABSTRACT

Atmospheric nitrogen deposition induces a forest carbon sink across broad parts of the Northern Hemisphere; this carbon sink may partly result from slower litter decomposition. Although microbial responses to experimental nitrogen deposition have been well-studied, evidence linking these microbial responses to changes in the degradation of specific compounds in decaying litter is sparse. We used wet chemistry and Fourier transform infrared spectroscopy (FTIR) methods to study effects of chronic simulated nitrogen deposition on leaf litter and fine root chemistry during a three-year decomposition experiment at four northern hardwood forests in the north-central USA. Leaf litter and fine roots were highly different in initial chemistry, such as concentrations of acid-insoluble fraction (AIF, or Klason lignin) and condensed tannins (CTs). These initial differences persisted over the course of decomposition. Gravimetrically-defined AIF and lignin/carbohydrate reference IR peak ratios both provide evidence that lignin in fine roots was selectively preserved under simulated nitrogen deposition. Lignin/carbohydrate peak ratios were strongly correlated with AIF, suggesting that AIF is a good predictor of lignin. Because AIF is abundant in fine roots, slower AIF degradation was the major driver of the slower fine root decomposition under nitrogen enrichment, explaining 73.5% of the additional root mass retention. Nitrogen enrichment also slowed the loss of CTs and proteins in fine roots. Nitrogen additions initially slowed the loss of AIF, CTs, and proteins in leaf litter, which was comparatively low in AIF, but these effects disappeared at the later stage and did not affect leaf litter mass loss during the experiment. Our results suggest that decomposition of chemical classes subject to oxidative degradation, such as lignin and CTs, is generally inhibited by nitrogen enrichment; but whether this inhibition eventually slows litter mass loss and leads to organic matter accumulation depends on the initial quantities of these classes in litter.

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## 1. Introduction

Anthropogenic production of reactive nitrogen (Nr) has rapidly increased over the last century and exceeds natural production of Nr (Ciais et al., 2014). A large portion of the Nr created by human activity is added to terrestrial ecosystems via atmospheric deposition, leading to a substantial increase in inputs of Nr across wide areas of Europe, North America, and Asia (Holland et al., 2005; Gruber and Galloway, 2008; Liu et al., 2013). Because carbon (C) and N are coupled in nearly all

fundamental biological metabolic pathways, and because N availability limits plant productivity in most terrestrial ecosystems (Chapin et al., 2011), Nr deposition alters the biogeochemical cycling of C and other elements.

There have been numerous reports of increased forest C sequestration in Western Europe and North America as a result of Nr deposition (e.g., Nadelhoffer et al., 1999; Sutton et al., 2008; Thomas et al., 2010). Chronic N deposition experiments have shown that N additions can increase tree growth and soil organic C (Hyvönen et al., 2008; Pregitzer et al., 2008; Zak et al., 2008; Frey et al., 2014), although some of the increase in tree growth can be offset by higher rates of tree mortality (Magill and Aber, 2000; Frey et al., 2014). Increases in soil organic C have been generally associated with slower decomposition of plant residues and soil organic

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matter, rather than greater litter input (Zak et al., 2008; Janssens et al., 2010; Xia et al., 2017).

Although N additions can slow litter decomposition, it is unclear whether this is a broad effect or is specific to individual chemical fractions within plant residues. The broad suppression hypothesis (H1) suggests that N additions inhibit microbial activity in a non-specific way. Several mechanisms may be responsible for the relatively non-specific detrimental effects of N additions on microbial activity. For example, introduction of excess ions by N enrichment could increase osmotic pressure in the soil solution, which could be toxic to microorganisms (Söderström et al., 1983). Nitrogen additions may also lead to soil acidification, loss of cation nutrients, and increased aluminum toxicity, each of which could negatively affect microbial growth (Vitousek et al., 1997; Högborg et al., 2006). Nitrogen additions decreased total microbial biomass by up to 35% and heterotrophic soil respiration by up to 15% across a broad range of terrestrial ecosystems, regardless of the form in which N was supplied (Treseder, 2008; Janssens et al., 2010; Ramirez et al., 2012; Frey et al., 2014).

Alternatively, preservation of specific chemical fractions within decomposing litter by N deposition would be in line with observations that N additions selectively suppresses the activity of oxidative extracellular enzymes involved in lignin degradation, such as phenol oxidase and peroxidase (Carreiro et al., 2000; DeForest et al., 2004; Freedman et al., 2016; Sun et al., 2016). Also, in northern hardwood forests simulated N deposition has been reported to down-regulate expression of the *lcc* gene that is responsible for synthesis of phenol oxidase (laccase, Edwards et al., 2011). In contrast, N additions generally increased or caused little change in cellulolytic enzyme activities (Carreiro et al., 2000; DeForest et al., 2004; Edwards et al., 2011; Sun et al., 2016). Because N additions exhibited differential effects on these extracellular enzymes, an alternative hypothesis (H2) is that simulated N deposition selectively preserves lignin relative to polysaccharides in decomposing litters. Although microbial responses to experimental N deposition have been well-studied, evidence for slower degradation of specific chemical compounds in decaying litter is still sparse.

We tested these two hypotheses by conducting a three-year study of leaf litter and fine root decomposition at four northern hardwood forests in the north-central USA that have received N additions simulating enhanced atmospheric deposition since 1994. To characterize chemical changes during litter decay for both leaf litter and fine roots, we used a broad set of wet chemistry methods. In addition, we used Attenuated Total Reflection (ATR)-Fourier transform infrared spectroscopy (FTIR) to characterize the

degradation of lignin relative to that of polysaccharides. At these study sites, long-term simulated N deposition has increased organic carbon (C) in soil organic horizon and surface mineral soil by ~ 26% (Pregitzer et al., 2008), altered extracellular enzyme activity and microbial community composition (DeForest et al., 2004; Edwards et al., 2011; Freedman et al., 2016), as well as slowed fine root mass loss (Xia et al., 2017), while plant litter production and leaf litter mass loss were unaltered (Zak et al., 2008; Xia et al., 2017). This long-term field experiment provides an opportunity to understand whether observed increases in soil C accumulation result from the selective preservation of individual organic compounds in decaying litter materials. We have previously reported that leaf litter and fine roots were the two dominant litter types at our sites and that their initial chemical compositions were very different (Xia et al., 2015). Another objective of this study was to determine whether the large initial chemical difference between these two litter sources persists or disappears during decomposition, a question that could shed light on the origin of chemical heterogeneity within soil organic matter (Wickings et al., 2012).

