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Earthworm effects on the incorporation of litter C and N into soil organic matter in a sugar maple forest

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Abstract. To examine the mechanisms of earthworm effects on forest soil C and N, we double-labeled leaf litter with ¹³C and ¹⁵N, applied it to sugar maple forest plots with and without earthworms, and traced isotopes into soil pools. The experimental design included forest plots with different earthworm community composition (dominated by *Lumbricus terrestris* or *L. rubellus*). Soil carbon pools were 37% lower in earthworm-invaded plots largely because of the elimination of the forest floor horizons, and mineral soil C:N was lower in earthworm plots despite the mixing of high C:N organic matter into soil by earthworms. Litter disappearance over the first winter–spring was highest in the *L. terrestris* (T) plots, but during the warm season, rapid loss of litter was observed in both *L. rubellus* (R) and T plots. After two years, 22.0% ± 5.4% of ¹³C released from litter was recovered in soil with no significant differences among plots. Total recovery of added ¹³C (decaying litter plus soil) was much higher in no-worm (NW) plots (61–68%) than in R and T plots (20–29%) as much of the litter remained in the former whereas it had disappeared in the latter. Much higher percentage recovery of ¹⁵N than ¹³C was observed, with significantly lower values for T than R and NW plots. Higher overwinter earthworm activity in T plots contributed to lower soil N recovery. In earthworm-invaded plots isotope enrichment was highest in macroaggregates and microaggregates whereas in NW plots silt plus clay fractions were most enriched. The net effect of litter mixing and priming of recalcitrant soil organic matter (SOM), stabilization of SOM in soil aggregates, and alteration of the soil microbial community by earthworm activity results in loss of SOM and lowering of the C:N ratio. We suggest that earthworm stoichiometry plays a fundamental role in regulating C and N dynamics of forest SOM.

Key words: aggregate; C:N ratio; decomposition; litter; *Lumbricus*; stoichiometry.

INTRODUCTION

Soil organic matter (SOM) plays a variety of important roles in forest soils: storing carbon, maintaining fertility, and promoting favorable structure and porosity. Forest soils form a major global pool of C and release of that C as CO₂ could influence greenhouse warming of the climate. Forest SOM also stores large amounts of N and the behavior of this N pool in the face of continuing high atmospheric deposition of reactive N and other environmental changes remains inadequately understood (Aber et al. 2003, Pardo et al. 2011). Plant litter is a principal source of forest SOM and, in many acidic forest soils, plant litter accumulates on the soil surface because soil faunal groups that mix litter into soil are depauperate.

Earthworms play a particularly prominent role in processes of soil formation (Edwards and Bohlen 1996). However, many northern forests in North America were historically devoid of native earthworms because of limited colonization since the last glacial maximum (James 1995). In recent decades, exotic earthworm species from Europe and Asia have been introduced into North American forests and are gradually colonizing across a wide geographic area (Hendrix et al. 2008, Addison 2009). The invasive earthworm community can consume and mix into mineral soil both fresh litter and preexisting surface SOM. The earthworm activity also appears to promote the microbial mineralization of SOM in mineral soil (Alban and Berry 1994, McLean and Parkinson 1997, Li et al. 2002), potentially reducing soil C storage. Conversely, earthworms can also promote the formation of water-stable aggregates in soil and consequent stabilization of SOM (Tisdall and Oades 1982, Bossuyt et al. 2004). Hence, Lavelle et al. (1998) suggested that following earthworm invasion of forest soil, carbon storage should initially decline with

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the elimination of the forest floor horizons and subsequently increase as SOM is stabilized by earthworm activity. The net effect of earthworm invasion on forest soil carbon storage may vary depending upon pre-invasion soil pools (Bohlen et al. 2004), the time interval since invasion (Huang et al. 2010), and also earthworm community composition. These factors may help to explain why previous studies have reported decreases (Alban and Berry 1994, Bohlen et al. 2004), increases (Wironen and Moore 2006) or no change (Hale et al. 2005) in forest soil C pools following earthworm invasion.

By disrupting preexisting soil dynamics earthworm invasion may provide basic insights into the process of SOM development and the evolution of element stoichiometry in the soil ecosystem. The mechanisms underlying soil C to N stoichiometry have been the subject of much research and speculation since the original proposal of Waksman (1924) that soil microbial communities play a fundamental role in regulating soil C:N. In theory, the ratio of nutrient acquisition by the microbial community should link soil nutrient availability to microbial stoichiometry (Sinsabaugh and Moorhead 1994, Cleveland and Liptzin 2007). How would earthworm invasion be expected to alter this relationship? Resource supply to the detrital community is depleted in N relative to C so that detritivores may be N limited (Martinson et al. 2008). Maintenance of homeostatic C:N in detritivores in general and especially in earthworms has been widely observed (Pokarzhevskii et al. 2003, Marichal et al. 2011). Digestion in earthworms is operated by microorganisms (Trigo et al. 1999), and earthworms are capable of regulating their gut flora (Curry and Schmidt 2007), which may help to maintain this homeostasis. Thus, the gut microflora of worms may account for the disconnection between litter and SOM stoichiometry (Marichal et al. 2011). Previous studies of earthworm invasion in temperate forests have also demonstrated that the C:N ratio of surface horizons typically increases, in part because earthworms feed preferentially on lower C:N tissues, leaving behind high C:N structural components (Bohlen et al. 2004, Wironen and Moore 2006, Filley et al. 2008).

The effects of earthworms on soil properties and dynamics vary with the species composition of the earthworm community (Ponge et al. 1999, Suárez et al. 2004, Hale et al. 2005). Earthworms commonly are classified into distinct functional groups based upon feeding and burrowing habits: epigeic earthworms (e.g., *Dendrobaena octaedra*), feed on surface litter and mix it superficially into the soil; endogeic species (e.g., *Aporrectodea* spp., *Octolasion tyrtaeum*) live and feed belowground; and anecic species (*Lumbricus terrestris*) form vertical burrows but feed on surface litter and make prominent midden piles (Edwards 2004). The common forest invasive earthworm, *Lumbricus rubellus*, is considered an epi-endogeic species as it feeds on surface litter and also mixes this organic matter into the

upper mineral soil (Addison 2009). Species from these functional groups may form a loose consortium that facilitates colonization, as they often coexist in recently invaded forest soils (Suárez et al. 2006a, Mathieu et al. 2010).

In central New York State, exotic earthworms have invaded northern hardwood forests and are profoundly influencing forest ecosystem processes including soil C and N dynamics (Bohlen et al. 2004). The composition of the earthworm community in these forests varies spatially (Suárez et al. 2006a), in part because of landscape-scale variation in colonization sources (Suárez et al. 2006c) and possibly the time interval since the invasions began. In particular, some areas lack earthworms, some are dominated by *L. terrestris*, and other areas lack this anecic species and are dominated by the epi-endogeic *L. rubellus*. Field and laboratory studies have illustrated the contrasting feeding behavior and soil effects of these two common species (Shipitalo et al. 1988, Hale et al. 2005, 2008, Suárez et al. 2006b). We took advantage of this variation in earthworm community composition to explore the mechanisms of earthworm effects on soil C and N dynamics in sugar-maple-dominated forests. By double labeling sugar maple leaf litter with ^{13}C and ^{15}N we attempted to trace litter C and N into mineral SOM pools and to quantify the effects of earthworms on this process. We hypothesized that (1) earthworms would greatly accelerate the transformation of litter C and N into SOM; (2) this effect would be greater in plots dominated by the anecic earthworm *L. terrestris* than the epi-endogeic *L. rubellus*; (3) earthworm feeding activity would incorporate litter C and N into soil aggregates; and (4) by altering microbial activity, earthworms would affect soil C storage and C:N stoichiometry of mineral SOM.

