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Comparison of the chemical alteration trajectory of *Liriodendron tulipifera L.* leaf litter among forests with different earthworm abundance

Timothy R. Filley,[1] Melissa K. McCormick,[2] Susan E. Crow,[1] Katalin Szlavecz,[3] Dennis F. Whigham,[2] Cliff T. Johnston,[4] and Ronald N. van den Heuvel[2,5]

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[1] To investigate the control of earthworm populations on leaf litter biopolymer decay dynamics, we analyzed the residues of *Liriodendron tulipifera L.* (tulip poplar) leaves after six months of decay, comparing open surface litter and litter bag experiments among forests with different native and invasive earthworm abundances. Six plots were established in successional tulip poplar forests where sites varied in earthworm density and biomass, roughly 4–10 fold, of nonnative lumbricid species. Analysis of residues by diffuse reflectance Fourier transform infrared spectroscopy and alkaline CuO extraction indicated that open decay in sites with abundant earthworms resulted in residues depleted in cuticular aliphatic and polysaccharide components and enriched in ether-linked lignin relative to open decay in low earthworm abundance plots. Decay within earthworm-excluding litter bags resulted in an increase in aliphatic components relative to initial amendment and similar chemical trajectory to low earthworm open decay experiments. All litter exhibited a decline in cinnamyl-based lignin and an increase in nitrogen content. The influence of earthworm density on the chemical trajectory of litter decay was primarily a manifestation of the physical separation and concentration of lignin-rich and cutin-poor petioles with additional changes promoted by either microorganisms and/or mesofauna resulting in nitrogen addition and polysaccharide loss. These results illustrate how projected increases in invasive earthworm activity in northern North American forests could alter the chemical composition of organic matter in litter residues and potentially organic matter reaching the soil which may result in shifts in the aromatic and aliphatic composition of soils in different systems.

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1. Introduction

[2] Both substrate quality, i.e., plant nutrients and biopolymer composition, and local environmental conditions, i.e., temperature and moisture, have been considered as the primary controls of the path of litter decomposition by microbial agents [*Moore et al.*, 1999]. Numerous studies have shown strong correlations between plant chemistry (e.g., lignin, nitrogen, and Ca^{2+} content) and the rate of litter decomposition across forest floors [e.g., *Berg et al.*, 1996; *Lorenz et al.*, 2000; *Hobbie et al.*, 2006]. In northern North American (NA) forests there is, however, an increasing awareness of the role that detritivore macroinvertebrates, specifically earthworms, have on litter decay dynamics and the associated nature of stabilized soil organic matter [*Bohlen et al.*, 2004a; *Reich et al.*, 2005; *Brussaard et al.*, 2007; *Heneghan et al.*, 2007]. The study of the earthworm-litter-soil system is particularly relevant today as most identified earthworm species in northern NA forests are introduced from Europe and Asia [*Hendrix and Bohlen*, 2002; *James and Hendrix*, 2004]. It is anticipated that over the next few decades they will expand farther into northern forests driven by both global, e.g., rising surface temperature, and local, e.g., soil transport, discarded fishing bait, land use change, factors [*Hendrix and Bohlen*, 2002].

[3] A well-documented effect of earthworm introduction into forests with no native earthworm populations is the overall depletion of organic horizons and forest floor litter [*Alban and Berry*, 1994; *Gundale*, 2001; *Bohlen et al.*,

[1]Department of Earth and Atmospheric Sciences and the Purdue Climate Change Research Center, Purdue University, West Lafayette, Indiana, USA.
[2]Smithsonian Environmental Research Center, Edgewater, Maryland, USA.
[3]Department of Earth and Planetary Sciences, Johns Hopkins University, Baltimore, Maryland, USA.
[4]Department of Agronomy (Crop, Soil, and Environmental Sciences), Purdue University, West Lafayette, Indiana, USA.
[5]Now at Landscape Ecology, Institute of Environmental Biology, Utrecht University, Utrecht, Netherlands.

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2004b; *Hale et al.*, 2005; *Eisenhauer et al.*, 2007]. Mesocosm and field manipulation studies have demonstrated that earthworm activity can dramatically influence the decay rate, physical breakdown, and translocation of litter below ground into stabilized structures above the background microbial action [*Blair et al.*, 1995; *Cortez*, 1998; *Seeber et al.*, 2006; *Hedde et al.*, 2007; *Heneghan et al.*, 2007]. Additionally, the abundance and specific feeding habits of earthworms strongly influence the nature and rate of nutrient cycling and soil organic matter dynamics [*Scheu and Wolters*, 1991; *Bohlen et al.*, 2004c]. However, while much is known of the impact of earthworm invasion on litter decay and nutrient cycling in general, their role in the alteration of plant biopolymers is rarely determined and past research has primarily focused on cast-stabilized fractions [e.g., *Guggenberger et al.*, 1996; *Bossuyt et al.*, 2005].

[4] The impact of earthworms on the relative abundance and chemical alteration of plant biopolymers such as lignin, condensed and hydrolysable phenolics, suberin, and cutin is important because the chemistry of the degraded residue can impact its subsequent utilization by microbes and other micro- and mesofauna [*Field and Lettinga*, 1992; *Satchell*, 1967; *Nicolai*, 1988; *Hendriksen*, 1990] and potentially influence soil organic matter dynamics. The fate of these biopolymers are of particular interest to soil scientists as they are considered to be major contributors to stabilized soil carbon [*Baldock and Preston*, 1995; *Nierop*, 1998; *Nierop et al.*, 2003; *Mikutta et al.*, 2006]. While earthworms are thought to be unable to degrade lignin, they readily degrade and digest carbohydrates and proteins [*Brown and Doube*, 2004; *Nakajima et al.*, 2005]. In addition, cuticular and suberinitic materials may be degraded by earthworms through the action of enzymes such as serine-proteases which have been shown to have esterase activity [*Nakajima et al.*, 2005]. Although earthworms contain a complex assemblage of microbes in their digestive tract, which aid them in organic matter decomposition, there are indications that their internal bacterial and fungal composition, along with their subsequent enzyme activity, reflects that of the ingested soil and litter microbes [*Lattaud et al.*, 1998; *Li et al.*, 2002]. The actual composition of these hosted microorganisms is crucial as both fungi and bacteria are capable of utilizing polysaccharides and cutin but the decomposition of lignin and many condensed phenolics are restricted to certain groups of fungi (see reviews in the work of *Eriksson et al.* [1990]).

[5] Therefore, whether or not lignin, cutin or suberin as classes of biopolymers are considered relatively resistant to decay in a forest floor and candidates to accumulate in surface soils should be strongly a function of the ability of the litter and soil microbes and animals to express the necessary decomposer enzymes (e.g., ligninases for lignin decomposition). By most accounts earthworms should have an important impact on this balance.

[6] In this study we sought to demonstrate through field-based litter manipulations at a forested site in eastern Maryland how differences in earthworm abundances will cause a common litter amendment to decay along different chemical pathways. Our study plots were established in six stands that varied in earthworm abundance and successional stage where, in general, old successional sites exhibited low earthworm abundances compared to young successional sites. Our goal was to inform research about the different potential fates of plant biopolymers in litter when earthworms are present and how soils may inherit distinctly different chemical signatures from the same litter source in the same overall regional environment based upon differences in localized macroinvertebrate detritivory. Although earthworm abundances and stand successional age are linked in this system, our litter bag experiments that exclude earthworm activity, allow us to distinguish differences in stand age and earthworm ecology as the major control on resultant litter biopolymer decay trajectory. Herein, we speculate that the action of earthworms may be a major control on the overall aromaticity or aliphaticity of resultant soil organic matter.

2. Methods

2.1. Study Site

[7] The 2886-hectare Smithsonian Environmental Research Center (SERC) lies along the Western Shore of Maryland on the Rhode River estuary (http://www.serc.si.edu/). The forested area at SERC is a combination of stands at various successional stages from agricultural abandonment or logging over the last 200 years with minor areas that have no record of ever being cleared. Experimental decay plots were established in stands of different ages that also varied in earthworm abundance [*Szlavecz and Csuzdi*, 2007]. Since the old sites have a relatively higher abundance of oak and hickory species, which are high in tannin content and low in N and are known to be less palatable for earthworms, *Szlavecz and Csuzdi* [2007] proposed that earthworm distributions reflected this difference.