## 2. Methods and materials

### 2.1. Site description

The four study sites are located along a north-south latitudinal gradient in the Great Lakes region of North America (Table 1). These ecosystems are dominated by sugar maple (*Acer saccharum* Marsh.), and are floristically and edaphically matched, similar in stand composition (82± 4%, basal area in sugar maple), age (105 ± 3 years), stand structure, and soil properties (Table 1). Across the latitudinal range of the experiment, there are gradients in both climate and ambient N deposition, which ranges from 6.8 kg N ha<sup>-1</sup> yr<sup>-1</sup> (wet plus dry) at the most northerly site to 11.8 kg N ha<sup>-1</sup> yr<sup>-1</sup> at the most southerly site (Table 1). Until a shift toward increased NH<sub>4</sub><sup>+</sup> deposition and decreased NO<sub>x</sub> emissions in the previous decade, NO<sub>3</sub><sup>-</sup> was the dominant form of N deposition (Talhelm et al., 2012). Soils are sandy (Kalkaska series, Typic Haplorthods) and pH values range from 4.4 to 4.7 in the top 10 cm of mineral soil. Within each of these forests, six 30-m x 30-m plots were established in 1994, with each plot surrounded on all sides by a 10-m wide buffer that received the same treatment as the main plot. Three plots at each site receive ambient N deposition plus an experimental addition of N (30 kg N ha<sup>-1</sup> yr<sup>-1</sup> as NaNO<sub>3</sub> in six equal increments across the growing season), a rate similar to those occurring in some areas of Europe and China (Holland et al., 2005; Liu et al.,

**Table 1**  
Characteristics of northern hardwood forest study sites in lower and upper Michigan, USA.

Site characteristic	Site A	Site B	Site C	Site D
Latitude (N)	46°52'	45°33'	44°23'	43°40'
Longitude (W)	88°53'	84°51'	85°50'	86°09'
Mean annual precipitation (mm) <sup>a</sup>	873	871	888	812
Growing season precipitation (mm) <sup>b</sup>	401	388	393	379
Mean annual temperature (°C) <sup>a</sup>	4.7	6.0	6.9	7.6
Growing season temperature (°C) <sup>b</sup>	15.0	16.0	16.2	16.8
Total basal area (m <sup>2</sup> ha <sup>-1</sup> ) <sup>c</sup>	34	31	32	33
Sugar maple basal area (%) <sup>c</sup>	86	86	83	75
Ambient wet + dry N deposition (kg N ha <sup>-1</sup> yr <sup>-1</sup> ) <sup>d</sup>	6.8	9.1	11.7	11.8
Soil texture, 0–10 cm depth (%sand-%silt-%clay) <sup>a</sup>	75–22–3	89–9–2	89–9–2	87–10–3
Soil texture, 10–70 cm depth (%sand-%silt-%clay) <sup>a</sup>	84–11–5	88–7–5	91–6–3	92–5–3

Sites A, B, C, and D are located in Houghton, Emmett, Wexford, and Oceana counties, respectively, from north to south.

<sup>a</sup> Pregitzer et al. (2008).

<sup>b</sup> Burton et al. (2012).

<sup>c</sup> Burton et al. (2000).

<sup>d</sup> MacDonald et al. (1992).

2013), and approaching that expected by 2050 in large regions of Europe (Galloway et al., 2004).

## 2.2. Litter sampling and decomposition study

Sugar maple leaf litter was collected in the autumn of 2010 from litter traps that are randomly located in each plot following the protocol of Pregitzer et al. (2008). Fine roots were excavated from the top 10 cm soil, including the O<sub>e+a</sub>, at six to ten random locations within the 10-m buffer zone surrounding each plot in October 2010 (autumn) and May 2011 (spring). After being removed from soil, roots were identified to the genus *Acer* by morphological characteristics, washed, and flash frozen in liquid N<sub>2</sub> before being transported back to the University of Idaho on dry ice for further processing. We collected fresh fine roots instead of dead root litter because it was impractical to identify large enough quantities of recently-senesced fine roots to conduct a three-year decomposition study. Nutrient resorption and other biochemical changes during senescence are less understood in roots than in leaves (Comas et al., 2000). Compared to the drastic changes in leaf biochemistry during senescence, previous studies observed either no nutrient resorption or considerably less nutrient resorption in fine roots (Nambiar, 1987; Gordon and Jackson, 2000). We excluded white and turgid fine roots during processing to minimize the difference between the roots we sampled and root necromass, as root pigmentation has been shown to relate to decomposition patterns (Goebel et al., 2011). Because sugar maple leaf litter accounted for ~77% of leaf litter flux at these sites, and the genus *Acer* contributed up to 90% of overstory basal area and 83% of woody seedling groundcover stems (Talhelm et al., 2013), the leaf litter and fine roots we sampled are broadly representative of the litter produced in these forests.

We isolated first to third branch orders of fine roots following the procedures described in Pregitzer et al. (2002) for this decomposition study. The distal three orders of roots consistently exhibit similar anatomy, lack secondary growth (Guo et al., 2008), and have a fast turnover rate (Valenzuela-Estrada et al., 2008; Xia et al., 2010). Although these distal roots do not necessarily account for a large portion of total root standing biomass, they are the major driver of root production and N turnover due to their short lifespan (Xia et al., 2010; McCormack et al., 2015). Conventionally defined “fine roots” (<2 mm in diameter) often contain a significant portion of roots that have undergone secondary thickening and have a longer lifespan (Xia et al., 2010; McCormack et al., 2015).

About ~1 g leaf litter or fine roots were sealed into 20 cm × 20 cm polyester litterbags. The mesh sizes of litter bags were 20 μm on the bottom and 300 μm on the top. The bottom mesh allowed the fungal hyphae to penetrate into the bags while minimizing the physical loss of decomposing plant debris (Hobbie, 2005); the top mesh size permitted entry of most soil micro-fauna, but likely excluded entry of larger soil animals (Bradford et al., 2002). Thus, this study focused on the decomposition driven mostly by microorganisms, and caution remains as to the potential effects of soil animals.

Litter bags were returned to each of the four sites for the three-year decomposition study starting in July 2011. Leaf litter was deployed *in situ* to represent native leaf litter fall. Because the litter traps did not yield enough leaf litter mass from some plots, leaf litter collected from the three plots receiving the same treatment at each site was homogenized before placement into litterbags. The homogenized leaf litter from three plots of a treatment at each site were deployed to those three plots. Leaf litter bags were placed flat on the top of the forest floor (O horizon surface), while additional leaf litter bags were also placed at the interface between mineral soil and organic soil horizons (O/A interface) to rule out environmental factors as a source of differences in leaf litter and fine root

decomposition. For fine roots, we collected enough initial material for a reciprocal deployment between ambient and N added plots to distinguish between initial substrate effects and the effect of externally-supplied N. Specifically, besides being deployed in the plots from which they were collected, fine roots were also placed in the plots of the alternate treatment at the same site. All root bags were placed at the O/A interface. Because root mortality occurs relatively evenly throughout growing season at these sites (Burton et al., 2000), both autumn and spring roots were decomposed to better characterize root decomposition patterns. Autumn and spring roots of a treatment type were decomposed in separate litterbags in a plot, yet the final mass remaining and chemistry of that plot was calculated as the average of these two litterbags. Taken together, we had six types of litterbags (two contained leaf litter and four contained fine roots) in each plot at each site, with each type having 18 bag replicates per plot. We deployed 2592 litterbags in total.

We harvested three of each litter bag type from each plot after periods of one month to three years. Harvested bags were flash-frozen in liquid N<sub>2</sub> and transported to the University of Idaho on dry ice. We removed sample material from the litter bags, cleaned them of soil, new root growth, and animal necromass using forceps. Decomposing samples were freeze-dried and weighed. Mass loss for a litter bag was calculated as the difference between the oven-dry mass loss and chemical traits were reported on the ash-free basis. Plots are the replicates in the data analysis, so we averaged the mass remaining (%) of three bag replicates from each plot.