METHODS

Study site.—The research was conducted at Cornell University's Arnot Forest located in Tompkins County, central New York State, USA (42°15' N, 76°40' W). This forested landscape is typical of the glaciated Allegheny Plateau where nonnative earthworms have widely colonized (Suárez et al. 2006c). For a detailed site description see Fain et al. (1994). Briefly, the study plots were located at 600–620 m elevation in forests dominated by *Acer saccharum* Marsh. The stands are mature, second-growth forests originating following clearcut harvest in the 1870s. Basal area ranges from about 30–35 m²/ha and canopy height 23–25 m. Soils are acidic Dystrochrepts (pH 4.5–5.0) derived from glacial till overlying Upper Devonian shales. Clay content of the <2 mm fraction ranges from 24–28% in the 0–10 cm soil and sand content from 13–17%. Soils are stony, averaging 22% by volume coarse fraction (>2 mm) in the 0–10 cm soil. The climate is temperate continental with mean temperature of –4°C in January and 22°C in July, and mean annual precipitation of 90 cm, evenly distributed through the year. In the absence of

earthworms, sugar maple leaf litter has been observed to decay with an average exponential decay coefficient (k) value of 0.49 yr^{-1} (Suárez et al. 2006b).

Invasive earthworms are common in the Arnot Forest (Bohlen et al. 2004), and the composition of the earthworm assemblage varies markedly across the landscape (Suárez et al. 2006a). We selected nine study plots arranged in three blocks across a topographic gradient from mid slope to upper slope to ridge top. Three plots in each topographic position were in areas lacking earthworms (no worm, NW) and six in areas with well established earthworm assemblages of differing composition, dominated either by *Lumbricus rubellus* or *Lumbricus terrestris* (hereafter “rubellus” [R] and “terrestris” [T] plots). These differences were readily apparent by visual inspection because of the distinctive burrows and midden piles produced by *L. terrestris*. Quantitative sampling of earthworm communities in these plots was conducted in mid- May 2008 and mid-October 2008 and 2009 using a “hot” mustard extraction method (Lawrence and Bowers 2002). Powdered hot mustard was mixed with water at a concentration of 10 g/L and stored for at least 2 h. Four quadrats (0.25 m^2) were sampled in each plot in May 2008 and two or three in October 2008 and 2009.

Isotopic labeling of leaf litter.—Sugar maple leaf litter was labeled with ^{13}C and ^{15}N and applied to 0.5-m^2 quadrats in the field study plots. The process for labeling leaf litter in enclosed chambers is described in detail in Horowitz et al. (2009). Leaf litter was collected from the labeling chambers, returned to the laboratory, and air dried to constant moisture content. Litter was thoroughly mixed and subsamples taken for chemical analysis. The atom % ^{13}C in the labeled litter was $1.2608\% \pm 0.0041\%$ and atom % ^{15}N was $1.5823\% \pm 0.0409\%$; the bulk C:N of the leaf litter (42.1) was somewhat higher than the atom excess $^{13}\text{C}:^{15}\text{N}$ (34.0; see *Methods: Isotope calculations*).

Field plot establishment and sampling.—In each of the nine sugar-maple-dominated plots, eight 0.5-m^2 quadrats were established in fall 2007. Fresh litter from 2007 was removed from each quadrat, and a coarse-mesh nylon screen (hole size = 6 cm^2) was positioned on the underlying soil and anchored at the corners. About 200 g (weighed to $\pm 0.01 \text{ g}$) of isotope-labeled litter was added to each 0.5-m^2 quadrat to roughly match leaf litterfall in the study area (400 g/m^2 ; Fisk et al. 2004). A second coarse-mesh screen was positioned on the added litter and anchored to confine the litter and prevent mixing of fresh litter with labeled litter.

The field quadrats were destructively sampled on 21 May 2008, 10 October 2008, and 13 October 2009. Two quadrats from each stand were chosen randomly for harvest on each date. First, the corner anchors on the screens were removed and all the litter remaining between the two screens was collected. In the no-worm plots (NW) the underlying forest floor horizons ($\text{O}_e + \text{O}_a$) were collected by excavating with hand spades to the

top of the mineral soil; the distinction between forest floor and A horizon was usually clear, being marked by high stone content in surface mineral soil. In the R and T plots, which lacked forest floor, the top 0–2 cm of the A horizon of the mineral soil was excavated from the entire quadrat. Mineral soil was then cored to 20 cm depth at six to eight points using 5 cm diameter, split-PVC corers; previous observations indicated that earthworms rarely penetrate beyond 20 cm depth in these soils (Bohlen et al. 2004). In the NW plots, soil samples from six to eight cores were composited for each quadrat by 5 cm depth increment. In the R and T quadrats, the upper-most core section was 2–5 cm (0–2 cm removed) and then 5 cm depth increments were collected to 20 cm and pooled across cores. In addition, in the T plots we sampled discrete earthworm midden piles and cored around burrows (these were absent from R plots), again pooling several samples across each quadrat. After soil sampling was complete, earthworms were extracted for isotope analysis from the remaining soil using the hot mustard method. All samples were returned to the laboratory for processing the same day as collected.

Lab processing of samples.—Litter and $\text{O}_e + \text{O}_a$ samples were weighed moist, and a subsample was taken for moisture determination by reweighing after oven drying to constant mass at 70°C . The subsample was stored for chemical analysis. A second subsample of $\text{O}_e + \text{O}_a$ was taken for microbial biomass and related measurements and stored at $\sim 2^\circ\text{C}$ until analyzed within 48 h. For mineral soil cores, coarse fragments ($>1 \text{ cm}$) were removed and the rest of the bulk sample was weighed moist. Subsamples were taken for moisture determinations, aggregate fractionation, isotope analysis, and microbial biomass (and related) measurements. The subsamples were either stored at 2°C (for microbial biomass) or dried to constant mass at 70°C (for moisture determination) and sieved to remove the $>2 \text{ mm}$ fraction. Earthworms were sorted by taxonomic group and the anterior ends (15 segments) were dissected and cleaned of gut contents for tissue isotope analysis.

Samples from the May 2008 and October 2009 collections were processed for analysis of isotope enrichment of soil aggregate fractions. Because of the low isotope enrichment of soils below 5 cm depth, we confined this analysis to the surface soil layers (0–2, 2–5 cm). Subsamples of air-dried soils were wet sieved, following methods described by Elliot (1986), which divides a soil into three size classes: macroaggregates ($>250 \mu\text{m}$), microaggregates (53 to $250 \mu\text{m}$), and the silt and clay fraction ($<53 \mu\text{m}$). An 80-g subsample was placed on a $250\text{-}\mu\text{m}$ sieve and slaked in deionized water for 5 minutes before sieving. A low density fraction that floated above the sieve was removed and retained. The sieving process involves moving the sieve up and down gently by hand for a total of 50 cycles over a 2-minute period. Material remaining on the sieve was washed into a preweighed pan and saved, while material passing

through the 250- μm sieve was transferred to a 53- μm sieve for further fractionation by the same procedure. The macroaggregate fraction was further divided into the same three size classes as described by Six et al. (2000). Briefly, a 10-g portion of oven-dried (60°C) macroaggregates was slaked in deionized water for 5 minutes. The sieving process included 50 stainless-steel bearings (4 mm diameter) to break up the macroaggregates while a continuous stream of water flushed the entrained microaggregates through the sieve. The process was repeated on the 53- μm sieve, resulting in three size fractions: coarse POM (>250 μm), entrained microaggregates (53 to 250 μm), and internal OM and silt and clay (<53 μm) held within macroaggregates.

Litter, forest floor, soil, and earthworm samples were finely ground and homogenized for isotope analysis. The elemental and isotopic (^{13}C , ^{15}N) composition of samples was measured on a Finnegan isotope ratio mass spectrometer at the Cornell Stable Isotope Laboratory for litter, forest floor, and soil, and at the analytical chemistry laboratory of the University of Georgia Odum School for Ecology for earthworms. Appropriate standards were included for normalization correction, instrument linearity and precision purposes. Samples were run in batches with expected similarity of isotope enrichment to avoid sample carryover errors.