[8] The plots described herein were situated in stands that ranged from 50 to 70 years since abandonment (young successional sites) and 150 years or more since abandonment (old successional sites). The SERC forests are of the tulip poplar association [*Parker et al.*, 1989; *Brown and Parker*, 1994] with young forests dominated by tulip poplar (*Liriodendron tulipifera L.)*, sweetgum (*Liquidambar styraciflua L.)*, red maple (*Acer rubrum L.)*, and beech (*Fagus grandifolia Ehrh)* and old forests dominated by tulip poplar, beech, and several oak (*Quercus, sp.p)* and hickory (*Carya, spp.)* species.

[9] Soils at all study sites are fine sandy loams of the Collington (Fine-loamy, mixed, active, mesic Typic Hapludults) and Monmouth (fine, mixed, active, mesic Typic Hapludults) Series that have formed on sedimentary soils from the Pleistocene Talbot formation [*USDA*, 1968]. Land-use history includes plantation agriculture of a variety of crops that resulted in only minor differences in mineralogy and soil chemistry [*Pierce*, 1974]. The average soil pH is 5.71 + 0.12 (S.E.) in mature forests and 5.09 + 0.06 (S.E.) in successional forests [*Correll*, 1974]. Leaf input rates at SERC range typically from 330 $g/m^2/y$ to 450 $g/m^2/y$ with high and low amounts ranging from 272$g/m^2/y$ to 525$g/m^2/y$ (G. Parker, unpublished data). Mean rainfall in the region is 114.6cm and mean annual temperature is 13°C (D. Correll, T. Jordan, and J. Duls, unpublished data) which was consistent with the 2004–2005 experiment and sampling period.

2.2. Manipulation Plots

[10] In May 2004, six 3 × 3 m plots were established within a 2 km^2 area in SERC; three in old successional forest stands and three in young successional forest stands, which previous work had shown, corresponded to sites of respectively low and high earthworm abundance [*Szlavecz and Csuzdi*, 2007]. Each 3 × 3 m plot was further divided into a 0.5 × 0.5 m grid. Each grid square had a 25 cm diameter subplot placed in the center surrounded by a perforated, 5 cm high plastic ring made of 10 cm wide wallbase, cut in half lengthwise. As part of a larger investigation into the effect of tulip poplar wood and leaf amendments on the soil environment, each ringed subplot was randomly assigned to receive replicate decay treatments of ~1000 cm^3 chipped tulip poplar wood, ~1000 cm^3 crushed tulip poplar leaves, or no amendment. An additional control treatment with natural leaf fall was established adjacent to established subplots. Amendments were added to each ringed subplot annually since 2004. The data from the 2004 open decay experiment are presented herein. A common tulip poplar amendment was chosen because tulip poplar is the dominant tree species (by biomass) for all of the study stands, regardless of successional age. The focus of the present manuscript is only on the subplots associated with the leaf addition treatment.

[11] Freshly fallen, intact tulip poplar litter was collected by hand in the fall of each year prior to spring addition, air dried at approximately 20°C and stored over winter before addition in the following May. After amendment each plot was screened with 1.25 cm mesh screen suspended above the plot surface by approximately 5 cm in the late summer/early fall to minimize mixed litter fall. The litter fall collected off of the screen was added to the control treatments in late November. In this way we ensured that all chemical modifications were on a common litter addition and the trajectory of biopolymer alteration could be easily assessed. Each subplot was sampled by hand using forceps and inspected, so as to not include animal droppings and casts, from a uniform area within each ring. These subsamples were then combined for each plot, dried, and ground for processing.

[12] In an attempt to separate the role of macroinvertebrates from the role of fungi, bacteria, and micro- and mesofauna in litter decay at SERC, three 10 cm × 15 cm litter bags, screened with fiberglass window screen to 1 mm, and containing 10 g of tulip poplar litter were also set out within the plots. The mesh was of a sufficient size to exclude all macrofauna contributions. The bags were loaded with the same amendment material as the open decay experiments and fixed to the soil surface in April of 2005 and harvested in November of that year. Upon harvest litter material from each bag was dried in a convection oven at 50°C for 48 h and weighed. Any soil adhering to the outside of the litter bag was carefully removed and no adhering soil was observed on leaf material. The three bags from each plot were then combined and subsampled for biopolymer and chemical analysis.

2.3. Biopolymer and Elemental Chemical Analysis

[13] Initial leaf amendment and decayed material was dried in a convection oven at 50°C and powdered to a flour consistency with a liquid N_2 SPEX CertiPrep (Metuchen, NJ, USA) freezer mill to ensure homogeneity in analysis. Intact leaves were dissected with a razor to separate petioles from leaf body then weighed and ground separately. The relative dry weight proportion of petiole to leaf (n = 20) was 10.4% ± 0.6 for a sampling of the tulip poplar used herein. However, a wide variation in this proportion was observed ranging from 7.3% to 19.0%. It is important to consider that this variation has implications for the mean biopolymer chemistry of litter fall and ultimate residue if petiole and leaf body have distinct chemistries and represent different proportions of the final residue. Plant residues were analyzed for organic carbon (C) and nitrogen (N) content using a Carlo Erba 1108 elemental analyzer. Alkaline cupric-oxide (CuO) oxidation [*Hedges and Mann*, 1979; *Goñi and Hedges*, 1990] was used to assess content of extractable lignin phenols and substituted hydroxy fatty acids from cutin. The biopolymer extractions utilized Monel reaction vessels (Prime Focus, Inc. Seattle, WA, USA). Ethyl vanillin and DL-12 hydroxystearic acid were added as internal recovery standards (IRS) for lignin and SFA, respectively, post reaction and prior to extraction.

[14] Lignin phenols were quantified by analysis of the trimethylsilane (TMS) derivatives of vanillyl (V-lignin)-based (i.e., vanillin, acetovanillone, vanillic acid); syringyl (S-lignin)-based (i.e., syringealdehyde, acetosyringone, syringic acid); and cinnamyl (C-lignin)-based (i.e., *p*-hydroxycinnamic acid and ferulic acid) monomers using extracted ion internal calibration curves. The TMS derivatives of nine SFA were assessed by extracted ions based upon similar proxy standard calibration curves relative to the IRS DL-12, hydroxystearic acid. These nine SFA included 16-hydroxyhexadecanoic acid, hexadecanoic diacid, 18-hydroxyoctadec-9-enoic acid, 9,16 & 10,16 dihydroxyhexadecanoic acid, 9-octadecene-1,18-dioic acid, 7&8-hydroxyhexadecane dioic acid, 9,10,18-trihydroxyoctadec-12-enoic acid, and 9,10,18-trihydroxyoctanoic acid. As SFA proxy standards were used, calibration curves represent reasonable assessments of concentration by selected ions. A Hewlett-Packard (5971) quadrupole mass spectrometer interfaced to a 5890 series II gas chromatograph was used in the quantification. Duplicate CuO analyses were performed for each sample. Additionally, the leaf and petiole were analyzed separately for their biopolymer composition. In general, mean standard reproducibility of the analytical method for individual lignin phenol compounds ranged between 2 and 5% and from 2 and 9% for hydroxyl fatty acids in lab reference NIST standard peach leaf.

[15] In this study the SV-lignin, which is the sum of vanillyl and syringyl monomers, (frequently termed $\Lambda 6$ in mg/100 OC), SVC-lignin, which is the sum of vanillyl, syringyl, and cinnamyl monomers, (frequently termed $\Lambda 8$ in mg/100 mg OC), and the sum of substituted fatty acids (ΣSFA in mg/100 mg OC) are the terms used to express the relative concentration of extractable lignin phenols and cutin, respectively. Additionally, because the relative abundance of acid (Ac) and aldehyde (Al) based monomers, e.g., vanillic acid to vanillin (Ac/Alv) and syringic acid to syringealdehyde (Ac/Al_S) increases with increasing microbial degradation of plant tissue, these ratios have been used to infer lignin degradation state [*Hedges et al.*, 1988].

Table 1. Earthworm Species Composition, Density (Individuals*m^{-2}), Total Density, and Biomass (g*m^{-2}) in Old (Low Earthworm Abundance), and Young (High Earthworm Abundance), Successional Forests at the Smithsonian Environmental Research Center (SERC)[a]

Species	Ecological Category	Old Forests	Young Forests
Lumbricus rubellus (Hoffmeister, 1843)	Epi-endogeic	2.2 (1.6)	24.0 (9.2)
Lumbricus friendi (Cognetti, 1904)	Anecic	0.9 (0.9)	0.9 (0.4)
Octolasion lacteum (Örley, 1881)	Endogeic	0.4 (0.4)	18.2 (11.7)
Allolobophora caliginosa (Savigny, 1826)	Endogeic	0.0 (0.0)	1.8 (1.2)
Aporrectodea rosea (Savigny, 1826)	Endogeic	0.0 (0.0)	1.8 (1.8)
Eisenoides loennbergi (Michaelsen, 1894)	Endogeic	6.7 (6.7)	0.0 (0.0)
Lumbricidae juv. (excl. *Lumbricus*)		3.6 (1.8)	22.2 (3.6)
Lumbricus sp. juv.		4.0 (4.0)	60.4 (6.0)
Total density[b]		17.8 (13.2)	130.2 (17.1)
Total biomass[c]		10.0 (6.5)	41.3 (1.2)

[a]Numbers are mean of the three plots at each forest type; standard deviations are in parentheses.
[b]Here $p < 0.01$ (Student's t-test, two tailed).
[c]Here $p < 0.001$.