## 2.3. Chemical analysis

All samples were pulverized in a Wig-L-Bug grinder (Dentsply-Rinn, Elgin, IL) before chemical analysis. We determined concentrations of total C and N, acid-insoluble fraction (AIF), acid-soluble fraction (ASF), nonstructural carbohydrates (NSCs), soluble phenolics, condensed tannins (CTs), soluble proteins, and total lipids, for initial and harvested materials. We have previously reported that leaf litter and fine roots were highly different in initial chemical characteristics (Xia et al., 2015; also see Table 2). We note AIF has been assumed to represent lignin, but may also contain other complex substrates such as cutins, suberins, and CT-protein complexes (Preston et al., 2009); ASF primarily consists of cell-wall polysaccharides, along with other compounds (e.g., phenolics and proteins) linked to cell wall via ester bonds (Preston et al., 2000).

The details of wet chemical methods were reported in Xia et al. (2015). Briefly, total C and N were analyzed with an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA). Extractive-free fraction was obtained with a sequential extraction procedure (Friend, 1992; Booker et al., 1996). This fraction was subsequently divided into AIF and ASF using a two-phase H<sub>2</sub>SO<sub>4</sub> hydrolysis adapted from Booker et al. (1996). For NSCs, we used phenol-sulfuric acid analysis to determine sugar concentrations (Chow and Landhäusser, 2004). Starch was determined colorimetrically with a peroxidase-glucose oxidase (PGO)/o-dianisidine reagent after α-amylase/amyloglucosidase digestion (Chow and Landhäusser, 2004). Soluble phenolics were determined with Folin-Ciocalteu reagent based on catechin standards (Booker et al., 1996). Condensed tannins were determined with acid-butanol assay (Booker et al., 1996) with standards prepared from fresh apples (Li et al., 2010). We used Coomassie protein Bradford Reagent (Thermo Fisher Scientific Inc. Rockford, IL) to measure concentrations of soluble proteins. Total lipids were extracted and determined according to Bligh and Dyer (1959).

The pulverized samples were also analyzed using ATR (Smart-Performer, ZnSe crystal) on a ThermoNicolet Avatar 370 FTIR spectrometer. We collected infrared spectra for initial materials and

**Table 2**  
Chemical characteristics (%) and litter quality indices (as ratios) of the initial litters collected from ambient and NO<sub>3</sub><sup>-</sup> added plots.

Chemical traits	Abbr.	Site A				Site B				Site C				Site D			
		Leaf litter		Fine roots		Leaf litter		Fine roots		Leaf litter		Fine roots		Leaf litter		Fine roots	
		Ambient	NO <sub>3</sub> <sup>-</sup>	Ambient	NO <sub>3</sub> <sup>-</sup>	Ambient	NO <sub>3</sub> <sup>-</sup>	Ambient	NO <sub>3</sub> <sup>-</sup>	Ambient	NO <sub>3</sub> <sup>-</sup>	Ambient	NO <sub>3</sub> <sup>-</sup>	Ambient	NO <sub>3</sub> <sup>-</sup>	Ambient	NO <sub>3</sub> <sup>-</sup>
Extractive fraction	EXT	39.30	43.45	17.51 (1.18)	18.09 (0.51)	37.00	39.33	15.68 (0.50)	13.96 (0.22)	38.84	39.07	15.90 (1.71)	13.76 (1.11)	39.51	36.72	16.58 (0.90)	16.68 (2.35)
Acid-insoluble fraction	AIF	16.18	14.66	42.71 (1.09)	45.49 (1.76)	15.05	14.08	45.34 (0.49)	45.72 (0.82)	14.44	14.22	45.90 (1.37)	46.72 (0.72)	14.74	14.67	46.42 (2.36)	45.16 (1.27)
Acid-soluble fraction	ASF	44.52	41.89	39.79 (1.07)	36.43 (1.64)	47.95	46.59	38.98 (3.14)	40.32 (2.75)	46.73	46.71	38.20 (1.62)	39.62 (4.32)	45.75	48.60	37.00 (4.96)	38.17 (1.92)
Soluble phenolics	PHE	13.61	15.09	3.88 (0.21)	4.03 (0.11)	13.53	11.60	3.24 (0.24)	2.99 (0.34)	13.29	10.60	4.11 (0.73)	3.16 (0.28)	11.82	10.69	4.57 (0.47)	4.79 (0.96)
Condensed tannins	CTs	9.08	6.92	14.83 (0.77)	15.82 (1.13)	5.22	3.54	11.70 (1.25)	10.49 (0.50)	6.11	4.19	13.02 (2.54)	9.90 (1.50)	4.48	5.08	14.82 (1.02)	13.36 (2.42)
Lipids	LIP	8.50	8.28	3.72 (0.29)	3.54 (0.33)	7.67	6.98	3.72 (0.21)	3.18 (0.43)	7.64	6.88	3.07 (0.56)	3.31 (0.32)	8.17	8.91	3.38 (0.27)	3.60 (0.07)
Non-structural carbohydrates	NSCs	5.26	7.14	2.09 (0.08)	2.10 (0.32)	4.98	6.10	1.73 (0.24)	1.71 (0.10)	5.88	4.91	1.74 (0.07)	1.66 (0.29)	4.76	5.53	1.94 (0.23)	1.92 (0.52)
Soluble proteins	PRO	2.02	1.48	3.20 (0.07)	3.30 (0.26)	0.78	0.73	3.24 (0.25)	2.61 (0.28)	1.20	1.25	3.58 (0.72)	2.80 (0.28)	0.91	1.34	3.12 (0.34)	3.08 (0.39)
Nitrogen	N	0.79	0.83	1.35 (0.02)	1.55 (0.06)	0.66	0.97	1.74 (0.11)	1.72 (0.06)	0.64	0.83	1.66 (0.05)	1.78 (0.04)	0.73	0.85	1.46 (0.15)	1.51 (0.12)
Carbon/N	C/N	63.33	61.32	37.38 (0.59)	33.66 (1.43)	71.22	50.27	29.05 (1.80)	29.32 (1.02)	77.39	58.33	31.33 (1.01)	29.06 (0.63)	68.68	58.11	35.75 (3.99)	34.59 (2.98)
AIF/N		20.38	17.74	31.73 (0.68)	29.42 (1.44)	22.65	14.46	26.30 (1.92)	26.63 (1.31)	22.60	17.08	27.84 (1.61)	26.36 (0.27)	20.29	17.22	32.32 (4.78)	30.19 (3.20)
Lignocellulose index	LCI	0.27	0.26	0.52 (0.01)	0.56 (0.02)	0.24	0.23	0.54 (0.01)	0.53 (0.01)	0.24	0.23	0.55 (0.01)	0.54 (0.01)	0.24	0.23	0.56 (0.03)	0.54 (0.01)

Values of leaf litter are homogenized leaf litter combined from three ambient or NO<sub>3</sub><sup>-</sup> amended plots at each site. Values of fine roots are means with SD in parentheses for three ambient or NO<sub>3</sub><sup>-</sup> amended plots (n = 3), which have been documented in Xia et al. (2015). Lignocellulose index is calculated as AIF/(AIF + ASF).

decomposed materials from the final harvest (3 years). For each sample, we recorded 64 scans (4000–400  $\text{cm}^{-1}$  wavenumber) at a resolution of 4  $\text{cm}^{-1}$ . A blank spectrum of air was taken as background measured before each sample and automatically subtracted from each sample spectrum. The spectra were rubber-band baseline corrected (Baker et al., 2014) in a consistent manner for leaf litter and fine root samples, and normalized using OMNIC software v9.0. Peak heights were measured against the baseline to represent the intensity for the bands of interest.