For inorganic N and microbial biomass measurements, mineral soil samples were pooled within quadrats into 0–10 and 10–20 cm depth increments. Inorganic N (NH_4^+ and NO_3^-) was extracted from soil with 2M KCl, followed by colorimetric analysis on a flow injection analyzer. Microbial biomass C and N content was measured using the chloroform fumigation-incubation method (Jenkinson and Powlson 1976), and isotope enrichment following the procedures detailed by Fahey et al. (2011). KCl-extracted samples were prepared for ^{15}N analysis by diffusing inorganic N onto acidified disks (Stark and Hart 1996), which were subsequently analyzed at the University of California Davis Stable Isotope Lab on a Europa Integra isotope ratio mass spectrometer (Sercon, Cheshire, UK) with an integral combustion unit. CO_2 samples from incubations were analyzed for ^{13}C at the same facility.

Isotope calculations.—We present ^{13}C and ^{15}N isotope values using various standard conventions (Unkovich et al. 2001). To illustrate differences across reference soils, earthworms, and soil aggregate fractions, we present δ values, expressed per mil (‰) relative to ^{13}C and ^{15}N standards. For isotopic tracing, we use units of atom percent, i.e., the absolute number of atoms of a given isotope in 100 atoms of total element. For mass balance calculations, we present isotopic values in terms of atom percent excess ^{13}C and ^{15}N ; these values represent the atom percent of the isotopic tracer by subtracting out the atom percent of natural abundance isotopes in each pool. Pools of ^{13}C and ^{15}N in litter on each quadrat were calculated at time zero and at the time of plot collection as the product of dry mass, carbon concentration, and

isotopic atom % (^{13}C and ^{15}N). The release of the isotope from each plot during decay was estimated as the difference between initial and final isotope pools in litter; these values were used to calculate the percentage of isotope recovered in underlying soil.

Calculation of isotope pools and fluxes requires accurate and precise estimates of reference (pre-treatment) soil mass and bulk density, element contents, and isotope natural abundance (Nadelhoffer and Fry 1994). Soil mass, bulk density, and coarse fragment content were determined in each plot by the soil pit excavation method (Rowell 1994). Four soil pits (0.2 $\times$ 0.2 m) were excavated to 20 cm depth at random locations in each plot in summer 2007. First, forest floor was excavated to the top of the mineral soil, as for the experimental quadrats. The mineral soil was excavated in 5 cm depth increments. Samples were returned to the laboratory and weighed in bulk. Coarse fragments and roots were removed and weighed. A subsample was weighed moist, dried to constant mass, reweighed, and then sieved to separate the <2 mm fraction. Samples of large roots and coarse fragments were retained for measurement of bulk density of these components of soil volume. The fine (<2 mm) fractions were stored and processed for elemental and isotope analysis, including microbial biomass.

Isotopic enrichment of forest floor, mineral soil, inorganic N pools, and microbial biomass was calculated relative to the mean natural abundance in samples from the four soil pits in each stand. We calculated the initial total pool of ^{13}C and ^{15}N in each soil layer in each stand from the mean atom % isotope natural abundance, mean total element (C or N) concentration, and dry mass (based on bulk density) of the fine soil fraction (<2 mm) for each depth. Similarly, we calculated the final isotope pool for each quadrat at the time of collection from isotope enrichment and element concentration, assuming bulk density and fine fraction content were equivalent to the plot-level values. The differences between initial and final isotopic pool estimates for each depth, component, and quadrat were used to calculate percentage recovery of excess tracer isotopes. Finally, we present isotope recovery in various pools in two ways: relative to the mass of excess isotope added to each plot with the labeled litter, and relative to the mass of excess isotope released from the litter at each collection date.

Statistical analysis.—We used a mixed ANOVA model (SAS proc mixed) to test for differences in measured response variables among worm treatments (NW, T, and R plots) and soil depths. Random effects in the model included plot whereas worm treatment, soil depth, and date were considered fixed effects. In addition, slope position (mid slope, upper slope, ridge top) of each plot was included as a fixed effect. A two-way ANOVA was used to compare soil C and bulk density across plots and to compare isotope enrichment across earthworm species. Distribution of isotopic enrichment across aggregate size classes was analyzed using ANOVA for repeated measures. In all cases, the

TABLE 1. Density and ash-free biomass (AFDM) of earthworms in May 2008, October 2008, and October 2009 in plots dominated by *Lumbricus rubellus* and *L. terrestris* in Arnot Forest, New York, USA.

Dominant worm in plot	<i>L. terrestris</i>	<i>L. rubellus</i>	<i>Lumbricus</i> immature	<i>Octolasion tyrtaeum</i>	<i>Aporrectodea</i> spp.	Other immature	Total
May 2008							
Density (no./m ²)							
<i>L. rubellus</i>	0 A	14.3 (3.4) A	96.3 (14.8)	14.0 (4.6) A	0.7 (0.4) A	16.3 (4.6)	141.7 (19.1)
<i>L. terrestris</i>	6.7 (1.6) B	6.0 (1.9) B	59.0 (7.1)	3.3 (1.4) B	4.0 (1.3) B	18.3 (4.1)	97.3 (10.8)
Biomass (g/m ²)							
<i>L. rubellus</i>	0 A	1.07 (0.29)	1.12 (0.21)	0.41 (0.14)	0.03 (0.02) A	0.13 (0.05) A	2.76 (0.47) A
<i>L. terrestris</i>	2.06 (0.71) B	0.54 (0.30)	3.39 (0.86)	0.48 (0.12)	0.48 (0.12) B	0.66 (0.18) B	7.10 (0.25) B
October 2008							
Density (no./m ²)							
<i>L. rubellus</i>	0 A	18.5 (5.6)	58.0 (7.3)	6.0 (3.0)	9.5 (3.7)	18.5 (5.1)	110.5 (12.3)
<i>L. terrestris</i>	8.0 (2.6) B	5.7 (2.3)	37.7 (6.7)	2.8 (1.4)	2.3 (1.7)	20.6 (3.6)	77.2 (9.8)
Biomass (g/m ²)							
<i>L. rubellus</i>	0 A	2.33 (0.71)	2.19 (0.47)	0.19 (0.08)	0.23 (0.10)	0.30 (0.08)	5.24 (0.94)
<i>L. terrestris</i>	2.36 (1.04) B	0.68 (0.23)	2.58 (0.40)	0.06 (0.04)	0.17 (0.17)	1.04 (0.33)	6.89 (1.30)
October 2009							
Density (no./m ²)							
<i>L. rubellus</i>	0 A	16.0 (3.9)	27.3 (12.7)	15.3 (4.9)	0 A	8.0 (3.3)	66.7 (35.3)
<i>L. terrestris</i>	10.0 (5.2) B	10.7 (5.0)	12.7 (4.5)	6.0 (8.0)	4.0 (3.3) B	12.7 (4.5)	56.0 (18.8)
Biomass (g/m ²)							
<i>L. rubellus</i>	0 A	2.18 (0.78)	1.43 (0.58)	0.62 (0.48)	0 A	0.12 (0.08)	4.36 (1.91)
<i>L. terrestris</i>	4.32 (2.03) B	1.35 (0.83)	1.70 (0.72)	0.35 (0.53)	0.50 (0.38) B	0.43 (0.15)	8.66 (2.64)

Notes: Within columns, significant differences ($P < 0.05$) in density or biomass between plots within dates are indicated by different letters. Values are means; standard errors are in parentheses.

residuals were tested for normality using a Shapiro-Wilks test and homogeneity of variance was examined visually by plotting the predicted and residual values. Data were transformed when necessary to meet assumptions of normality and homoscedasticity.