[16] Diffuse Reflectance Fourier transform infrared spectroscopy (DR-FTIR) was used to analyze ground surface litter from open decay experiments, leaf bag, and initial leaf body and petiole samples using a Perkin Elmer Model 2000 GX FTIR spectrophotometer (Norwalk, CT) equipped with a liquid nitrogen cooled MCT detector and a KBr beamsplitter. The system employed an EasiDiff accessory (Pike Technologies, Madison, WI). Typically, 15 mg of air-dried sample was mixed with 285 mg of spectral grade KBr (Pike Technologies, Madison, WI, lot # 43004) using an agate Wig-L-Bug for 30 seconds (Densply Rinn, IL, model # 3110-3A). The sample-KBr mixture was placed in 9 mm sample cups. Reference FTIR spectra were obtained from spectral-grade KBr that was subjected to the same physical mixing conditions.

[17] For each sample a total of 64 individual scans, which were then signal averaged, were obtained using an optical resolution of 4 cm^{-1} in the region from 4000 to 580 cm^{-1}. These were processed using the Kubelka-Munk transformation using Grams/32 AI Version 6.0 (Galactic software, Salem, NH). The mean spectra for the old and young successional sites were obtained by taking the spectral average of the three replicates which also allowed for evaluation of changes between sites though spectral subtraction.

2.4. Earthworm Sampling

[18] Earthworms were sampled in early June 2005 in three locations around each plot, 1–2 m from a different edge using standard formalin techniques [*Raw*, 1959]. First, a 50 cm × 50 cm quadrat was laid down, then surface litter was removed and 20 L 0.2% formalin was used to flush out earthworms (added over the course of ~20 min such that it did not flow out of the quadrat). This sampling was performed in the context of a long term fall and spring earthworm monitoring project, discussed in more detail in following sections. It has shown clear patterns of earthworm density, biomass and distribution, stable at a decadal timescale, in keeping with the hypothesis tested herein, and the data are provided to show that our plots lie spatially within this context of earthworm dynamics. Earlier assessments [*Szlavecz and Csuzdi*, 2007] as well as occasional digging around the plots previously showed that native endogeic species (e.g., Diplocardia spp.) are absent from these areas. These species are a concern, because the formalin technique tends to underestimate their abundance [*James*, 1990]. All worms emerging within the quadrat were collected and weighed, killed in ethanol, fixed in 4% formalin, and preserved in 75% ethanol. Adults were identified to species, juveniles to the smallest possible taxonomic category (genus or family). Identification and nomenclature followed *Csuzdi and Zicsi* [2003].

2.5. Soil Microbial Enzyme Activity

[19] To examine the effects of leaf amendments on soil microbial communities, we first examined two extracellular enzymes related to breakdown of cellulose and phenols, β-1,4-glucosidase, and polyphenol oxidase, respectively, in fresh soils using an adaptation of the method described by *Sinsabaugh et al.* [1991]. Soils were sampled in October 2004 using a 2.5 cm diameter and 5 cm deep core. One core was collected from each of 18 subplots in each of the 6 sites. Each core was homogenized and passed through a 2 mm sieve to remove large particulate organic matter and improve reproducibility. All enzyme analyses were performed on fresh soil less than 24 hours after collection. Approximately 2 g of fresh soil was mixed in a commercial kitchen blender in 50 mM acetate buffer (per liter: 4.374g sodium acetate trihydrate and 1.1 ml glacial acetic acid). 0.75 ml of this homogenate was mixed with 0.75 ml of each substrate solution (5mM pNP-beta-glucopyranoside for the β-1,4-glucosidase assay and 5 mM pyrogallol for the polyphenoloxidase assay). Differences in activity between amendment treatments and sites were tested using ANOVA with Earthworm Abundance as a fixed main effect and site nested within Earthworm Abundance (Systat v. 10.2).

3. Results

3.1. Earthworm Diversity and Abundance

[20] At SERC, plots yielded from 1 to 4 different earthworm species with all three major earthworm ecological categories, epigeic, endogeic, anecic [*Bouché*, 1977] although individual plots may not show species in each feeding habit (Table 1). All species belonged to the Lumbricidae family, and all but one were nonnative species. The only native earthworm, *Eisenoides loennbergi*, was found in one of the old successional sites where overall numbers and biomass of earthworms were low. *Lumbricus rubellus* and *Octolasion lacteum* were the most abundant species in the young successional sites and likely greatly impact leaf litter

Table 2. Chemical Composition and Concentration of Biopolymers Extracted by Alkaline CuO Oxidation in Fresh and Degraded Open Surface Litter Amendments in Old, Low Earthworm Abundance (LEW), and Young, High Earthworm Abundance (HEW) Successional Forests at the Smithsonian Environmental Research Center (SERC)[a]

Sample	SV-Lignin	SVC-Lignin	ΣSFA	C/V	S/V	Ac/Al(v)	Ac/Al(s)	SFA/SV-Lignin	Mass Loss	Wt% C	Wt% N
	mg/100 mg OC										
Whole Leaf	8.12	8.42	4.62	0.15	3.02	0.13	0.14	0.56	-	45.3	0.9
Petiole	11.4	11.6	3.31	0.06	3.2	0.11	0.11	0.29	-	44.2	0.3
Leaf Body	3.04	3.55	7.11	0.54	2.18	0.2	0.25	2.33	-	43.7	1.1
LEW sites -open litter-	5.90 *(0.82)*	6.40 *(0.76)*	7.70 *(0.56)*	0.23 *(0.04)*	2.20 *(0.31)*	0.20 *(0.30)*	0.17 *(0.05)*	1.3 *(0.32)*	-n.d.-	37.4 *(3.5)*	1.4 *(0.2)*
HEW sites -open litter-	13.10 *(2.0)*	13.30 *(2.00)*	2.50 *(0.09)*	0.05 *(0.01)*	2.50 *(0.31)*	0.13 *(0.01)*	0.13 *(0.03)*	0.2 *(0.09)*	-n.d.-	37.7 *(3.9)*	1.0 *(0.2)*
Probability LEW vs. HEW (Student's t test, two-tailed)	0.005	0.004	0.001	0.002	0.033	0.01	0.04	0.003		0.9	0.1
LEW sites -litter bag-	4.6 *(0.17)*	4.90 *(0.17)*	11.30 *(0.82)*	0.33 *(0.04)*	2.90 *(0.03)*	0.18 *(0.01)*	0.14 *(0.01)*	2.48 *(0.27)*	23.1 *(3.5)*	42.7 *(1.4)*	1.1 *(0.0)*
HEW sites-litter bag-	5.2 *(0.86)*	5.60 *(0.87)*	11.40 *(1.3)*	0.31 *(0.04)*	2.90 *(0.09)*	0.14 *(0.02)*	0.14 *(0.02)*	2.10 *(0.57)*	24.7 *(3.4)*	39.9 *(0.5)*	1.1 *(0.1)*
Probability LEW vs. HEW (Student's t test, two-tailed)	0.3	0.3	0.5	0.3	0.9	0.02	0.8	0.3	0.6	0.04	0.5

[a]Values for LEW and HEW sites represent means of three plots each and each plot is composed of triplicate experiments, physically pooled then analyzed. Standard error (SE) among field replicates are in parentheses.

disappearance and surface soil mixing. *Lumbricus friendi* is an anecic species that burrows vertically and inhabits mineral soil while feeding on litter on the soil surface. The native *E. loennbergi* and *O. lacteum* are endogeic worms that move horizontally and ingest organic as well as mineral soil. The abundant *L. rubellus* is an epi-endogeic species that feeds on leaf litter and surface organic matter and also lives close to the surface. Only a subset of the species reported by *Szlavecz and Csuzdi* [2007] was present in these samples. The sampling carried out in that study included more seasons and, more importantly, more vegetation types and microhabitats.