The IR peak near 1510  $\text{cm}^{-1}$  has been assigned to aromatic skeletal vibration in lignin and is considered the most characteristic lignin reference in IR spectra (Faix, 1991; Pandey and Pitman, 2003; Radotić et al., 2012). The relative intensity of this peak ( $I_{1510}$ ) against carbohydrates characteristics bands have successfully predicted lignin concentrations in wood, which was confirmed by acetyl bromide methods (Rodrigues et al., 1998; Pandey and Pitman, 2004). Differences between decomposed and initial substrates in the ratios of lignin/carbohydrate characteristic peak intensity ( $\Delta I_{L/C}$ ) were used to quantify the selective degradation of lignin against polysaccharides by white-rot fungi in wood chemistry studies (Pandey and Pitman, 2004; Fabiyi et al., 2011). Here,  $\Delta I_{L/C}$  is calculated as follows (Pandey and Pitman, 2004; Fabiyi et al., 2011):

$$\Delta I_{L/C} = \frac{I_{L,d}}{I_{C,d}} - \frac{I_{L,und}}{I_{C,und}}$$

where  $I_{L,d}$ ,  $I_{C,d}$ ,  $I_{L,und}$ ,  $I_{C,und}$  represent the intensity of lignin reference peak, each of the carbohydrate reference peaks for decomposed litters (3 years) and those for undecomposed/initial substrates respectively. Higher values of  $\Delta I_{L/C}$  indicate that more lignin is preserved relative to carbohydrates during litter decomposition.

#### 2.4. Data analysis

We used the fraction of the original amount of a chemical class remaining to describe chemical changes during litter decomposition. The fraction remaining ( $F_t$ ) for a certain chemical class was calculated as follows:

$$F_t = \frac{C_t M_t}{C_0}$$

where  $C_0$ ,  $C_t$  are substrate concentrations for a certain chemical class in initial and decomposed litters respectively, and  $M_t$  is the percentage mass remaining. Data analyses were conducted using SAS software (version 9.3; SAS Institute Inc. Cary, NC). We tested the effects of site ( $df = 3$ ), simulated N deposition ( $df = 1$ ), and their interactions on the proportion of mass remaining, fraction remaining of each chemical class, as well as the values of  $\Delta I_{L/C}$ , with a two-way ANOVA for leaf litter and fine roots. Because fine roots were decomposed reciprocally between ambient and N added conditions, we further analyzed decomposition metrics of fine roots with factorial arrangements of substrate source and external N availability. We conducted a mixed linear model analysis (Proc mixed, Littell et al., 2006) to test if substrate source (litter collected from N-amended vs. ambient,  $df = 1$ ), external N availability (decomposed in N-amended vs. ambient,  $df = 1$ ), and study sites ( $df = 3$ ) affected the chemical fraction remaining at different time points for fine roots. Site and external N treatment and their interactions ( $df = 3$ ) were tested on the whole-plot experimental units, while substrate source was the within-plot fraction. The chemistry of initial and decomposed leaf litter and fine roots at individual harvest points were characterized by principle

components analysis (PCA) on the concentrations of major chemical classes, as an attempt to visualize chemical shifts during decomposition.

A partial correlation analysis on fraction remaining of different chemical classes was performed to reveal how chemical fluxes may couple with each other during decomposition. The partial correlation analysis controlled the effects of mass remaining to remove the monotonic correlations between mass remaining and the fraction remaining of nearly all chemical classes. In addition, a correlation analysis was performed to investigate the relationship among the ratios of lignin/carbohydrates FTIR peak intensities, AIF concentrations, and AIF/ASF ratios. Both Pearson's product-moment correlations and Spearman rank-order correlations were estimated in this study. We present Spearman rank-order correlations when the assumptions for Pearson correlations did not hold for the data. Data were log-transformed when needed to improve homogeneity of variance and normality before being analyzed.

### 3. Results

#### 3.1. Chemical dynamics during decomposition

Leaf litter, either decomposed on the O horizon surface or at the O/A interface, had greater mass loss than fine roots, with only 15.1–24.6% mass remaining at the end of this study (Tables 3 and 4). During the initial decomposition, there was substantial loss of soluble phenolics, condensed tannins, and NSCs from leaf litter, with approximately 60–90% of initial amount of these chemical classes either leached or degraded within three months (Table 3). At the end of this study, less than 6% of the initial amount of these chemical classes remained (Table 3). By contrast, acid-insoluble fraction (AIF), soluble protein, and N accumulated (i.e. fraction remaining >100%) or were retained for the first year of decomposition, and about half of the initial amount of these components remained in leaf litter at the end of this study (Table 3).

In comparison with leaf litter, fine roots showed a slower loss of soluble phenolics, condensed tannins, and NSCs, and did not exhibit an increase of AIF, protein, and N during the first three months (Table 4). Fine roots maintained considerably larger fractions of AIF, ASF, soluble phenolics, condensed tannins, NSCs, and lipids than leaf litter throughout this study, with 24.1–69.3% of the initial amount for these chemical classes still remaining in the third year (Table 4).

The PCA based on tissue chemistry indicated that during decomposition, the chemistry of leaf litter and fine roots converged along PC1 axis, which is associated with relative labile components such as soluble phenolics, NSCs, proteins, and N (Fig. 1). However, the large difference in initial chemistry between leaf litter and fine roots along PC2 axis persisted through the end of the third year of decomposition, with PC2 axis negatively correlated with AIF, condensed tannins (CTs), and positively correlated with ASF and lipids (Fig. 1). Fine roots maintained higher concentrations of AIF and CTs than leaf litter throughout the decomposition period (data not shown).

The partial correlation analysis showed that a number of chemical fluxes exhibited significant correlations with each other, with their directions and magnitude often different between leaf litter and fine roots (Table S1). The most consistent of these effects was that the change of phenolics was positively correlated with that of CTs for both leaf litter and fine roots ( $R > 0.451$ ,  $P < 0.001$ ). Also, the change of CTs were positively correlated with that of soluble proteins for both leaf litter and fine roots, with this correlation the strongest in fine roots ( $R = 0.400$ ,  $P < 0.001$ ). However, there was a correlation between phenolics and proteins that was positive in fine roots, but negative in leaf litter (Table S1); this is

**Table 3**

Fraction remaining (%) of original mass and individual chemical classes in leaf litter decomposed on the top of the forest floor (O horizon) and at the interface between mineral soil and organic soil horizons (O/A interface) for three months, one year, or three years under ambient conditions or with simulated N deposition across four northern hardwood forests.