RESULTS

Earthworms and soils.—Earthworm censuses conducted in the plots in May 2008 and October 2008 and 2009 indicated significant differences in the composition of earthworm communities among the three plot types: no-earthworm (NW), *L. rubellus* (R), and *L. terrestris* (T). In all R and T samples immature *Lumbricus* were most abundant (Table 1). No *L. terrestris* adults were observed in R plots whereas several large *L. terrestris* adults were collected in most of the T plot samples. Although *L. rubellus* adults were observed in both the R and T plots, they were two to three times more abundant in the R plots ($P < 0.01$). The endogeic earthworms, *Aporrectodea* spp. and *Octolasion tyrtaeum*, were commonly observed in both R and T plots, both adults and juveniles. Density of *O. tyrtaeum* was two to four times higher in R plots than T plots ($P < 0.01$). Earthworm biomass was significantly higher in the T than the R plots in May, but no significant differences were observed in October. Biomass was dominated by *L. rubellus* and juvenile *Lumbricus* spp. in the R plots and by *L. terrestris* and juvenile *Lumbricus* spp. in the T plots (Table 1) with lesser contributions from the endogeic species owing to their small size. No earthworms were observed in the NW plots in May 2008 and

October 2009, but a few juvenile *Lumbricus* individuals were observed in two of the NW plots in October 2008.

Earthworms had clear effects on soil properties in the sugar-maple-dominated forest ecosystems at Arnot Forest. In particular, earthworms largely eliminated the forest floor ($O_e + O_a$) horizons. Depth patterns of mineral soil bulk density appeared to differ between plots, but because of high variation these differences were not statistically significant (Table 2). Organic C concentrations and C:N ratios in mineral soil were significantly lower in earthworm-invaded than no-worm sites and consistently lower (though not significantly) in R than T plots (Table 2). Soil C pools (to 20 cm depth) were significantly lower (about 30–45%) in earthworm-invaded sites, largely as a result of the elimination of forest floor horizons (Fig. 1). Although soil C content appeared to be slightly higher in T than R plots (Fig. 1), these differences were not statistically significant. Patterns of soil N content (data not shown) mirrored those of C, but differences among plots were not statistically significant ($P = 0.079$). Finally, microbial biomass in mineral soil was significantly higher in earthworm-invaded than NW plots; for example, in 0–5 cm depth of reference soils, microbial biomass C averaged 1.28 ± 0.14 mg/g soil in worm vs. 0.71 ± 0.09 mg/g soil in no-worm sites.

Litter decomposition.—During the first six months of litter decay (November–May), dry mass and carbon loss from litter in T plots greatly exceeded that in NW and R plots, reflecting high overwinter litter mixing activity of earthworms in the T plots (Fig. 2). Litter carbon loss after six months was similar in NW and R plots and

TABLE 2. Mineral soil bulk density, carbon concentration, C:N ratio, and natural abundance of ^{13}C and ^{15}N in no-worm (NW), *L. rubellus*-dominated (R), and *L. terrestris*-dominated (T) study plots in Arnot Forest, New York (prior to adding labeled litter).

Soil depth (cm)	Bulk density (g/cm^3)			Carbon (%)			C:N (mass basis)	
	NW	R	T	NW	R	T	NW	R
0–5	0.51 (0.04)	0.49 (0.04)	0.62 (0.04)	9.31 (1.14) A	5.75 (0.70) B	6.79 (0.71) B	15.9 (0.68)	13.3 (0.75)
5–10	0.66 (0.05)	0.58 (0.04)	0.60 (0.05)	3.25 (0.45)	2.63 (0.51)	4.12 (0.61)	14.1 (0.59)	11.6 (0.92)
10–15	0.77 (0.08)	0.76 (0.07)	0.60 (0.05)	2.68 (0.35) A	1.56 (0.14) B	1.93 (0.10) AB	14.4 (0.78) A	10.1 (0.50) B
15–20	0.76 (0.07)	0.87 (0.07)	0.71 (0.10)	2.20 (0.26) A	1.43 (0.10) B	1.71 (0.14) AB	13.5 (0.64) A	10.0 (0.45) B

Notes: Values are means; standard errors are in parentheses. Within rows, values for C:N and percentage carbon with different letters were significantly different ($P < 0.05$; 12 samples per treatment).

there was only minor visual evidence of earthworm activity in the R plots, despite abundant earthworm populations (Table 1). During the warm season (May–October), high earthworm activity resulted in rapid C loss from litter in the R and T plots (Fig. 2); in the latter, only a residue of petioles and large leaf veins remained after one year. After one year, the percentage of carbon remaining in the litter differed significantly among plots ($P < 0.001$; ANOVA model results in Appendix A) in the rank order NW (62%) > R (25%) > T (4.5%). After two years, none of the original litter was visible in the earthworm-invaded plots. In contrast, decomposition was surprisingly slow in the NW plots during the second year; 48.1% of initial ^{13}C remained on the plots in October 2009.

Nitrogen concentration in decaying litter increased significantly during the first six months of decay. In the NW and R plots this resulted in a net gain in total N content of litter, about 21% higher than the initial litter N pool (Fig. 2). In contrast, in the T plots, net loss of N from litter was observed, with about 53% of the initial litter N pool remaining by May, a significant difference ($P < 0.01$) from the NW and R plots. Continued net N accumulation was observed in decaying litter during the first summer in NW plots, but rapid N release occurred in all the earthworm plots with highest rates of loss in the R plots (Fig. 2). During the second year of decay in the NW plots, litter N content declined significantly but still remained higher than the initial litter N mass ($114.5 \pm 6.6\%$ of original; Fig. 2).

Loss of ^{13}C from decaying litter closely paralleled total C (Fig. 2) during the first year, as ^{13}C concentration of litter did not change significantly in any of the plots. In contrast, significant decline of ^{15}N enrichment was observed in all the plots and litter types (Fig. 2), despite the observed accumulation of total N. Hence, while N was being transported into the decaying litter from the environment, a substantial amount of the original N in the litter tissue was lost. For example, after one year, litter in the NW plots lost about 25% of its initial ^{15}N while gaining an additional 29% of total N. The effect of earthworms on ^{15}N loss from the litter was highly significant ($P < 0.001$; Appendix A), with the magnitude of release after one year increasing in the order NW (25%) < R (79%) < T (97%) plots.

Isotope recovery in soil.—As evidenced by enrichment above natural abundance levels, ^{13}C and ^{15}N from decaying litter were detected in soils on all three collection dates (Table 3). In May 2008, ^{13}C enrichment was observed to 10–15 cm depth in the NW and R plots and to 20 cm depth in the T plots. The highest enrichment was observed for midden piles in the T plots, and soil from earthworm burrows in these plots was more enriched than bulk soil; for example, after one year burrow soil exhibited about three-fold higher ^{13}C enrichment than bulk soil (Appendix B; $P < 0.001$).

The most informative comparisons among earthworm plots and dates are based upon percentage recovery of ^{13}C and ^{15}N released from litter (“soil recovery”; Fig. 3). On this basis in May, ^{13}C recovery in the soil profile (to 20 cm depth) ranged from 36% to 80% across the plots, with significantly ($P < 0.05$) lower recovery in NW than the T and R plots (Fig. 3). The same between-plot patterns were observed after one year and percentage recoveries (16–35%) were significantly lower than in May. No significant effects of topographic position were observed. A highly significant earthworm by soil depth interaction (Appendix A) resulted from the deeper transport of ^{13}C in the T than the R and NW plots. After two years of decay, soil recovery of the ^{13}C released from litter ($22.0\% \pm 5.4\%$) was fairly similar in all the earthworm treatments (Fig. 3).

Not surprisingly, percentage recovery of ^{15}N released from litter was generally higher than for ^{13}C (Fig. 3). In contrast to ^{13}C , significantly lower ^{15}N recovery was observed in the T than the R and NW plots after one year of decay. After two years, recovery in soil of the ^{15}N released from litter ranged from 35% (T plots) to 52% (R) and 56% (NW), but these differences were not statistically significant.

We also calculated the percentage recovery of ^{13}C and ^{15}N on the basis of the total amount added in labeled litter to each quadrat (total recovery, i.e., including that remaining in the partially decayed litter). Total recovery differed significantly for both ^{13}C and ^{15}N among earthworm treatments. After two years, total recovery of ^{13}C in litter plus soil was much higher ($P < 0.01$) in the NW (73%) than the earthworm-invaded plots (28%). Similarly, total recovery of ^{15}N was much higher ($P < 0.01$) in the NW (100%) than the earthworm plots (42%).