[21] Mean total density per plot ranged between 4.0 and 22.7 individuals/m^2 and 114.7 and 145.3 individuals/m^2 for old and young sites, respectively. Biomass values varied between 4.1 and 16.9 g/m^2 (old sites) and 40.0 and 42.4 g/m^2 (young sites). In our study plots both total density (individuals/m^2) and total live biomass (g/m^2) were significantly higher in the young sites then in the old sites (Table 1, two-tailed t-tests, $p < 0.001$ and $p < 0.01$ for density and biomass, respectively). Both adult ($p < 0.01$) juvenile ($p = 0.01$) earthworm densities were higher in the young plots. The 2005 earthworm sampling was consistent with the results of [*Szlavecz and Csuzdi*, 2007] which conducted sampling at several young and old successional sites at SERC in 1998, 1999, and 2006: young successional forests at SERC had consistently higher earthworm density and biomass, from here out referred to as "high earthworm sites" (HEW sites) than old successional forests, from here out referred to as "low earthworm sites" (LEW sites).

3.2. Surface Soil Microbial Enzyme Activity

[22] Polyphenol oxidase activity (μmol/hr) was greater in surface soils from HEW (0.78 ± 0.06) than LEW (0.63 ± 0.06) sites ($r^2 = 0.135$, p (2-tailed) = 0.015). Similarly, the activity of β-1,4-glucosidase (μmol/hr) was greater in soils from HEW (0.34 ± 0.03) than LEW (0.27 ± 0.02) sites ($r^2 = 0.114$, p (2-tailed) = 0.006). The relative activities between these two enzymes among the sites showed no distinction with earthworm density or biomass among sites.

3.3. Mass Changes and Elemental C and N

[23] Visual inspection of open surface plots indicated that by 6 months of decay much of the soft leaf tissue had been degraded in HEW sites, with the remaining litter residue as petioles, blackened and smaller in size than starting amendments. The LEW sites still maintained much of the added litter obscuring the mineral soil below, while the HEW sites commonly exhibited areas of exposed mineral soil. No attempt was made to quantify the overall mass loss in open experiments to avoid disturbing fungal activity but as petioles, on average represent on 10% of litter mass, we estimate up to 90% of the added litter was removed in the HEW sites. For the litter bag experiments, the HEW and LEW sites showed equivalent mass losses within experimental error at 23.1 and 24.7%, respectively (Table 2).

[24] For the initial tulip poplar amendment, petioles and leaf body had near equivalent wt % C but petioles had only one third the content of N at 0.34 wt % as compared to 1.05 wt % in the leaf body (Table 2). Litter bag decay resulted in changes for both C and N from the initial whole leaf values with C exhibiting a drop on average of 5.7 to

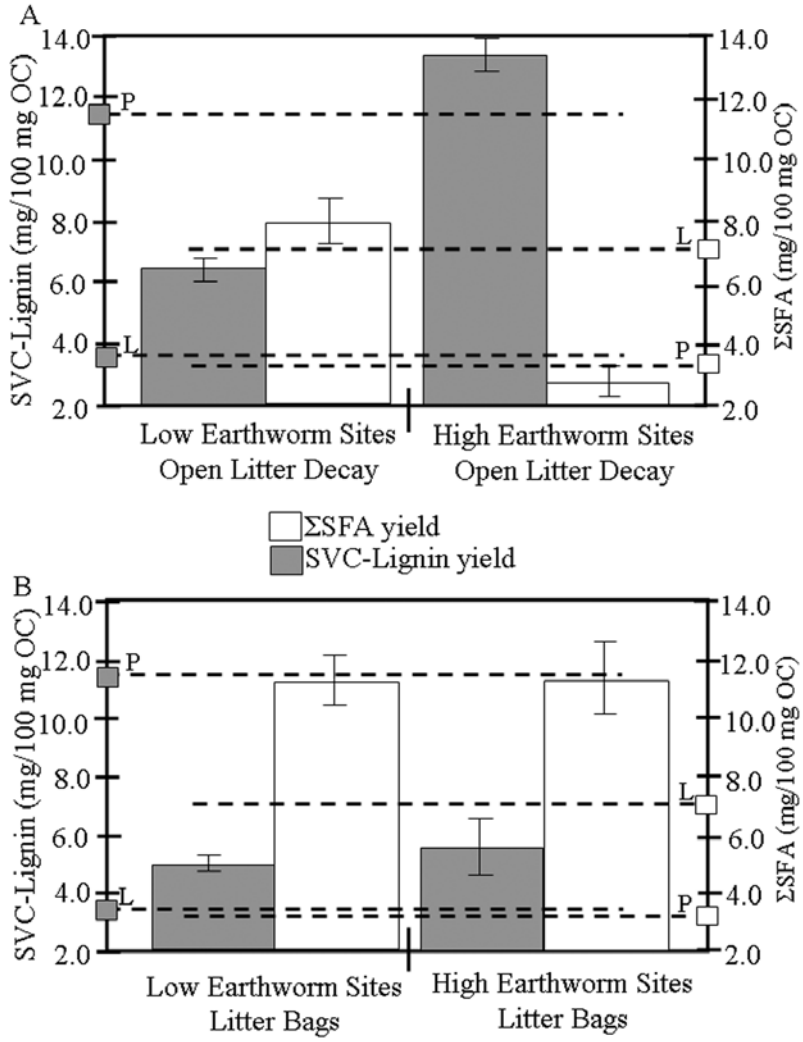

Figure 1. Chemical composition and concentration of CuO extracted lignin phenols and substituted fatty acids in fresh and degraded litter amendment experiments in high earthworm abundance (HEW) (sites 4–6) and low earthworm abundance (LEW) (sites 1–3) sites at the Smithsonian Environmental Research Center (SERC). The sum of 8 lignin phenols (SVC-lignin) and substituted fatty acids (ΣSFA) from cutin in open decay experiments (a) and litter bags (b) are shown. These values for isolated leaf body and petioles from the initial amendment are illustrated by the solid and open squares, respectively, along with the stippled horizontal lines.

11.9% from initial C content and the N increased by up to 22.3% of initial values. The greatest changes in C were seen in the open decay experiments where residue's C content dropped on average by up to 17% of initial content. The greatest change in N was observed for the LEW open decay experiments, where, on average, they increased by nearly 52% above the initial amendment. For the open decay experiments there was no distinction among the LEW and HEW sites with respect to residue wt % C but the mean LEW wt % N values were significantly greater than the HEW residues. In contrast, the litter bag residues exhibited no distinction in wt % N while the HEW sites resulted in a significantly lower C content (Table 2).

3.4. Biopolymer Characteristics in Fresh and Degraded Litter

3.4.1. SVC-Lignin and ΣSFA Analysis of Litter

[25] The leaf body and petioles of the initial amendment were different in both SVC-lignin and ΣSFA composition and concentration (Table 2). Petioles were relatively enriched in SVC-lignin (11.6 mg/g OC) and depleted in ΣSFA (3.31 mg/g OC) with respect to the leaf body (3.55 and 7.11 mg/g OC, respectively). Additionally, the C/V and S/V ratios were distinct between the two components with petioles being nearly devoid of C-lignin but relatively enriched in S-lignin (Table 2). It is interesting to note that by these parameters the petioles more resembled the general characteristics of woody tissue lignin [*Hedges and Mann*,