Litter traits	Treatment	O horizon			O/A interface		
		3 mon	1 yr	3 yr	3 mon	1 yr	3 yr
Mass	Ambient	64.9 (2.7)	45.7 (2.9)	24.6 (3.6)	52.3 (4.5)	30.2 (2.9)	15.0 (4.2)
	NO <sub>3</sub> <sup>-</sup>	64.6 (3.3)	45.3 (3.9)	25.4 (5.2)	51.9 (3.1)	30.1 (4.0)	15.1 (3.3)
AIF	Ambient	145.8 (11.2)	113.0 (9.7)	65.9 (11.6)	127.5 (18.5)	76.5 (7.0)	37.0 (11.5)
	NO <sub>3</sub> <sup>-</sup>	156.4 <sup>**</sup> (6.6)	116.6 (12.2)	69.9 (16.3)	136.3 (15.6)	83.2 (12.3)	41.2 (10.2)
ASF	Ambient	62.6 (5.2)	43.6 (5.2)	22.8 (3.1)	51.4 (5.2)	29.3 (5.1)	14.8 (3.9)
	NO <sub>3</sub> <sup>-</sup>	61.8 (5.6)	46.0 <sup>†</sup> (5.4)	24.8 (6.6)	50.0 <sup>†</sup> (6.2)	28.8 (4.8)	14.5 (2.9)
PHE	Ambient	20.0 (4.3)	6.0 (2.0)	1.4 (0.5)	7.8 (2.4)	2.03 (0.6)	0.7 (0.2)
	NO <sub>3</sub> <sup>-</sup>	20.3 (5.6)	6.0 (2.1)	1.8 (0.8)	8.6 (2.0)	2.37 (1.0)	0.9 (0.3)
CTs	Ambient	27.5 (5.8)	14.3 (4.3)	4.8 (1.8)	14.7 (7.1)	5.1 (2.4)	2.8 (1.6)
	NO <sub>3</sub> <sup>-</sup>	41.0 <sup>**</sup> (14.3)	16.5 (7.3)	5.4 <sup>†</sup> (2.8)	20.9 <sup>**</sup> (6.2)	7.3 (6.0)	3.1 (1.3)
NSCs	Ambient	16.7 (3.4)	10.3 (2.5)	4.6 (1.1)	11.1 (1.8)	5.8 (1.4)	3.1 (1.1)
	NO <sub>3</sub> <sup>-</sup>	13.1 <sup>**</sup> (2.5)	7.8 <sup>***</sup> (2.5)	4.3 (1.1)	9.0 <sup>***</sup> (2.0)	4.5 <sup>**</sup> (1.4)	2.4 (1.0)
LIP	Ambient	52.2 (5.7)	31.6 (5.0)	14.9 (2.7)	38.7 (5.1)	20.7 (3.0)	9.3 (3.5)
	NO <sub>3</sub> <sup>-</sup>	53.2 (5.5)	30.0 (7.2)	16.9 (3.4)	38.8 (4.2)	19.6 (4.4)	10.4 (4.2)
PRO	Ambient	105.8 (30.1)	101.4 (25.8)	59.2 (15.9)	93.4 (23.6)	73.4 (21.8)	43.9 (11.7)
	NO <sub>3</sub> <sup>-</sup>	129.9 <sup>**†</sup> (34.0)	103.8 (25.0)	67.6 (15.8)	98.4 <sup>†</sup> (23.1)	69.1 <sup>†</sup> (13.2)	45.8 (16.3)
N	Ambient	111.4 (21.6)	112.2 (11.7)	78.2 (13.0)	99.0 (17.2)	76.3 (12.3)	49.2 (13.0)
	NO <sub>3</sub> <sup>-</sup>	101.8 (10.2)	100.0 <sup>**†</sup> (16.9)	69.2 (17.1)	87.6 <sup>***†</sup> (7.8)	65.6 <sup>***†</sup> (10.5)	40.1 (7.6)

Values are shown as means (SD) of fraction remaining of mass and chemical classes in leaf litter decomposed in each plot of ambient or NO<sub>3</sub><sup>-</sup>-amended across four study sites (n = 12). \*, \*\*, \*\*\* denote significant main effects of simulated N deposition at  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ . <sup>†</sup> refers to a significant interaction of site and N at  $P < 0.05$ . See Table 2 for abbreviations.

consistent with a difference between leaf litter and fine roots in the chemical composition of the total phenolic pool (Xia et al., 2015).

### 3.2. Effects of simulated nitrogen deposition

We used the fraction of the initial amount of overall mass and mass of each chemical class remaining at a time point to investigate the effects of N additions on litter decomposition. Although simulated N deposition did not affect leaf litter mass loss during this study ( $P > 0.693$ , Table 3), the effects of N additions on leaf litter chemical changes were manifested in the early stage of

decomposition. After three months, N additions significantly increased the remaining fraction of AIF ( $F = 11.47$ ,  $P = 0.004$ ), condensed tannins ( $F = 9.04$ ,  $P = 0.008$ ), and soluble protein ( $F = 9.61$ ,  $P = 0.007$ ) for leaf litter decomposed on the O horizon surface. In contrast, N additions accelerated the loss of NSCs ( $F = 14.05$ ,  $P = 0.002$ ). The accumulation of protein due to N additions observed at the third month occurred at sites A and B, but not at sites C and D (Site  $\times$  N,  $F = 7.73$ ,  $P = 0.002$ ). These N addition effects on initial leaf litter decomposition disappeared at the third year (Table 3). The effects of simulated N deposition on leaf litter decomposed at the O/A interface were similar to those on leaf litter decomposed on the O horizon surface, with the initial effects of N additions disappearing in the later stages of decomposition (Table 3). Added N decreased the fraction remaining of leaf litter N at all sites but site A, leading to a significant site  $\times$  N treatment interaction within the first year of decomposition for leaf litter decomposed both on the O horizon surface and at the O/A interface ( $P < 0.009$ , Table 3).

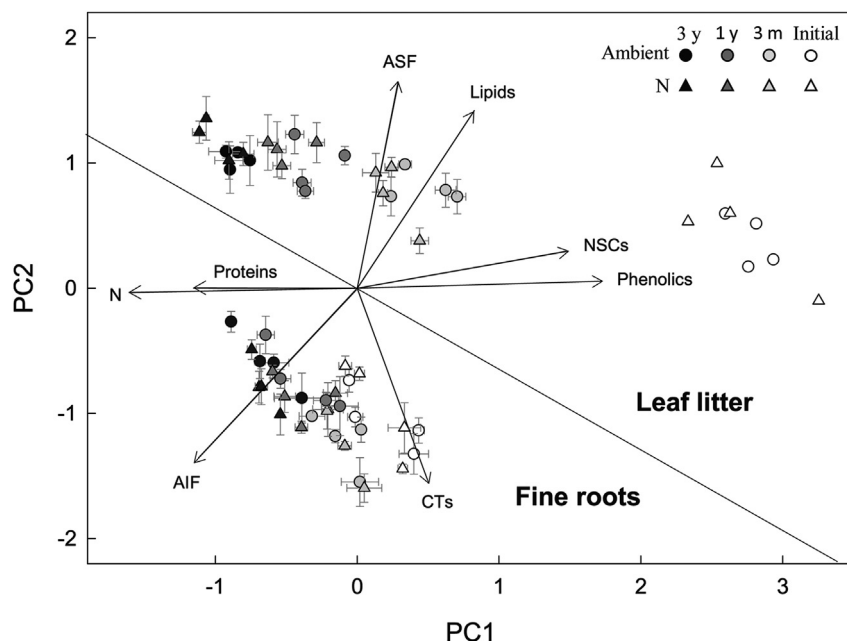
In contrast, N additions increased fine root mass remaining at the end of this study (51.3%–56.4%,  $F = 21.23$ ,  $P < 0.001$ , Table 4). However, this trend did not occur at site C (site  $\times$  NO<sub>3</sub><sup>-</sup>:  $F = 2.72$ ,  $P = 0.079$ ). As observed in leaf litter, N additions increased fraction remaining of CTs ( $F = 7.09$ ,  $P = 0.017$  at three months;  $F = 9.72$ ,  $P = 0.007$  at three years) and soluble proteins ( $F = 12.53$ ,  $P = 0.003$  at three months;  $F = 7.48$ ,  $P = 0.015$  at one year;  $F = 10.06$ ,  $P = 0.006$  at three years) in fine roots. The slower loss of CTs and proteins in fine roots due to N additions was consistent across all sites and persisted throughout the study (Table 4). Added N significantly increased AIF remaining in fine root mass at the third-year harvest (60.1–69.3%,  $F = 32.25$ ,  $P < 0.001$ , Table 4). At the ecosystem scale, we have previously documented that simulated N deposition retained more decomposing root mass at three out of the four study sites (by 27.3–41.1 g m<sup>-2</sup>, sites A, B, and D) via slowing late-stage fine root decomposition (Xia et al., 2017). Combining the fraction of AIF remaining at the end of the third year, initial AIF concentration, and the annual litter input data for fine roots (documented in Xia et al., 2015), we estimated that the slower loss of AIF in fine roots observed in this study contributed 73.5  $\pm$  5.8% of additional root mass retention caused by N additions.