TABLE 2. Extended.

C:N (mass basis)	$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)		
T	NW	R	T	NW	R	T
14.1 (0.63)	−25.941 (0.128)	−25.938 (0.138)	−26.104 (0.074)	7.988 (0.567)	5.928 (0.540)	6.790 (1.378)
13.3 (0.60)	−25.583 (0.107)	−25.437 (0.145)	−25.829 (0.100)	12.206 (0.815)	10.045 (0.608)	10.099 (1.846)
11.8 (0.37) AB	−25.391 (0.091)	−25.185 (0.116)	−25.593 (0.091)	12.587 (0.558)	11.839 (0.355)	15.876 (3.286)
11.1 (0.48) B	−25.205 (0.168)	−25.235 (0.125)	−25.471 (0.087)	13.988 (0.677)	11.747 (0.306)	15.636 (3.267)

Isotopes in soil aggregate fractions.—We quantified isotopic enrichment in seven soil fractions for the surface mineral soils (0–5 cm) in the NW, R, and T plots collected in May 2008 and October 2009. Based on repeated-measures ANOVA, differences were observed among these fractions and the patterns of enrichment differed markedly between worm and no-worm plots (Fig. 4). In particular, in the R and T plots, the highest isotope enrichment was observed in the low-density organic matter fraction (R, $\delta^{13}\text{C} = -17.18 \pm 1.61$; T, $\delta^{13}\text{C} = 3.20 \pm 12.01$) that presumably represented litter fragments mixed into mineral soil by the earthworms. In contrast, this fraction had the lowest isotope enrichment in the NW plots ($\delta^{13}\text{C} = -26.72 \pm 0.25$) as it consisted mostly of unlabeled roots. Among the remaining aggregate fractions, the highest ^{13}C and ^{15}N enrichment in the no-worm plots occurred in the silt plus clay fractions, whereas these fractions had the lowest enrichment in the earthworm plots (Fig. 4). High enrichment in the earthworm plots was observed in free microaggregates and in particulate organic matter (POM) held within the macroaggregates. Presumably the latter represents incorporation of litter fragments into earthworm casts because in May 2008 earthworm middens in the T plots exhibited exceptionally high enrichment of POM held within macroaggregates (Fig. 4). In May 2008, patterns of isotopic enrichment in the R and T plots were qualitatively similar although the magnitude of enrichment was much lower in the R plots; by October 2009, they were very similar, and the highest enrichment in earthworm plots was observed in POM and microaggregates held within macroaggregates.

Isotope recovery in microbial biomass.—Both ^{13}C and ^{15}N were detected in microbial biomass above natural abundance to a soil depth of 10 cm in all the plots on all three dates. No ^{13}C enrichment of microbial biomass was observed in 10–20 cm soil in any of the NW plots, whereas very slight enrichment was observed at this depth in two of the R and T plots. Because of the reduced sample size associated with sample pooling and relatively high variation, differences in microbial ^{13}C between treatments were not statistically significant; however, some notable patterns did emerge. For example, recovery of ^{13}C in microbial biomass was consistently low in surface soil (0–2 cm) of the T plots during the first year (Fig. 5), despite the high ^{13}C recovery in that soil layer (Fig. 3). This result primarily reflected relatively low microbial biomass in T plots as

^{13}C concentration of microbial biomass was nearly identical in 0–2 cm soil of R and T plots.

Percentage recovery in microbial biomass of ^{13}C released from litter ranged from 1.3% to 4.5% in May 2008 (Fig. 5). These values declined significantly ($P = 0.013$) through time and were consistently (but not significantly) lower in the T than R plots. In May, recovery of ^{15}N in microbial biomass (3.5 to 15.5%) was much higher than for ^{13}C and it decreased ($P = 0.06$) through time (Fig. 5). During the first year microbial ^{15}N recovery was significantly lower ($P = 0.03$) in the T than NW and R plots in surface soil, reflecting the same pattern as for SOM and ^{13}C . Although we rarely detected ^{13}C in microbial biomass at 10–20 cm depth, small amounts of ^{15}N were recovered in microbial biomass at that depth in T plots in May 2008 and in all the plots after one year and two years.

Earthworm isotopes.—Earthworms collected from reference areas outside the experimental quadrats exhibited $\delta^{13}\text{C}$ isotope natural abundances in their tissues comparable to the soils from which they were collected (e.g., soil $\delta^{13}\text{C} = -25\text{‰}$ to -26‰ , earthworm $\delta^{13}\text{C} = -24.47\text{‰}$ to -25.67‰). The lower values of $\delta^{15}\text{N}$ for earthworms than soil (soil $\delta^{15}\text{N} = 6\text{‰}$ to 16‰ , earthworm $\delta^{15}\text{N} = 0.319\text{‰}$ to 3.967‰) reflect the lower

FIG. 1. Soil carbon pools in 0–20 cm soil of earthworm-free sites and sites with invasive earthworm communities dominated by *Lumbricus rubellus* or *L. terrestris* at Arnot Forest, New York, USA. Error bars indicate standard errors. Bars with different letters are significantly different ($P < 0.05$).

FIG. 2. Loss of carbon, nitrogen, ^{13}C , and ^{15}N from sugar maple litter incubated in no-worm, *Lumbricus rubellus*-dominated, and *L. terrestris*-dominated plots at Arnot Forest, New York. Error bars indicate $\pm\text{SE}$.

$\delta^{15}\text{N}$ of forest floor horizons (0.385‰) and leaf litter (−1.790‰) that the earthworms consume. Isotope natural abundances of endogeic earthworms were significantly higher than for the litter-feeding *Lumbricus* species, reflecting the higher values in mineral soil than

forest floor and litter and the high proportion of mineral SOM in the endogeic earthworm diets.

Earthworms collected from the experimental plots were highly enriched in both ^{13}C and ^{15}N compared with natural abundance values (Appendix C). Much higher

TABLE 3. Accumulation of excess ^{13}C and ^{15}N in soils (above natural abundance) of quadrats with labeled leaf litter added and differing earthworm communities, in Arnot Forest, New York.

Depth (cm)	^{13}C (mg/m ²)			^{15}N (mg/m ²)		
	May 2008	October 2008	October 2009	May 2008	October 2008	October 2009
No-worm plots						
Oa	9.66 (2.11)	4.92 (0.72)	17.22 (3.30)	6.08 (0.99)	2.07 (0.22)	6.78 (0.77)
0–5	14.56 (4.57)	12.22 (2.80)	9.90 (3.85)	1.62 (0.39)	7.13 (1.05)	2.43 (0.39)
5–10	17.19 (8.80)	1.39 (0.83)	2.69 (1.41)	0.36 (0.14)	3.00 (0.21)	1.14 (0.66)
10–15	0	0	0	0	0	0.12 (0.12)
Total	41.41 A	18.53 A	29.81 A	8.06	12.20	10.52 A
<i>Lumbricus rubellus</i> plots						
0–2	28.60 (7.52)	30.83 (6.61)	30.98 (2.39)	3.89 (0.82)	8.30 (1.57)	7.56 (0.34)
2–5	10.88 (3.82)	33.14 (4.65)	44.29 (11.07)	3.40 (1.24)	10.67 (1.46)	12.19 (2.47)
5–10	5.51 (1.35)	8.93 (2.72)	7.33 (5.43)	0	5.97 (2.86)	4.96 (2.32)
10–15	7.90 (7.31)	1.43 (0.90)	1.19 (0.64)	0	1.18 (0.27)	0.32 (0.18)
15–20	0	5.05 (1.55)	1.08 (0.46)	0	0	0
Total	52.89 A	79.38 B	84.87 B	7.29	26.12	25.03 B
<i>Lumbricus terrestris</i> plots						
Middens	26.84 (9.69)	4.30 (0.92)	ns	3.35 (1.17)	0.70 (0.14)	ns
0–2	50.67 (5.40)	12.48 (2.00)	21.00 (4.36)	7.89 (1.01)	2.77 (0.35)	5.18 (1.07)
2–5	38.05 (5.49)	41.32 (10.16)	39.17 (9.66)	4.15 (1.56)	9.12 (1.40)	8.09 (1.64)
5–10	28.78 (4.92)	15.37 (3.29)	32.12 (17.05)	1.35 (0.93)	3.15 (0.83)	3.09 (1.67)
10–15	8.17 (1.73)	6.43 (1.21)	1.52 (0.86)	0.25 (0.20)	0.76 (0.23)	0.36 (0.12)
15–20	4.77 (1.73)	5.18 (1.76)	1.79 (0.18)	0	0	0.24 (0.12)
Total	157.28 B	85.08 B	95.60 B	16.99	16.50	16.96 AB

Notes: Values are means; standard errors are in parentheses; ns means not sampled. Within columns, totals with different letters are significantly different ($P < 0.05$; 3 plots per treatment) for a particular date. Values for *L. terrestris* plots include both burrow and bulk soil.