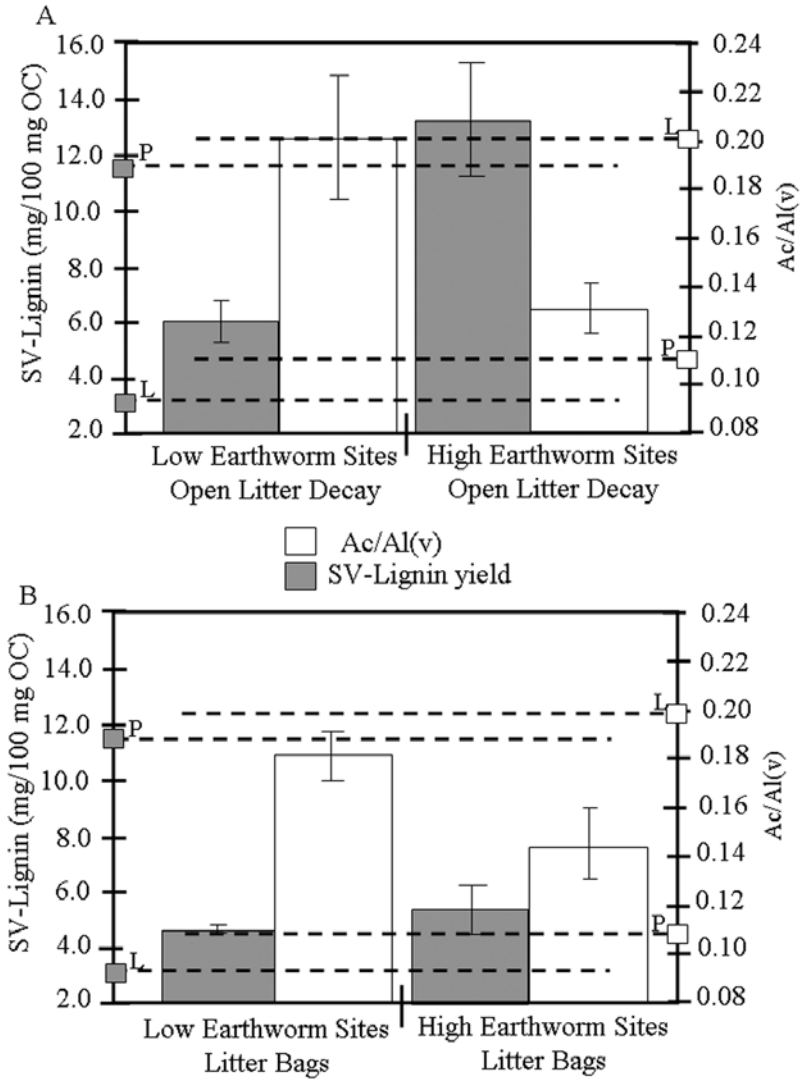

Figure 2. Chemical composition and concentration of CuO extracted lignin phenols and substituted fatty acids in fresh and degraded litter amendment experiments in high earthworm abundance (HEW) and low earthworm (LEW) sites at SERC. The 6 carbon-carbon and ether-linked lignin phenols (SV-lignin) and the relative oxidation state of vanillyl lignin (Ac/Alv) in open decay experiments (a) and litter bags (b) experiments are shown. These values for isolated leaf body and petioles from the initial amendment are illustrated by the solid and open squares, respectively, along with the stippled horizontal lines.

1979] than leaf matter they are derived from. Petioles also exhibited lower Ac/Al(s,v) ratios than the leaf body tissue. In all, the differences in the chemistry of soft leaf (leaf body) tissue and petiole were sufficiently large that they could provide a distinct chemical character permitting their separate tracking into the residues. Undegraded whole leaf chemistry (Table 2) was intermediate between the composition of leaf body and isolated petioles. However, the actual values for unfragmented litter will change depending upon the relative abundance of vascular tissue in the leaf body and the size of the petioles. Because of this, subsequent comparisons (i.e., Figures 1–5) of initial SVC-lignin and ΣSFA chemistry to degraded residues are made with respect to isolated petioles and leaf body as the ultimate end member source.

[26] The residues from the open decay experiments had chemically distinct biopolymer composition and concentration depending upon whether they were placed in the HEW or LEW sites (Table 2). The SVC-lignin content of the decayed residue, normalized to OC content, in HEW sites increased in concentration to actually exceed what is expected of pure petioles and became enriched in SVC-lignin with respect to old sites (Table 2, two-tailed t-tests, $p = 0.004$). The LEW sites exhibited only a small increase with respect to the average initial leaf chemistry (Table 2) resembling content mostly of the leaf body (Table 2 and Figure 1a). The ΣSFA, however, in HEW sites became depleted with respect to both initial amendment components, the pure petioles, and LEW sites, while LEW sites exhibited an increase in ΣSFA, exceeding the pure leaf body in content.

[27] In open decay experiments, the often used proxy for SVC-lignin oxidation, Ac/Al(v), was elevated in LEW sites with respect to HEW sites (two-tailed t-tests, $p = 0.01$), which actually exhibited a drop from the initial

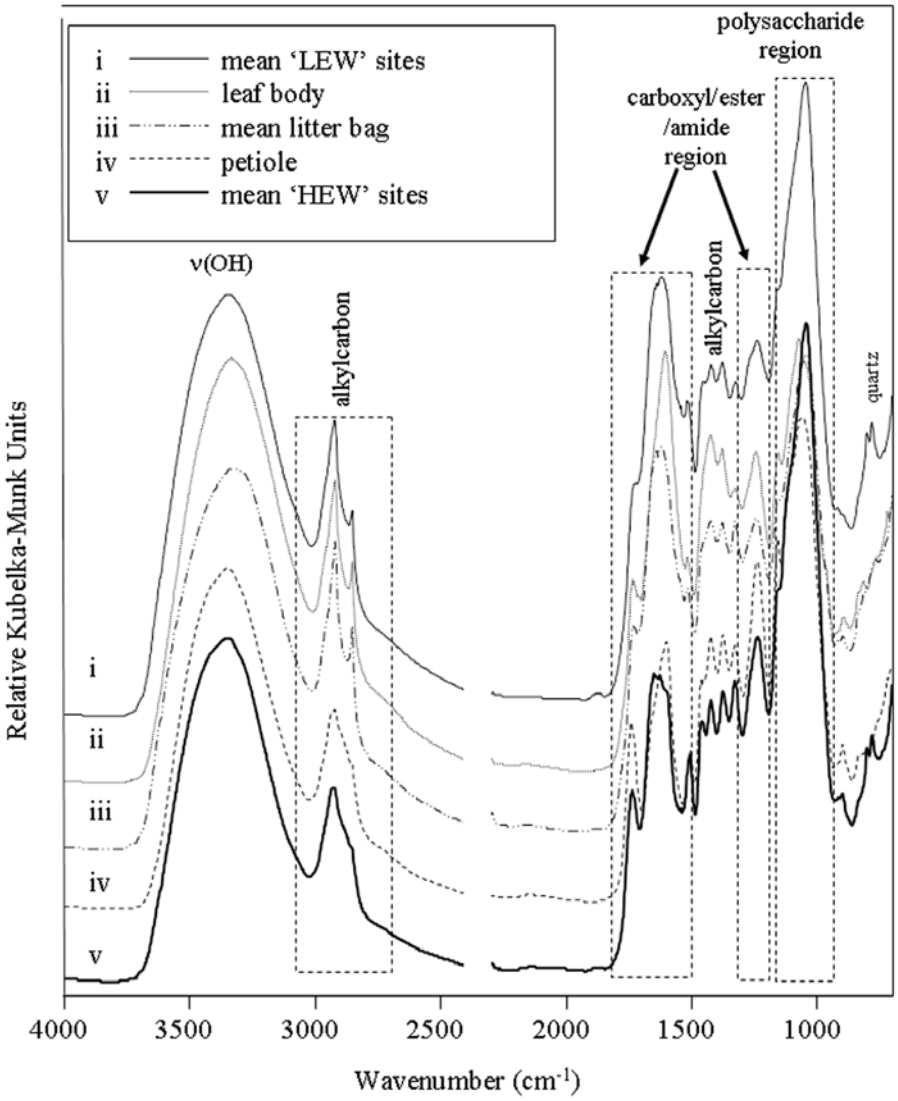

Figure 3. Diffuse reflectance FTIR spectra of open litter samples, leaf body only, petiole and litter bag obtained in the 4000 to 700 cm^{-1} region from the open litter amendment experiments in (i) low earthworm (LEW) (spectral mean of LEW sites), (iii) litter bag (spectral mean of sites 1–6), and (v) high earthworm abundance (HEW) (spectral mean of HEW sites) sites at SERC. For comparison, spectra of leaf body only (ii) and petiole samples (iv) are also included.

amendment but remained above the isolated petiole values (Table 2). Syringyl-based lignin also showed a modest increase in Ac/Al(s) in the open decay experiment LEW sites (Table 2). Overall, the pattern for the open decay experiments was that Ac/Al(s,v) increased with decreased SV-lignin content (Figure 2a).

[28] Changes also occurred to SVC-lignin molecular composition in the open decay experiments, such that the C/V values among the residues were distinct between HEW and LEW sites (Table 2, two-tailed t-tests, $p = 0.002$) with HEW sites exhibiting a near total loss of C-lignin. The HEW sites trended toward C/V values nearly identical to that found in isolated petioles while the LEW sites had C/V values near to the leaf body. The S/V values decreased for both HEW and LEW sites with respect to the initial amendments.