**Table 4**

Fraction remaining (%) of original mass and individual chemical classes in fine roots after three months, one year, or three years of decomposition under ambient conditions or with simulated N deposition across four northern hardwood forests.

Litter traits	Treatment	Fraction remaining (%)		
		3 mon	1 yr	3 yr
Mass	Ambient	82.6 (3.0)	70.9 (4.1)	51.3 (2.5)
	NO <sub>3</sub> <sup>-</sup>	83.4 (1.8)	72.8 (4.4)	56.4 <sup>***</sup> (3.2)
AIF	Ambient	99.5 (4.9)	82.4 (7.9)	60.1 (4.6)
	NO <sub>3</sub> <sup>-</sup>	99.3 (4.8)	84.1 <sup>†</sup> (6.2)	69.3 <sup>***</sup> (4.4)
ASF	Ambient	64.1 (5.4)	61.6 (5.8)	45.9 (3.3)
	NO <sub>3</sub> <sup>-</sup>	64.9 (6.6)	61.1 (3.9)	46.4 (4.3)
PHE	Ambient	54.6 (5.4)	40.1 (8.8)	24.1 (5.2)
	NO <sub>3</sub> <sup>-</sup>	56.0 (9.8)	42.4 <sup>†</sup> (5.7)	30.0 (10.8)
CTs	Ambient	49.7 (5.9)	35.5 (6.0)	26.3 (4.6)
	NO <sub>3</sub> <sup>-</sup>	56.7 <sup>**</sup> (8.4)	39.5 (5.1)	30.7 <sup>**</sup> (5.5)
NSCs	Ambient	42.9 (9.6)	36.5 (7.3)	29.4 (3.8)
	NO <sub>3</sub> <sup>-</sup>	44.4 (10.1)	36.0 (10.0)	33.4 (7.1)
LIPD	Ambient	71.3 (23.6)	59.6 (16.7)	43.8 (8.8)
	NO <sub>3</sub> <sup>-</sup>	77.3 (13.0)	52.7 (19.5)	50.3 (7.6)
PRO	Ambient	49.0 (4.5)	51.1 (7.2)	43.8 (5.9)
	NO <sub>3</sub> <sup>-</sup>	55.2 <sup>**</sup> (5.4)	57.4 <sup>*</sup> (8.4)	52.3 <sup>**</sup> (6.9)
N	Ambient	89.8 (5.3)	84.8 (9.0)	69.1 (6.8)
	NO <sub>3</sub> <sup>-</sup>	86.3 (4.2)	80.9 (5.8)	72.3 (5.9)

Values are shown as means (SD) of the fraction remaining of mass and chemical classes in fine roots decomposed in each plot of ambient or NO<sub>3</sub><sup>-</sup>-amended across four study sites (n = 12). \*, \*\*, \*\*\* denote significant main effects of simulated N deposition at  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ . <sup>†</sup> refers to a significant interaction of site and N at  $P < 0.05$ . See Table 2 for abbreviations.

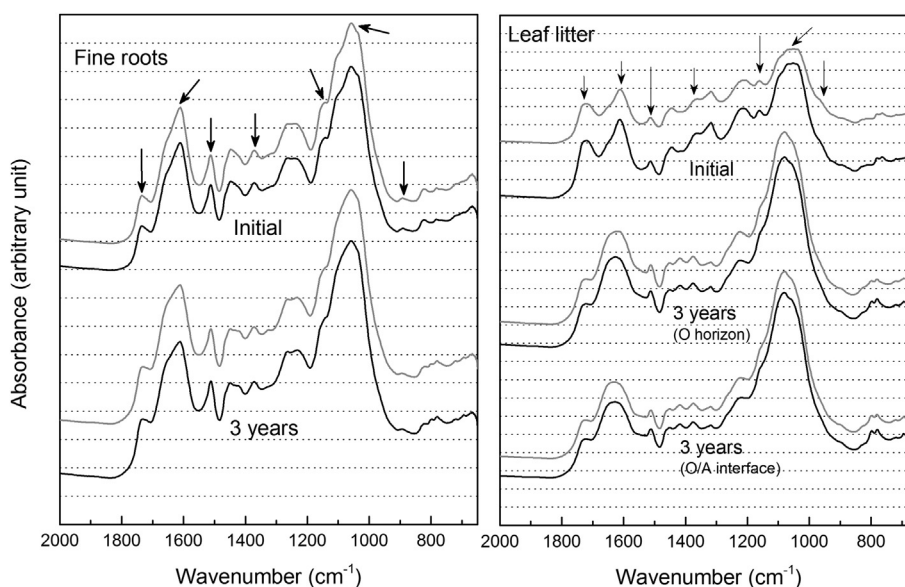


**Fig. 1.** PCA based on the concentrations of major chemical classes in initial leaf litter and fine roots, and the decomposed materials at each of the harvests. Principal components explain a variance of 51.4% (PC1) and 26.6% (PC2). Each data point is the mean with SD along both axes across three replicate plots of ambient or N-amended for each study site ( $n = 3$ ).

The reciprocal deployment of fine root bags between ambient and N-addition plots makes it possible to disentangle the changes caused by initial substrate differences from those caused by increased external N availability. Externally-supplied N significantly increased the AIF fraction remaining in fine roots at the end of the study ( $F = 33.16$ ,  $P < 0.001$ , Table S2), and this pattern persisted across four forests and fine roots collected in ambient or N-amended plots. Substrate source had minor effects on AIF, both positive and negative, that were site-specific (Site  $\times$  N:  $F = 5.01$ ,  $P = 0.012$ , Table S2). Both externally-supplied N and substrate source tend to increase the fraction remaining of CTs and proteins ( $P < 0.082$ , data not shown).