FIG. 3. Percentage recovery in soil of ¹³C and ¹⁵N released from sugar maple litter in no-worm, *Lumbricus rubellus*-dominated, and *L. terrestris*-dominated plots at Arnot Forest, New York. Samples were collected after six months (May 2008), one year (October 2008), and two years (October 2009) of litter decomposition. Error bars indicate standard errors. Different letters indicate significant differences ($P < 0.05$) among earthworm plots for particular dates.

enrichment was observed for the *Lumbricus* spp. than for the endogeic species during the first year but these values were similar after two years. Percentage recovery in earthworms of ¹³C and ¹⁵N released from litter was calculated in the same manner as for soil and microbial biomass pools. In May 2008, percentage recovery of ¹³C in earthworm biomass ranged from 0.51% to 0.63% across plots and was similar between R and T plots (Appendix C). Percentage ¹³C recovery in earthworms appeared to decline by October 2008 (0.4%) but remained similar in the second year. Not surprisingly, percentage recovery of ¹⁵N in earthworms was much higher than for ¹³C, ranging from 2.5% to 3.3% in spring 2008 and generally declining thereafter (Appendix C).

Stoichiometry of C and N recovery.—The C:N ratio of mineral soil was lower in earthworm-invaded than in no-worm plots (Table 2). Similarly, the C:N ratio of microbial biomass was significantly lower in surface soils (0–10 cm) of R (4.25 ± 0.53) and T (4.90 ± 0.42) than in NW plots (7.30 ± 0.66). The C:N ratio of

earthworm biomass exhibited strong homeostasy, ranging from 3.72 to 4.05 across taxa (slightly lower for endogeic than *Lumbricus* spp.) and differing by less than 2.5% across plots within taxa.

We calculated the C:N ratio of new soil organic matter (SOM), earthworms, various aggregate fractions, and microbial biomass, all derived from the labeled plant litter, based on the ¹³C and ¹⁵N enrichment above natural abundance; inorganic ¹⁵N values were subtracted from total N before calculating C:N in soils. We note that the bulk C:N of the leaf litter (42) was higher than the excess ¹³C:¹⁵N in the labeled litter (34). Generally consistent and sometimes significant patterns of variation in new SOM C:N were observed (Fig. 6). In particular, C:N values of new SOM were significantly lower in the O_e + O_a horizon of NW than in surface soil (0–2 cm) of R and T plots. Among aggregate fractions C:N of new SOM adsorbed to silt plus clay (C:N = 2.7–3.1) was significantly lower than for aggregates (C:N = 3.8–4.6) with the highest values for POM held within

FIG. 4. Enrichment of ^{13}C in six aggregate fractions of surface mineral soil in no-earthworm plots in May 2008 and October 2009; in plots dominated by *Lumbricus rubellus* and *L. terrestris* in 2008; average of *L. rubellus* and *L. terrestris* plots (worm) in 2009; in earthworm middens of *L. terrestris* plots in 2008, and ^{13}C to ^{15}N ratio in these aggregate fractions, in Arnot Forest, New York. Abbreviations are: macro, macroaggregates; micro-free, free microaggregates; POM-macro, particulate organic matter in macroaggregates; micro-macro, microaggregates in macroaggregates; S + C macro, silt + clay in macroaggregates. Error bars indicate standard errors.

aggregates (C:N = 4.9). After one year, new microbial biomass C:N in surface soil was significantly ($P < 0.05$) higher for NW than R and T plots (Fig. 6), reflecting the pattern for overall microbial biomass C:N. No significant effects of collection date were observed. Finally, earthworm biomass exhibited lower ^{13}C : ^{15}N ratios, ranging from 1.2 to 2.2 across species, dates, and plots.

DISCUSSION

Earthworms alter soil properties as a result of their feeding, burrowing, and casting activities (Edwards and Bohlen 1996). Earthworm invasion of cold temperate forests can eliminate surface organic horizons (forest floor), alter the depth distribution of carbon in soil (Hale et al. 2005), and sometimes reduce soil C storage (Alban and Berry 1994, Bohlen et al. 2004; but see Wironen and Moore 2006). The observations from the present study generally confirmed previous observations from the Arnot Forest study area (Bohlen et al. 2004) that earthworms significantly reduce soil C storage in this forest ecosystem, and they strongly supported the expectation that earthworms would accelerate heterotrophic processing of litter C and N (Wardle 2000); they also provided valuable insights into the complex mechanisms of earthworm influences on forest soil properties. We begin by evaluating earthworm effects on the processing of litter C and N, followed by interpretation of the implications for understanding C/N stoichiometry of soil

organic matter. Throughout, we refer to the effects of differing earthworm communities in terms of dominance by *Lumbricus rubellus* (R plots) or *L. terrestris* (T plots); although other differences in earthworm composition were observed between R and T plots (e.g., *Octolasion tyrtaeum*; Table 1), the high biomass and isotope enrichment of *Lumbricus* spp. (Appendix C) would suggest their key influence on litter processing.

Earthworm effects on litter C and N dynamics.—Our observations confirmed earlier work in this study area (Suárez et al. 2006b) and elsewhere (Staaf 1987), that earthworms accelerate the disappearance of leaf litter from temperate broadleaf forest floor. Moreover, we observed a striking and consistent difference between R plots and T plots. Accelerated litter disappearance in the R plots was delayed until the warm season (May–October; Fig. 2). This pattern was similar to that observed by Suárez et al. (2006b) during winter 2001–2002 in the same general area. In contrast, over half of the litter had disappeared by spring in all the T plots (Fig. 2) in the present study. One likely cause of these contrasting results would be some differences in cold season weather between 2001–2002 (Suárez et al. 2006b) vs. 2007–2008 that contributed to the high activity of *L. terrestris* in 2007–2008, because earthworm populations were roughly similar between the two studies (compare Table 1 vs. Suárez et al. 2006b). In fact, winter weather was actually warmer in 2001–2002 than 2007–2008 (data

FIG. 5. Percentage recovery in microbial biomass of ^{13}C and ^{15}N released from sugar maple litter in no-worm, *Lumbricus rubellus*-dominated, and *L. terrestris*-dominated plots at Arnot Forest, New York. Samples were collected after six months (May 2008), one year (October 2008), and two years (October 2009) of litter decomposition. Values for 0–2 cm in *L. terrestris* plots includes middens. Error bars indicate standard errors.

available online);⁷ however, the former year was marked by the delayed development of winter snowpack (no snow until January) likely leading to soil freezing (Groffman et al. 2001), which can decrease decomposition (Christenson et al. 2010), whereas a deep snowpack (15–20 cm) accumulated in early December 2007. Also, earthworm activity was observed in T plots on 9 January 2008 (T. Fahey, *personal observation*) during an exceptional warm spell that melted the snowpack. Apparently, the climatic effect on earthworm activity applies to *L. terrestris* but not *L. rubellus*. Tiunov et al. (2006) ranked the cold tolerance of common invasive earthworms based on laboratory assays and noted greater tolerance of *L. rubellus* than *L. terrestris*. However, the combined

effects of insulating snowpack and earthworm behavioral responses (e.g., deep burrowing) have not been evaluated.