[29] The residues from the litter bag decay experiments exhibited shifts in SVC-lignin and cutin, ΣSFA, concentration and composition from the initial amendment but unlike the open decay experiments both HEW and LEW sites exhibited similar decay trajectories (Figure 1b) and obtained nearly identical values. The SVC-lignin content in the decayed residue became enriched above leaf body and average whole leaf regardless of whether the plot was a LEW or HEW sites indicating a selective loss of other leaf chemicals. The HEW sites exhibited a moderately greater increase in SVC-lignin. The ΣSFA in the litter bag residue was highly enriched and exceeded even the cuticular-rich leaf body in both the HEW and LEW sites. As with the SVC-lignin and ΣSFA values, the Ac/Al(s,v) and SV-lignin showed only a small distinction between LEW and HEW sites (Table 2 and Figure 2b). Additionally, the Ac/Al(s,v) ratios of the litter bag residues were close to the initial ratio for the whole leaf body. The general trend of the HEW sites having relatively lower SVC-lignin and higher Ac/Al(s,v)

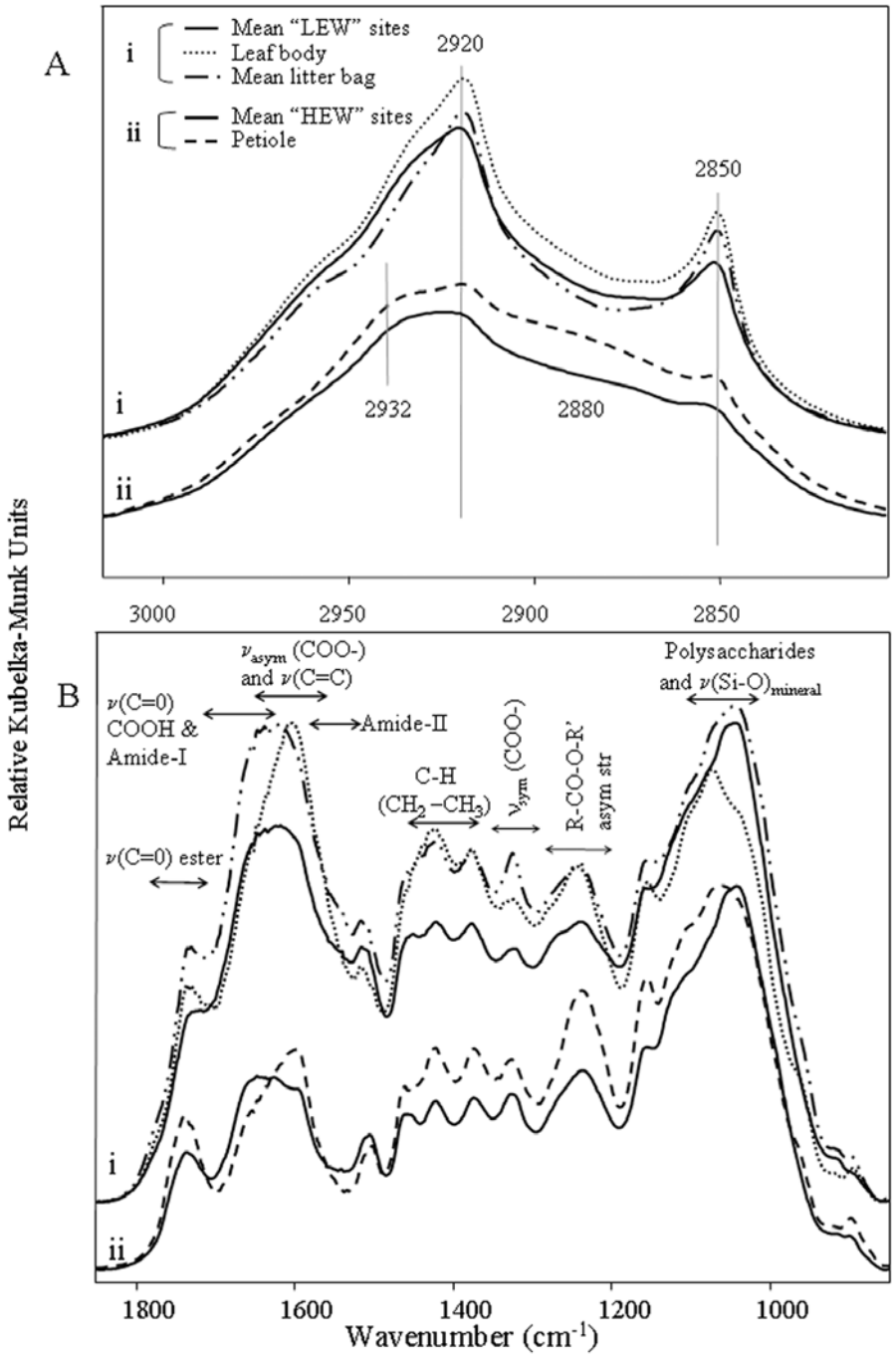

Figure 4. Expanded regions of the diffuse reflectance FTIR spectra shown in Figure 3. (a) Alkyl ν(C-H) absorption region from 3030 to 2800 cm^{-1}. Spectra are grouped into two groups based on the degree of apparent order of the ν(C-H) bands. The spectra comprising Group (A) correspond to highly ordered ν(C-H) groups. Spectra in this group include mean LEW surface litter, leaf body only, and mean leaf bag spectra. Spectra in Group B represent 'disordered' ν(C-H) components and include the mean HEW spectra as well as the petiole spectrum. (b) Spectra in the 1850 to 900 cm^{-1} region, and these spectra share the same legend as in Figure 4a.

still held but the differences were smaller with respect to the open decay experiments.

[30] The residues from the litter bag experiments exhibited changes to SVC-lignin molecular composition that were also evident as the C/V values were similar among LEW and HEW sites (Table 2) decreasing slightly from the initial but staying close to the values for whole leaf body. The S/V values changed very little in the bag experiments.

3.4.2. DR-FTIR Analysis of Litter

[31] The DR-FTIR spectra of surface litter, litter bag, leaf body and petiole samples are shown in Figure 3. FTIR spectroscopy provides a useful, nondestructive method of

characterizing organic polymers in soils and for studying organo-mineral complexes [*Cabaniss*, 1991; *Chapman et al.*, 2001; *Davis et al.*, 1999; *Galle et al.*, 2004; *Inbar et al.*, 1990; *Niemeyer et al.*, 1992; *Perez et al.*, 2004] as well as applications specific to the characterization of forest litter [*Gressel et al.*, 1995b, 1995a; *Haberhauer et al.*, 1998; *Haberhauer and Gerzabek*, 1999] and plant biopolymers [*Chefetz*, 2007]. For the organic constituents analyzed in this study, the major changes are observed in the C-H stretching region (ν(C-H)) in two distinct peaks at 2,920 and 2,850 cm-1, the C-H deformation of CH_2 or CH_3 groups in broad peaks at 1,470 and 1,380 cm-1, and a general broad ''fingerprint'' region from 1800 to 900 cm^{-1} which is characterized by many important biopolymer functional group classes (Figure 3). Spectra of the entire mid-IR region are shown in Figure 3 and the ν(C-H) and 'fingerprint' regions are shown in Figures 4a and 4b, respectively.

[32] The DR-FTIR spectra shown in Figure 3 are consistent with spectra of leaf litter samples [*Gressel et al.*, 1995b; *Haberhauer et al.*, 1998; *Haberhauer and Gerzabek*, 1999] and cutin biopolymer isolates [*Chefetz*, 2007] reported in the literature. The broad band centered around 3400 cm^{-1} in all samples corresponds to the ν(OH) region where sorbed water and surface ν(OH) groups absorb. The spectra of open decay experiments from the LEW sites have well-defined $\nu(C\text{-}H)_{sym}$ and $\nu(C\text{-}H)_{asymm}$ bands at 2850 and 2920 cm^{-1} (Figure 4a, group i) indicative of highly ordered aliphatic compounds. Similarly ordered aliphatics were observed in the litter bag and initial leaf body samples (Figure 4a, group i). The $\nu(C\text{-}H)_{sym}$ and $\nu(C\text{-}H)_{asym}$ bands of the leaf body sample showed the highest degree of order based on their band position and intensity indicative of leaf waxes and undegraded cutin materials consistent with published spectra for highly ordered waxes [*da Luz*, 2006]. The position of ν(C-H) bands corresponding to -CH_2 groups, in particular, have been found to reflect the overall order of the alkyl chains [*Greene and Bain*, 2005; *Merk et al.*, 1998; *Vaia et al.*, 1994].