### 3.3. ATR-FTIR analysis

The FTIR spectra of leaf litter and fine roots exhibited a typical broad and strong band for hydrogen bonded O–H stretch around  $3300\text{ cm}^{-1}$ , peaks for Csp<sup>3</sup>-H stretch in methyl and methylene groups at  $2922\text{ cm}^{-1}$  and  $2852\text{ cm}^{-1}$ , and a number of distinct peaks for plant tissue in the finger print region ranging from  $600$  to  $1800\text{ cm}^{-1}$  (Fig. 2). We identified the lignin reference peak at  $1512\text{ cm}^{-1}$  for aromatic skeleton vibration in lignin, while polysaccharide characteristic bands were identified at  $898\text{ cm}^{-1}$  for C–H deformation in cellulose,  $1160\text{ cm}^{-1}$  for glycosidic linkage (C–O–C) vibration in polysaccharides, and  $1370\text{ cm}^{-1}$  for C–H



**Fig. 2.** FTIR spectra of fine roots and leaf litter before decomposition and after three years of decomposition under ambient conditions (grey line) or with simulated N deposition (dark line). The spectra are shifted along the y-axis for clarity. Each spectrum represents the average of three replicates of an N treatment across four northern hardwoods forests ( $n = 12$ ). Arrows indicate the peaks at  $1738\text{ cm}^{-1}$ ,  $1620\text{ cm}^{-1}$ ,  $1512\text{ cm}^{-1}$ ,  $1370\text{ cm}^{-1}$ ,  $1160\text{ cm}^{-1}$ ,  $1060\text{ cm}^{-1}$  and  $898\text{ cm}^{-1}$  respectively for both leaf litter and fine root spectra.

deformation in various polysaccharides (Faix, 1991; Pandey and Pitman, 2003). The major peaks at 1738  $\text{cm}^{-1}$  and 1060  $\text{cm}^{-1}$  were also used as polysaccharide reference elsewhere, but not in this study. In plant litter, the peak near 1738  $\text{cm}^{-1}$  arises from C = O stretch in ester groups, typically from xylan (hemicellulose, Pandey and Pitman, 2003), as well as suberin in roots (Ferreira et al., 2013). Besides the peak at 1738  $\text{cm}^{-1}$ , other typical suberin features include major peaks at 2922 and 2852  $\text{cm}^{-1}$  that can be attributed to aliphatic chains in suberin (Rocha et al., 2001; Ferreira et al., 2013). In fine roots, the peak intensities of 1738  $\text{cm}^{-1}$  were highly correlated with other suberin characteristic peaks at 2922 and 2852  $\text{cm}^{-1}$ , but lacked correlation with polysaccharide peaks (Fig. S1), suggesting this peak is more associated with suberin than carbohydrates in fine roots. Leaf litter exhibited the opposite trend: the intensities at 1738  $\text{cm}^{-1}$  significantly correlated with those of polysaccharide peaks, but showed considerably weaker correlation with those at 2922 and 2852  $\text{cm}^{-1}$  (Fig. S1), indicating significant contribution from carbohydrates. The peak at 1060  $\text{cm}^{-1}$  typically arises from C–O stretch in polysaccharides. However, Si–O in soil minerals also contributes to this peak (Madejová, 2003). The decomposed samples contained more ash content than initial materials (data not shown). Consistent with this, decomposed leaf litter and fine roots exhibited strong bands near 1060  $\text{cm}^{-1}$ , along with the doublets near 790  $\text{cm}^{-1}$  characteristic of soil minerals (Madejová, 2003; Pedersen et al., 2011), indicating that these bands in decomposed litters had significant contribution from soil minerals.

We estimated the preservation of lignin relative to polysaccharides during decomposition by computing the lignin/carbohydrate peak intensity ratios for decomposed material minus those of the initial litters before decomposition ( $\Delta I_{L/C}$ ). We used peaks near 898  $\text{cm}^{-1}$ , 1160  $\text{cm}^{-1}$ , 1370  $\text{cm}^{-1}$  as polysaccharide reference peaks. These peaks are often used as polysaccharide characteristics peaks because they have no significant contribution from lignin (Pandey and Pitman, 2004). Generally, N additions increased  $\Delta I_{L/C}$  values (Table 5), indicating lignin was preserved against polysaccharides. Fine roots decomposed under simulated N deposition consistently had significantly higher  $\Delta I_{1511/1160}$ ,  $\Delta I_{1511/1370}$ , and  $\Delta I_{1511/\Sigma}$  across all study sites ( $P < 0.028$ ). In comparison, the responses in leaf litter to simulated N deposition were weaker and more variable among sites, as indicated by the significant interactions between site and N treatment (Table 5). Nitrogen additions generally increased leaf litter  $\Delta I_{1511/1370}$ , while the increases were the strongest at site A (Site  $\times$  N:  $F = 7.36$ ,  $P = 0.004$ , O horizon;  $F = 5.88$ ,  $P = 0.007$ , O/A interface). Nitrogen additions also increased

**Table 6**

Pearson's correlation coefficient matrix for lignin/carbohydrates FT-IR peak intensity ratios, AIF and AIF/ASF ratio.

	AIF	AIF/ASF	$I_{1511/898}$	$I_{1511/1160}$	$I_{1511/1370}$
AIF/ASF	0.939				
$I_{1511/898}$	0.667	0.749			
$I_{1511/1160}$	0.797	0.846	0.720		
$I_{1511/1370}$	0.893	0.901	0.615	0.855	
$I_{1511/\Sigma}$	0.901	0.935	0.734	0.950	0.969

All correlation coefficients were significant at  $P < 0.001$ . We used initial leaf litter and fine roots, and decomposed litters at the end of study to generate correlation coefficients ( $n = 106$ ).

leaf litter  $\Delta I_{1511/\Sigma}$  for leaf litter at the O/A interface at all sites except site C (Site  $\times$  N:  $F = 3.44$ ,  $P = 0.042$ ). All lignin/carbohydrate FT-IR peak ratios were significantly correlated with AIF and AIF/ASF ratios determined using gravimetric methods ( $P < 0.001$ , Table 6). Among four lignin/carbohydrate peak ratios, the values of  $I_{1511/\Sigma}$  had the highest correlation with both AIF ( $R = 0.901$ ,  $P < 0.001$ ) and AIF/ASF ratios ( $R = 0.935$ ,  $P < 0.001$ , Table 6). Because the peak at 1738  $\text{cm}^{-1}$  is primarily associated with suberin in fine roots, we also calculated the suberin/carbohydrate peak intensity ratios for decomposed material minus those of the initial litters before decomposition ( $\Delta I_{S/C}$ ). Nitrogen additions did not show consistent effects on  $\Delta I_{S/C}$  values in fine roots (Table S3).

#### 4. Discussion

We reported elsewhere that simulated N deposition significantly decreased fine root mass loss during decomposition at these sites (Xia et al., 2017). Using the same set of samples in Xia et al., 2017, we sought here to understand if simulated N deposition suppressed the decomposition of specific chemical fractions within fine roots. Consistent with Hypothesis 2, our measurements of the acid-insoluble fraction (AIF, or Klason lignin) and the lignin/carbohydrate reference FTIR peak ratios both provide evidence that simulated N deposition selectively preserved lignin relative to the rest of root mass (Tables 4 and 5). Also, lignin/carbohydrate FTIR peak ratios were highly correlated with AIF concentrations and AIF/acid-soluble fraction ratios (Table 6), suggesting AIF is a good indicator for lignin in the plant litters used in this study. Because fine roots were rich in AIF (>40%, Table 2), the inhibition of AIF decomposition by N additions was the major driver of slower fine root decomposition, contributing more than two-thirds of the

**Table 5**

The differences of ratios of lignin/carbohydrate reference peaks ( $\Delta I_{L/C}$ ) between decomposed (3 years) and initial litter under simulated N deposition across four northern forests.