After one year, litter disappearance on the R plots (75%; Fig. 2) was similar to observations of Suárez et al. (2006b), whereas it was much higher in the T plots (over 95%), continuing the pattern established in the cold season. Winter activity can be quite important as nutrients might be mineralized at a time when plants are not capable of taking them up, leading to increased hydrologic and/or gaseous losses (Judd et al. 2007). Winter climate may thus be a key regulator of earthworm effects on nutrient retention in forests. Indeed, this may account for the relatively low recovery of ^{15}N in the T plots in this study (Fig. 3).

As detailed by Fahey et al. (2011), the ^{13}C label was not uniformly distributed in the litter substrate, being

⁷ <http://climod.nrcc.cornell.edu/>

FIG. 6. $^{13}\text{C}:^{15}\text{N}$ ratio of new soil organic matter (SOM) and new microbial biomass derived from sugar maple litter in no-worm, *Lumbricus rubellus*-dominated, and *L. terrestris*-dominated plots at Arnot Forest, New York. Samples were collected after six months (May 2008), one year (October 2008), and two years (October 2009) of litter decomposition. Error bars indicate standard errors.

significantly more enriched in nonstructural than structural components. Nevertheless, the loss of ^{13}C from litter paralleled total C, both for NW and earthworm-invaded plots (Fig. 2). In the latter, this result reflects the fact that most of the litter disappearance was associated with particulate transport to soil, whereas in the NW plots, C loss from litter was dominated by leaching and decomposition (Fahey et al. 2011). Thus, it appears that the ^{13}C label was incorporated into the litter substrate in a way that did not differentially affect its transformation by these processes. As noted by Filley et al. (2008), processing of hardwood leaf litter by *L. rubellus*-dominated earthworm communities left a residue of leaf petioles on the soil surface that could alter the trajectory of SOM formation by differential decay of various organic fractions.

In general, the flux of N and ^{15}N from litter in the earthworm-invaded plots reflected patterns for C (Fig. 2). Total N and ^{15}N flux in the NW plots indicated coincident leaching of litter N (i.e., ^{15}N loss) and transport of exogenous N into decaying litter (i.e., increased total N). In the R plots, the N dynamics pattern matched the NW plots during the cold season, whereas loss of both total N and ^{15}N was observed in the T plots. Clearly, this contrast reflects the differences in earthworm feeding activity during winter between R and T plots, as noted above. Slightly (but significantly) higher ^{15}N loss from R than NW plots over winter probably reflects the effect of early spring earthworm activity on N leaching and particle transport from litter. During the warm season earthworm activity in both the R and T plots resulted in rapid loss of N, probably including both particle transport to mineral soil and

leaching. The rate of loss of both N and ^{15}N was higher in R than T plots in the first summer (Fig. 2), but the cumulative loss of N remained higher for the latter after one year. Filley et al. (2008) also demonstrated earthworm effects on N mobilization from litter, as well as the apparent fungal transport of N into structural tissues of low N concentration.

Recovery of litter ^{13}C in soil.—We were able to quantify precisely the transport of ^{13}C from litter to soil. Earthworm activity resulted in significantly higher percentage recovery in soil of carbon released from litter (Fig. 3). The two principal mechanisms that could contribute to this pattern are (1) earthworm mixing of largely undecayed particulate C into soil and (2) protection of C in soil aggregates created by earthworm feeding (Bossuyt et al. 2004). Overwinter in the absence of earthworms, leaching of soluble C from decaying leaf litter resulted in fairly uniform accumulation of ^{13}C in surface soil layers (Fig. 3) and this DOC probably was retained primarily by a physical adsorption/precipitation process (Kaiser et al. 1996, Kalbitz et al. 2000) as indicated by the highest ^{13}C enrichment of silt plus clay fractions (Fig. 4). In contrast, in the earthworm-invaded plots, earthworm feeding, burrowing, and casting activities transported particulate C into mineral soil. Overwinter, this process was most pronounced in the T plots, as earthworms remained active during the winter in the absence of soil freezing. In the absence of *L. terrestris* these earthworm-mediated processes overwinter were subdued, as much less particulate C was transported to soil by *L. rubellus*. Nevertheless, much higher ^{13}C recovery in microbial biomass (Fig. 5) and high ^{13}C enrichment of earthworm tissues (Appendix C) indicated relatively efficient assimilation of litter C by biota in the R plots. Perhaps *L. rubellus* fed on more labile C including that leached into surface mineral horizons.

Percentage recovery in soil of ^{13}C derived from litter declined dramatically during the first summer, presumably as a result of high soil microbial metabolism with warmer temperatures (Wei et al. 2010.). However, the percentage recovery of ^{13}C in microbial biomass also declined significantly ($P = 0.013$) during the summer, illustrating the rapid turnover of microbial C in soil (Wolters and Joergensen 1992). In the NW plots, the decline in soil ^{13}C must have resulted primarily from microbial utilization of sorbed DOC, supporting the contention of Guggenberger and Kaiser (2003) that most of this fraction remains relatively labile in the short term. In the earthworm-invaded plots it undoubtedly included microbial decay of particulate carbon as well as continued earthworm processing of soil. Low recovery of ^{13}C in microbial biomass of the T plots primarily reflected low microbial biomass at this time (rather than low ^{13}C enrichment). One possible explanation is that earthworm activity in the T plots stimulated higher specific activity and turnover of the microbial community. Dilution by mixing with unenriched soil, microbial

decay and possibly fungal transport could have contributed to this loss of ^{13}C . Utilization of burrows by *L. terrestris* promoted the accumulation of litter C on burrow walls (Appendix B) probably in the form of earthworm secretions (Brown et al. 2000). Tiunov and Scheu (1999) demonstrated that this process leads to higher microbial activity, especially for bacteria, on *L. terrestris* burrow walls. These observations of the dynamics of litter-derived C in middens and burrows illustrate the processes contributing to the development of the “drilosphere,” the earthworm-affected soil volume (Devliegher and Verstraete 1997).

At the end of the second year, percentage recovery of ^{13}C derived from litter remained high in the NW plots as considerable ^{13}C still remained in the leaf litter (Fig. 2) and averaged 27.6% in earthworm-invaded plots (Fig. 3) where all labeled litter had disappeared (Fig. 2). Using a similar approach, Rubino et al. (2010) also observed high recovery of litter C in soil. Our results demonstrate that, even with earthworm processing, much of the soil C derived from litter that is not catabolized during the first year of decay becomes relatively stable. In the presence of earthworms, the process of stabilization includes the formation of water-stable aggregates (Six et al. 2000). High ^{13}C recovery in macroaggregates and microaggregates (rather than silt plus clay) indicated the role of earthworm feeding and casting in protection of organic matter in these forest soils, paralleling observations from agricultural soils (Fonte et al. 2007). Six et al. (2000) proposed a hierarchical theory of aggregate formation, with microaggregates being incorporated within macroaggregates. After six months, we observed higher ^{13}C incorporation in free microaggregates than for those within macroaggregates (Fig. 4). This result may reflect the observation that the small forest earthworms produce smaller, more fragile, casts that disperse easily into free microaggregates (Blanchart et al. 2004), in contrast with the larger earthworms of agricultural and tropical soils. Also, the role of Ca^{2+} in binding fresh organic matter to clays in aggregates (Shipitalo and Protz 1989) might be diminished in these acid forest soils. After two years, all the aggregate fractions were roughly equally enriched in ^{13}C (Fig. 4), presumably reflecting continued mixing and earthworm ingestion of previously-formed microaggregates, both releasing microaggregates and forming new ones within new macroaggregates (DeGryze et al. 2006). Studies of the long-term fate of C and N in earthworm-affected forest soils is needed, but clearly the key role of their stabilization in microaggregates is indicated by the present study.