[33] In contrast to the LEW sites, the mean HEW spectrum (Figure 4a, group ii) has much broader ν(C-H) bands that are shifted to higher energy. Also, the HEW samples are nearly identical to the C-H bands of the tulip poplar petiole (Figure 4a, group ii). In the LEW site, the ν(C-H) signature of the litter residue reflects the spectra of both the litter bag and that of the leaf body. With high earthworm activity, however, the highly ordered ν(C-H) component, most likely due to the loss of cuticular waxes, is greatly diminished. Such changes were also observed in cutin isolate soil decay experiments [*Chefetz*, 2007].

[34] In the lower frequency spectral region from 1800 to 900 cm^{-1}, the spectral features of the litter and leaf tissue samples tend to be complex (Figure 4b) but exhibit distinct shifts associated with compound class absorbances found in leaves. General spectral assignments for bands in this region are included on Figure 4; however, band assignments are often complicated by the fact that many forms of carbon have strong absorption features in this region and bands can often be comprised of two structural moieties. In a comparison of LEW and HEW surface litter decay what is apparent is a loss of relative intensity in the carboxylic acid stretches, and potentially amide-I (mainly carbonyl stretch) and amide-II (mainly C-N stretch and N-H deformation) and the alcohol stretches potentially associated with polysaccharides in HEW relative to LEW sites. Overall, and consistent with the molecular data, the LEW and HEW surface litter residues have a relatively close correspondence with the leaf body and petiole of fresh tulip poplar, respectively. Leaf bag samples show a very close relationship to leaf body spectra, although differences are noted reflecting potentially a loss of carboxylic structures, a relative increase in esters, and a loss in polysaccharides. Some very interesting changes occur in the region of carbonyl stretching ν(C = O) region from 1750 to 1660, specifically with the ν(C = O) of esters occurring in the region from 1730 to 1750.

[35] We assign the well-defined band at 1738 cm^{-1} and the accompanying band at 1250 cm^{-1} to the R-CO-O-R' stretch of aliphatic ester bonds. These bands are most poorly resolved in the spectra of the surface litter decay at the LEW sites (Figure 4b, group i), but also lack sharpness in the leaf body and all litter bag samples. The spectra of these samples have much greater intensity in the region where R-COOH, which might correspond to leaf waxes, and amide-containing components. A comparison of the ''ester'' bands between HEW surface decay residue and petiole samples, which we consider to be the ultimate source of the residue at these sites (Figure 4b, group ii), indicates that there is a selective loss of these bonds in decayed residues, similar to the LEW surface residues but to a greater extent.

4. Discussion

[36] In forest systems where earthworm density and species abundance varies spatially it is anticipated that large differences in forest floor litter chemistry, physical appearance, and concentration will result [*Saetre*, 1998; *Hendriksen*, 1990; *Bohlen et al.*, 2004a]. Earthworms, often called ''keystone species'', can be disproportionately important relative to their biomass in ecosystem nutrient dynamics because of their specific feeding strategies, which not only degrade litter and soil organic matter and mix fresh litter into the mineral horizons but also fundamentally change the nature of decay by altering the relative abundances and activities of fungi and bacteria [*Brown and Doube*, 2004; *Groffman et al.*, 2004; *Li et al.*, 2002]. The biological implications of earthworm invasion are quite intricate as they can simultaneously promote the rapid disintegration and decomposition of litter [*Edwards and Bohlen*, 1996; *Li et al.*, 2002; *Liu and Zhou*, 2002] but also the preservation of litter components in stabilized microstructures [*Guggenberger et al.*, 1996; *Scheu and Wolters*, 1991; *Bossuyt et al.*, 2005] in their casts or as mineral-organic associations in soils. How a forest litter-soil system actually responds to changes in earthworm populations will be a function of the abundance and feeding ecology of the earthworms and the specific litter, microbial, and soil qualities at each site.

[37] Previous studies at SERC documented clear patterns in earthworm density, composition, and distribution with land use history and forest successional stage [*Szlavecz and Csuzdi*, 2007]. Forest stands that varied from recent successional stages (50–70 y since abandonment), mature successional stages (>150 y since abandonment), and sites that have never been logged exhibited relatively high, middle, and zero litter-degrading earthworm abundance,

respectively, with a majority of earthworm biomass and species as European introductions. The earthworm fauna at SERC exhibits all types of feeding habits [*Bouché*, 1977; *Lavelle et al.*, 2004].

[38] In the present study, litterbag and surface amendments were used to investigate how the biopolymer composition of tulip poplar leaves was altered by direct earthworm access compared to background decay due to microbial and mesofauna activity, intrinsic to both young and old forests. Differences in earthworm abundance between young and old forests did not have an important influence on ''background'' litter decay activity as demonstrated by similar mass losses and trajectory in biopolymer and elemental chemistry within litterbags across the sites (Table 2 and Figure 4), although there were differences in magnitude of soil enzyme activities. It appears that earthworm abundance did, however, have a major influence in the open decay experiments, serving to promote physical disruption of leaf components, selective chemical modifications of aliphatic structure, and enrichment of specific biopolymers from petioles in the decayed litter (Figures 1a, 2a, 4a, and 4b). Additionally, litter residues in both LEW and HEW sites increased in N content with a proportionally larger increase in the LEW sites. This is interesting, as the molecular, physical and spectroscopic data indicated that petiole organic matter dominated residues in the HEW sites and it would be anticipated that the overall N would have dropped in those settings. Clearly, there was a large movement of N, most likely of microbial origin, into these residues. This would be consistent with the typical lowering of C/N values found in most litter decay experiments [*Blair and Crossley*, 1988; *Dornbusch et al.*, 2002; *Berg and Laskowski*, 2006] and what might be the increased microbial action resulting from high earthworm activity [*Blair et al.*, 1995].

[39] Earthworm feeding experiments, both in situ and mesocosm-based, showed that comminution increased soil respiration rates and facilitated the removal of litter from the soil surface [*Hedde et al.*, 2007; *Scheu and Wolters*, 1991]. Earthworms, however, can have selective feeding strategies based upon litter chemistry and size of plant matter particles [*Curry and Schmidt*, 2007] and, therefore, it is reasonable to assume that the more rigid, lignin rich, and initially nitrogen poor components of leaves, such as the vascular tissue in petioles, would be selectively concentrated in litter after a season of selective earthworm feeding on more palatable leaf body components. The combination of selective shredding and removal and alteration of microbial activities and community structure would have cascading implications for the activity of secondary decomposers and the chemical nature of accumulated humus and SOM in earthworm impacted forests. As has been suggested in other studies, it may be just this interaction that makes interpretation of some comparative forest litter decay studies equivocal [e.g., *Lorenz et al.*, 2000].

[40] Several feeding experiments showed that earthworms, which are considered generalist consumers, will selectively feed on litter that is high in N and soluble carbohydrates, low in tannins and lignins, and generally has low C/N [*Satchell*, 1967; *Zicsi*, 1983; *Hendriksen*, 1990; *Schonholzer et al.*, 1998]. Preference can be achieved by selecting soft tissues over more rigid parts, more decomposed leaves over freshly fallen ones, or choosing leaf litter types of higher nutritional quality (such as tulip poplar over oak). Earthworm species with different feeding habits may behave differently in their selectivity. *Hendriksen* [1990] reported a strong correlation between preference and C/N ratio for litter-feeding *Lumbricus* spp., but not for endogeic species.

[41] The observed chemical changes in decayed residues from LEW and HEW sites, e.g., differences in S/V, C/V, Ac/Al(s,v), abundances of lignin and ΣSFA, and the loss of structural order seen in the DR-FTIR aliphatic, might also result from earthworm-induced modifications in enzyme activity through changes to the soil fungal and bacterial communities. It is reasonable to expect that both fungal and bacterial activities will be different between old and young sites. Indeed, the spectral changes in the aliphatic and carboxyl/ester regions of the FTIR of the HEW sites are quite similar to the decay seen in cutin isolates induced after 9 months of burial in soil [*Chefetz*, 2007]. Also, previous studies demonstrated that enhanced earthworm activity can increase respiration in casts, increase microbial activity and biomass, and decrease fungal hyphal networks [e.g., *Li et al.*, 2002; *Groffman et al.*, 2004]. At SERC soils in the young, earthworm-impacted forests were elevated in both β-1,4-glucosidase and polyphenol oxidase activity with respect to the old successional sites. Additionally, the molecular and spectroscopic data obtained from SERC could be interpreted in terms of the promotion of hydrolytic versus oxidative chemical paths because of earthworm action. For example, the relative importance of oxidative lignin decomposition to hydrolytic decomposition of ester linked compounds could be inferred by two parameters: (a) the ratio of ester-linked (C-lignin) to ether and carbon-carbon linked (V-lignin) lignin (C/V), (b) the ratio of total ester-linked ΣSFA to SV-lignin (Figure 5). Thus, increases in these relative parameters would indicate enrichment in esters through selective SV-lignin oxidation while a decrease in the parameters would indicate selective loss of ester-linked compounds. The values of these parameters for residues from the open experiments suggest that residues in the old sites with low-earthworm abundance may have undergone extensive lignin oxidative chemistry, depleting SV lignin and concentrating ester-linked compounds, while young successional sites with abundant earthworms are dominated by hydrolysis-based chemistry that results in relative loss of aliphatic and aromatic esters and the concentration of lignin. The trend of decreasing S/V during decay is consistent with other reported findings [*Hedges et al.*, 1988] which indicated that S-lignin was more easily removed during microbial decay with respect to V-lignin. Alternatively, one might speculate that the disruption and partial oxidation of the lignin biopolymer through microbial processing allowed V-lignin monomer units to be more easily released for analysis. These processes may occur as described but, as discussed previously, the simplest explanation for the majority of changes observed is a physical concentration of general vascular tissue or petioles over soft leaf tissue in areas with high earthworm activity.