Litter type	Treatment	$\Delta I_{1512/898}$	$\Delta I_{1512/1160}$	$\Delta I_{1512/1370}$	$\Delta I_{1512/\Sigma}$
Leaf litter (O horizon)	Ambient	2.96 (3.85)	0.755 (0.370)	1.12 (0.20)	0.465 (0.124)
	NO <sub>3</sub> <sup>-</sup>	5.45 (2.10)	0.897 (0.410)	1.27 <sup>†</sup> (0.20)	0.544 (0.091)
Leaf litter (O/A interface)	Ambient	2.08 (3.26)	0.723 (0.374)	1.18 (0.23)	0.468 (0.125)
	NO <sub>3</sub> <sup>-</sup>	4.20 (2.60)	0.994 <sup>*</sup> (0.264)	1.30 <sup>‡</sup> (0.26)	0.557 <sup>‡</sup> (0.095)
Fine roots	Ambient	7.98 (5.03)	0.435 (0.386)	0.274 (0.172)	0.208 (0.099)
	NO <sub>3</sub> <sup>-</sup>	7.61 (4.60)	0.783 <sup>*</sup> (0.374)	0.561 <sup>**</sup> (0.190)	0.340 <sup>**</sup> (0.099)

Values are shown as means (SD) of  $\Delta I_{L/C}$  values of ambient or NO<sub>3</sub><sup>-</sup>-amended plots across four study sites ( $n = 12$ ). Higher values of  $\Delta I_{L/C}$  indicate that more lignin is preserved relative to carbohydrates during litter decomposition. \*, \*\* denote significant main effects of simulated N deposition at  $P < 0.05$  and  $P < 0.01$ , respectively. † refers to a significant interaction of site and N at  $P < 0.05$ . The sum of intensity at 898  $\text{cm}^{-1}$ , 1160  $\text{cm}^{-1}$ , and 1370  $\text{cm}^{-1}$  was used as the carbohydrate reference when calculating  $\Delta I_{1512/\Sigma}$  values.

additional root detritus that accumulated under simulated N deposition at our study sites.

Because fine roots may contain a significant portion of suberin (~5–20%, estimated from Schreiber et al., 2005), and AIF may also be partially derived from suberin (Preston et al., 2009), it is possible that suberin also contributed to the additional root mass accumulation. However, the changes in the intensity of the peak associated with suberin in fine roots did not show consistent responses to simulated N deposition during decomposition (Table S3). Also, AIF isolated from fine roots in this study exhibited similar spectra with Kraft lignin (data not shown), with much weaker feature bands for suberin compared to the IR spectra of suberin-rich materials (e.g., birch bark IR spectra that showed strong peaks at 2920, 2851, and 1735 cm<sup>-1</sup>, Ferreira et al., 2013). We conclude that suberin was less important than lignin in explaining the accumulation of decomposing root mass under elevated N deposition.

Our observations of leaf litter and fine root decomposition help reconcile the contrasting effects of N additions on different litter materials. Knorr et al. (2005) conducted a meta-analysis on the effects of N additions on leaf litter decomposition and found that N additions inhibited decomposition when litter was lignin-rich, but stimulated or had neutral effects on decomposition when litter was low in lignin. In our study, simulated N deposition tended to decrease lignin degradation in leaf litter (Tables 3 and 5). However, such an effect did not lead to increased overall mass retention (Table 3), probably because AIF was low in leaf litter (14.4–16.8%, Table 2), and the small increase in AIF can be easily offset by increased decomposition of other chemical classes. We did observe that N additions significantly stimulated the initial degradation of non-structural carbohydrates in leaf litter (Table 3). Nitrogen additions have also been observed to stimulate degradation of cellulose via enhanced cellulose-degrading enzyme activity in other N enrichment studies (e.g., Carreiro et al., 2000; Sun et al., 2016). Consequently, although simulated N deposition tends to generally inhibit lignin degradation, the ability of this effect to meaningfully increase the amount of litter mass remaining (and thus cause the accumulation of soil organic matter) apparently depends on the quantity of lignin within the initial substrates.

The inhibition of lignin degradation by simulated N deposition is consistent with evidence from other studies that high levels of exogenous N suppress microbial metabolism of lignin. Under laboratory conditions, high N availability repressed metabolic activities associated with lignin degradation in the fungus *Phanerochaete chrysosporium* (Boominathan et al., 1990), while N limitation stimulated lignin-degrading activity (Reid, 1983). Previous work at our study sites observed that simulated N deposition suppressed the activity of the lignin-degrading extracellular phenol oxidase and peroxidase (DeForest et al., 2004; Freedman et al., 2016), and down-regulated expression of genes responsible for synthesis of lignin-degrading extracellular enzymes (Edwards et al., 2011). In a recent soil metagenomic analysis at these study sites, simulated N deposition shifted the saprotrophic microbial community towards bacterial metabolic pathways that have less oxidative power in lignin degradation than fungal pathways (Freedman et al., 2016).

Simulated N deposition also caused a slower loss of condensed tannins (CTs) and soluble proteins in both leaf litter and fine roots (Table 3, Table 4). Notably, CTs are structurally and biosynthetically related to lignin, and are also degraded by oxidative enzymes (Hagerman and Butler, 1991). The changes in proteins were positively correlated with that of condensed tannins during decomposition in both leaf litter and fine roots (Table S1). Condensed tannins can form recalcitrant complexes with proteins (Bravo, 1998). Greater proportions of CTs remaining may help stabilize proteins during decomposition, and thus lead to a slower loss of

proteins. Because CTs can be toxic and inhibit soil enzyme activity (Ushio et al., 2013), the slower degradation of CTs in soils may contribute to the general reduction of microbial biomass and heterotrophic respiration as has been observed in many N enrichment studies (Treseder, 2008; Janssens et al., 2010).

Lignin/carbohydrates IR peak rates exhibited high correlations with AIF and AIF/ASF (Table 6). Gravimetric AIF and ASF measurements obtained through sequential extraction have conventionally been regarded as lignin and holocellulose respectively (e.g., Taylor et al., 1989; Berg, 2000). The recalcitrance of AIF has been demonstrated by a number of reports that higher initial AIF contents or AIF/N ratios resulted in slower litter decomposition (Taylor et al., 1989; Berg, 2000; Adair et al., 2008). However, the ability of AIF to accurately predict lignin concentration is often questioned because AIF is not purely lignin, but a mixture of lignin and other complex substrates such as cutin, suberins, and CT-protein complexes (Preston et al., 2009). In this study, AIF was highly associated with lignin/carbohydrate reference band ratios, and the AIF/ASF ratio explained 87.3% of variation in  $I_{1511/\Sigma}$  values across leaf litter and fine roots. The strength of these relationships supports the idea that AIF is a good predictor of lignin in plant chemistry and litter decomposition studies.

Finally, this study supported the idea that differences in chemistry among substrate types persisted, rather than disappeared, during decomposition (Figs. 1 and 2). Whether chemical profiles of litter substrates converge, remain, or diverge during decomposition is a long-debated question that is important for understanding the chemical complexity of soil organic matter (Wickings et al., 2012). In our study, although leaf and fine root litter composition quickly converged for labile components such as soluble phenolics and NSCs, differences in more recalcitrant components such as lignin, polysaccharides, CTs, and lipids from initial materials persisted even after >80% of the initial leaf litter mass decomposed. Reports indicating that initial chemical profiles converged over the course of decomposition often compared litter types with smaller initial differences in lignin compared to those between leaf litter and fine roots in this study (e.g., Wallenstein et al., 2013, who compared aspen and pine leaf litters with lignin concentrations of 20% vs. 34%, respectively), or were based on elemental analysis (e.g., Moore et al., 2011). Our study implies that the large initial chemical differences between leaf litter and fine roots could continue to shape the roles of these major litter sources in belowground processes such as the development of soil food webs, carbon and nutrient cycling, and soil organic matter stabilization.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2017.04.011>.

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