Recovery of ^{15}N and interactions of C and N.—Previous studies have indicated that loss of soil C associated with earthworm invasion of forest is accompanied by retention of N, resulting in a lowering of mineral soil C:N ratio (Bohlen et al. 2004, Eisenhauer et al. 2007; Table 2). This response is somewhat surprising because the C:N ratio of litter and forest floor substrates

that earthworms mix into soil greatly exceeds that of the soil itself. The high C:N of the substrates utilized by detritivores results in N limitation of their enzymatic processes (Martinson et al. 2008). Fungal decomposers that dominate the detrital food chain in temperate forest in the absence of earthworms overcome this limitation in part by transporting N from N-rich soil horizons to the litter substrate (Hart and Firestone 1991), a process clearly indicated in the no-worm plots (Fig. 2; Fahey et al. 2011). Where abundant, earthworms assume dominance of the detrital food chain. Digestion of detrital substrates by earthworms is run by their gut microbial community (Trigo et al. 1999), and the worms apparently select these communities in a way that allows them to maintain strict stoichiometric homeostasis in face of varying substrate resource supply (Curry and Schmidt 2007). In theory, the stoichiometry of these detritivore communities plays a fundamental role in regulating the C:N of SOM, and recent studies indicate that stabilized SOM is derived mostly from microbial by-products (Simpson et al. 2007, Mambelli et al. 2011).

Our double labeling of plant litter applied to plots with and without earthworms provides evidence to further inform this theory. Microbial biomass in the earthworm-invaded plots exhibited a significantly lower C:N ratio than for the no-worm plots, and new microbial biomass derived from labeled leaf litter also had a lower ratio of $^{13}\text{C}:^{15}\text{N}$ in the presence than absence of earthworms (Fig. 6). This pattern may be explained in part by an earthworm-induced shift in the composition of the microbial community from fungal to bacterial dominance (Edwards 2004, Fierer et al. 2009). In our study area, bacterial:fungal ratios were significantly higher in earthworm-invaded than adjacent no-worm plots (Dempsey et al. 2011). Because bacteria generally have lower tissue C:N than fungi (Paul 2007), such a shift would be expected to result in reduced microbial C:N, thereby contributing to the lower microbial C:N in earthworm than NW plots.

Our observation that new SOM derived from the labeled litter exhibited significantly higher $^{13}\text{C}:^{15}\text{N}$ ratio in earthworm-invaded than no worm plots (Fig. 6) would appear to be at odds with the foregoing theory and with the observation of lower C:N of SOM in the earthworm-invaded plots. This paradox may be explained as a transient effect of earthworm mixing of high C:N particulate detritus into the mineral soil and its subsequent temporary protection in microaggregates and macroaggregates. In the absence of earthworms, leaching of soluble organic matter of relatively lower C:N than the litter solid phase (Qualls and Haines 1992, Kalbitz et al. 2000, Park and Matzner 2003) is the principal mode of transport from litter to soil. This low C:N DOC is adsorbed to silt + clay particles in mineral soil, which therefore exhibit relatively low $^{13}\text{C}:^{15}\text{N}$ ratio (Fig. 4). We propose that subsequent processing of particulate organic matter in the mineral soil by earthworms and the microbial community eventually

counteracts this initial effect, but longer term (i.e., beyond two years) observations are needed to demonstrate this proposed process.

The ratio of $^{13}\text{C}:^{15}\text{N}$ in the litter substrates was slightly lower (34) than bulk litter C:N (42), which might affect interpretation. However, the $^{13}\text{C}:^{15}\text{N}$ ratio of new SOM and of organic matter assimilated by microbial biomass and earthworms was consistently much lower than the C:N in those pools. This observation indicates that ^{15}N label was preferentially assimilated over ^{13}C label in comparison with overall assimilation of C and N from litter. Because the ^{13}C label was preferentially incorporated into nonstructural carbohydrates and hemi-celluloses in the litter substrate (Fahey et al. 2011), this observation indicates inefficient microbial assimilation of these labile substrates. Perhaps N limitation of microbial activity in the high C:N litter substrate results in "overflow metabolism," as suggested by Schimel and Weintraub (2003). In any case, the foregoing observations suggest that the overriding mechanism explaining the net effect of earthworm invasion on loss of forest soil C could be stoichiometric in nature; the N limited detritivore community is dominated by earthworms with strong stoichiometric homeostasis, resulting in a microbial community that mineralizes C while retaining N.

The most striking difference in the ^{15}N recovery between plots was the initially low values in the T plots (Fig. 3). Two possible explanations of this effect are (1) relatively low microbial immobilization of litter N and subsequent loss (e.g., by leaching or denitrification) and (2) relatively high plant root uptake. Elliott et al. (1990) reported a fivefold increase in denitrification from earthworm casts relative to soil, and the middens may have been denitrification hotspots in our study. Notably, ^{15}N in tissues of small plants rooted entirely in the quadrats was not significantly different between NW, R, and T plots (J. Maerz, *unpublished data*). In the NW and R plots, much of the soil ^{15}N recovery occurred in the surface soil layers (O_a or 0–2 cm) in May, but shifted to deeper soil during the summer (Fig. 3). Fahey et al. (2011) attributed this shift in the NW plots to a combination of plant root uptake in densely rooted surface soil and stabilization of organic N in mineral soil associated with microbial processing.

In the R and T plots, the pattern of distribution of ^{15}N among soil aggregate fractions mirrored those of ^{13}C (Fig. 6); the high enrichment in macroaggregates and free microaggregates indicates the role of earthworm feeding in stabilization of soil organic N. In contrast, in the no-earthworm plots, ^{15}N recovery was predominantly in the silt plus clay fraction, evidence for a sorption process of N retention. These observations indicate that earthworms fundamentally alter the process of organic N stabilization in forest soil. Sollins et al. (2006) showed that low C:N organic matter is stabilized on mineral surfaces of silt and clay particles with subsequent accretion of higher C:N compounds. As

noted above, earthworm processing of litter appears to incorporate organic matter into microaggregates and macroaggregates in the form of particulate organic matter rather than sorption on silt and clay (Fig. 6). Subsequent microbial transformation of the organic matter reduces its C:N ratio. The process of its longer term stabilization, presumably largely on silt plus clay particles, requires additional study.

Ecosystem scientists are wrestling with the complexity of the possible feedbacks among large-scale changes in several drivers of forest ecosystem dynamics—climate, atmospheric CO₂, N deposition, C sequestration (Thornley and Cannell 1996, Ollinger et al. 2003)—as well as invasive species (Ehrenfeld 2003). Our observations of the processing of C and N in plant detritus by invasive earthworms point toward the primary role of the stoichiometry of these dominant detritivores in regulating SOM dynamics. By altering the microbial community to maintain stoichiometric homeostasis, earthworms may enhance soil C mineralization and the retention of N as POM in soil aggregates. The abundance, composition, and seasonal activity of the earthworm community, including overwinter activity that depends on winter climate, will influence their overall effects. Moreover, variation in other key ecosystem properties—e.g., forest composition; forest floor thickness; soil pH, texture, and clay mineralogy; microbial community composition; and N deposition—can be expected to modify some of the processes we observed, especially earthworm feeding, DOC sorption, and aggregate dynamics, with possible consequences for the generality of the mechanism that we propose for earthworm effects on soil C and N. We emphasize that the effects we studied apply to aboveground detritus; a high proportion of forest soil C inputs is supplied belowground by root turnover and exudation, both of which are highly sensitive to the same global change factors (Norby and Jackson 2000). In fact, earthworms apparently play a significant role in root turnover, consuming live fine roots and mycorrhizae (Horowitz et al. 2009). The responses of northern forest ecosystems to global drivers should be evaluated in the context of these overriding effects of the detritivore community.

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SUPPLEMENTAL MATERIAL

Appendix A

ANOVA of isotope recovery in soils ([Ecological Archives A023-062-A1](#)).

Appendix B

Excess ¹³C content of earthworm burrows ([Ecological Archives A023-062-A2](#)).

Appendix C

Isotope enrichment and recovery in earthworms ([Ecological Archives A023-062-A3](#)).