[42] In stark contrast to the open experiments, the litter bag studies only showed a decay trajectory consistent with early decay paths, concentrating cutin esters and lignin at the expense of, possibly, polysaccharides, which were not

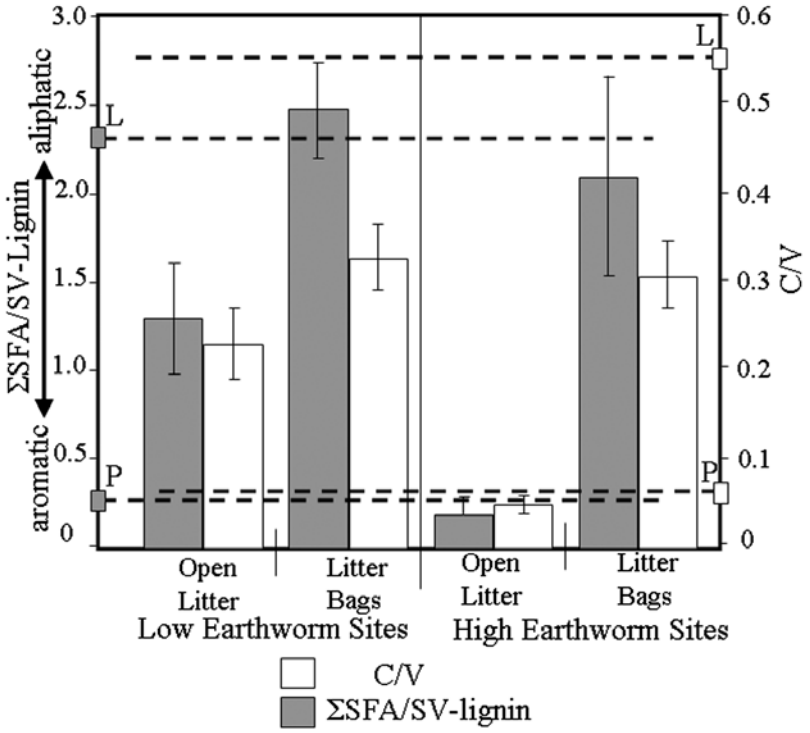

Figure 5. Comparison of the ratios of the CuO extractable substituted fatty acids (ΣSFA) (cutin derived) to SV-lignin and cinammyl lignin to vanillyl based lignin. Changes in the relative importance of oxidative lignin decomposition, resulting from the cleavage of lignin side chains on ether and carbon-linked monomers (SV-lignin), to hydrolytic decomposition, resulting from the decomposition of ester linked compounds (C-lignin) and ΣSFA, is inferred by position (higher values for oxidation and lower values for hydrolysis) ΣSFA/SV-lignin values.

directly measured but inferred based upon the drop in absorbance of the FTIR in the region 1720 to 1660 cm^{-1}. Therefore, litter bag experiments experienced chemical action similar to that observed for surface decay in old successional forest (LEW sites); all sites that had minimal earthworm mixing and impacts on soil microbial action (Figure 4b, group i, and Figure 5).

[43] Some litter decay studies do demonstrate that aliphatic components of leaves, waxes and cutin, increase in relative concentration during decomposition [e.g., *Lorenz et al.*, 2004; *Baldock and Preston*, 1995] and lead some to consider the abundance of cutin an important factor in palatability of litter [*Baldock and Preston*, 1995]. Indeed, the relative increase in aliphatic material with respect to lignin at depth in some soils has also been given as evidence of relative refractory nature of these biopolymers [e.g., *Nierop*, 1998].

[44] The fact that earthworm activity changes the chemical nature, not just the amount of decayed litter, has important biogeochemical implications for food web dynamics and soil organic matter stabilization processes since detritus remaining on the surface or translocated to the soil may have completely different nutritional value or physicochemical properties depending on earthworm abundance and feeding habit. This is a condition not typically addressed in studies of how nutrients are cycled in forests undergoing earthworm invasion [*Brussaard et al.*, 2007]. If the chemical differences observed for the tulip poplar leaf residues in this study are representative of how all litters across this forest respond then we might speculate that sites invaded by this assemblage of earthworms will develop surface residues low in aliphatics and cinnamyl lignin but high in ether-linked lignin and overall physical toughness. The overall change in nutrient status (C/N) and biopolymer composition should, then, have a cascading impact on the ability of microbes, fungi, mesofauna, and other macroinvertebrates to utilize the residue as a substrate and thus lead to an altered decomposer food web.

[45] The impact of earthworms on the overall content of soil organic matter and its stabilization has been investigated in many forest and grassland sites, and soil carbon generally tends to increase with worm invasion at the expense of litter floor [e.g., *Bohlen et al.*, 2004b] where the increase in SOM is most often attributed to enhanced mixing of litter material into the mineral soil. This physical activity has been demonstrated as a key aspect of the stabilization of fresh organic matter in physically protected casts or fecal pellets [*Cortez*, 1998; *Seeber et al.*, 2006; *Hedde et al.*, 2007]. Given the distinct earthworm activity and chemical degradation pathways observed among plots at SERC these sites may be ideal for investigating the soil-earthworm-litter system controls on the stabilization of organic matter in Eastern deciduous forest soils. Additionally, the fact that the residues have very distinct aliphatic/aromatic ratios indi-

cates that they may also have distinct physicochemical properties that will influence how they associate with mineral surfaces, soil aggregates, and soil water [*Sollins et al.*, 1996] and may help to explain observations of difference observed on mineral isolates from soils [*Sollins et al.*, 2006]. Such differences should have implications for soil carbon stabilization in these sites.

5. Conclusions

[46] 1. In a comparison of open surface decay and litter bag experiments in six forest stands at SERC it was demonstrated that earthworm activity controlled the biopolymer composition and concentration of decayed litter residues though physical separation of lignin-rich, cutin-poor petioles from cutin-rich, lignin-poor soft leaf tissue which was apparently consumed and incorporated into the soil.

[47] 2. Litter bag experiments, run for an equivalent time, resulted in residues most resembling the old successional forest stands, which had low earthworm abundance and bore little chemical similarity to the sites impacted by high earthworm activity.

[48] 3. We speculate that in forest systems earthworm activity may be a primary control on the relative concentration and translocation of aliphatic or aromatic-rich organic matter to the mineral soil which can then be mistakenly interpreted as an inherent property of the biopolymer for stabilization. Hence, discussions of the inherent stability of aromatics or aliphatics in soil organic matter at a particular location need to consider the role of macroinvertebrate activity.

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S. E. Crow and T. R. Filley, Department of Earth and Atmospheric Sciences and the Purdue Climate Change Research Center, Purdue University, 550 Stadium Mall Dr., West Lafayette, IN 47907-1397, USA. (filley@purdue.edu)

C. T. Johnston, Department of Agronomy (Crop, Soil, and Environmental Sciences), 915 W. State St., Purdue University, West Lafayette, IN 47907, USA.

M. K. McCormick and D. F. Whigham, Smithsonian Environmental Research Center, P.O. Box 28, Edgewater, MD 21037, USA.

K. Szlavecz, Department of Earth and Planetary Sciences, Johns Hopkins University, Baltimore, MD 21218-2681, USA.

R. N. van den Heuvel, Landscape Ecology, Institute of Environmental Biology, Utrecht University, P.O. Box 800.84, 3508 TB Utrecht, Netherlands.