Litter decomposition in earthworm-invaded northern hardwood forests: Role of invasion degree and litter chemistry

Andrew R. HOLDsworth, University of Minnesota, Conservation Biology Graduate Program, 187 McNeal Hall, 1985 Buford Avenue, St. Paul, Minnesota 55108, USA.
Lee E. FRElich & Peter B. Reich, University of Minnesota, Department of Forest Resources, 1530 N. Cleveland Avenue, St. Paul, Minnesota 55108, USA.

Abstract: The effects of invasive earthworms on decomposition are little known, and the controls of their effect on decomposition may be different than those of microbes. Sugar maple–dominated forests previously devoid of earthworms in the western Great Lakes region (USA) exhibit different degrees of earthworm invasion, presenting a natural experiment to study its effects on litter decomposition. We hypothesized that litter decomposition would depend on the degree of earthworm invasion, the presence or absence of worms of differing sizes, and initial litter chemistry. We established an experiment using fine- and coarse-mesh litterbags (to allow access by different-sized worms) to study decomposition of 3 different litters in mixture under different degrees of earthworm invasion in 12 Minnesota sites. The effect of earthworm invasion degree on litter decomposition varied by identity of the litter, mesh size, and time of litter collection. Decomposition of Quercus rubra, the litter with the lowest initial calcium concentration and highest lignin:nitrogen, was not significantly influenced by earthworm invasion degree. In contrast, decomposition of Tilia americana, the litter with the highest calcium concentration and lowest lignin:nitrogen, was fastest in coarse-mesh litterbags. After 15 months the mass of the highest-quality litters was highest in the fine-mesh bags of heavily invaded plots, suggesting that microbially mediated decomposition slowed where earthworms had removed the forest floor.

Keywords: calcium, decomposition, earthworm invasion, lignin:nitrogen, Lumbricidae.

Résumé : Les effets des vers de terre envahissants sur la décomposition sont peu connus et les contrôles de leurs effets sur la décomposition peuvent être différents de ceux des microbes. Les forêts dominées par l’érable à sucre de la région des Grands Lacs (États-Unis) étaient précédemment exemptes de vers de terre, elles sont maintenant exposées à différents niveaux d’invasion de vers de terre représentant une expérience naturelle pour en étudier les effets sur la décomposition de la litière forestière. Nous avons formulé l’hypothèse que la décomposition de la litière soit dépendante du niveau d’invasion de vers de terre, de la présence ou de l’absence de vers de tailles différentes et de la chimie de la litière initiale. Nous avons monté une expérience utilisant des sacs à litière possédant des mailles fines et grossières (pour permettre l’accès à des vers de différentes tailles) pour étudier la décomposition de mélanges de 3 types de litières différentes exposées à différents niveaux d’invasion de vers de terre dans 12 sites au Minnesota. L’effet du niveau d’invasion de vers de terre sur la décomposition de la litière différait selon le type de litière, la taille des mailles et le moment de la collecte. La décomposition de la litière de Quercus rubra (la litière ayant la concentration initiale de calcium la plus faible et le rapport lignine:azote le plus élevé), n’était pas significativement influencée par le niveau d’invasion de vers de terre. À l’opposé, la décomposition de la litière de Tilia americana (la litière ayant la concentration de calcium la plus élevée et le plus faible rapport lignine:azote), était la plus rapide dans les sacs à mailles grossières. Après 15 mois, la masse des litières de plus haute qualité était la plus grande dans les sacs à mailles fines pour les parcelles fortement envahies, suggérant ainsi que la décomposition microbienne a été ralentie là où les vers de terre ont retiré le sol forestier.

Mots-clés : calcium, décomposition, invasion de vers de terre, lignine:azote, Lumbricidae.

Northern temperate forests devoid of earthworms have a thick forest floor with well-developed O₂, O₃, and O₄ organic sub-horizons above the mineral soil (Bohlen et al., 2004c; Hale et al., 2005; Hobbie et al., 2006). This is the result of relatively slow fragmentation of leaf litter that largely occurs through physical processes and the activities of such macro- and meso-fauna as millipedes and isopods. Microbial biomass carbon and nitrogen, although less than in the mineral soil, is notable (Groffman et al., 2004), and total carbon, nitrogen, and C:N ratio decline with soil depth (Bohlen et al., 2004b). Earthworm invasion causes a cascade of changes in the forest floor and mineral soil that could influence litter decomposition. Invasive earthworms incorporate the forest floor into the mineral soil, reducing carbon storage and C:N ratios in the upper layers of the mineral soil (Bohlen et al., 2004b; Hale et al., 2005). Epi-endogeic species, such as Lumbricus rubellus, consume and move small litter fragments from the O₄ and O₃ sub-horizons into the upper mineral soil, while anecic species such as L. terrestris preferentially pull leaves with high calcium contents from the O₃ sub-horizon into their burrows (Holdsworth, 2006), leaving lower calcium litter on the mineral soil surface (Holdsworth, 2006; Suárez et al., 2006). Besides increased litter fragmentation, earthworms can enhance microbial processing of litter as it passes through their intestines (Edwards & Bohlen, 1996).

Earthworm invasion strongly influences the resource availability and microhabitat structure for the microorganisms that carry out decomposition (Bohlen et al., 2004c). The effect of these changes on microbial biomass varies. Earthworm invasion increased microbial biomass and respiration in the mineral soil while decreasing them in the forest floor and led to a net increase in total microbial biomass in northern hardwood forest of the northeastern United States (Groffman et al., 2004). In contrast, earthworm invasion decreased microbial biomass and respiration in both the litter layer and mineral soil in an aspen forest in the Canadian Rocky Mountains (Eisenhauer et al., 2007).

Invasive earthworms can also influence other members of the soil food web, such as microarthropods and microfungi. Where the endogeic species Octolasion tyrtaeum had invaded an aspen forest, microarthropod densities and the number of microarthropod species were significantly lower (Eisenhauer et al., 2007). Using a mesocosm study to evaluate the effects of Dendrobaena octaedra, McLean and Parkinson (1998) observed that its presence increased oribatid mite diversity and had variable effects on microarthropod abundances. McLean and Parkinson (2000) found that Dendrobaena octaedra decreased microfungal community richness and diversity. In general, the degree and character of these changes are shaped by the species composition of the invasive earthworm community, as well as by other factors such as land-use history (Bohlen et al., 2004a; Hale et al., 2005; Suárez et al., 2006; Eisenhauer et al., 2007).

Placing litterbags on the soil surface is a common technique used to measure decomposition. However, the removal of whole leaves and fragments from the litter layer by earthworms (or other macrofauna), and the invasion-induced changes to microbial biomass, C:N ratios, and the soil fauna community, complicate our predictions of litter decomposition in response to earthworm invasion. Furthermore, the controls of microbially mediated decomposition may be very different from what controls earthworms’ contribution to decomposition rates. There are many studies supporting lignin and/or nitrogen as the major controls of microbially mediated decomposition (Meentemeyer, 1978; McClaugherty & Berg, 1987; Hobbie, 1996). However, earthworms, especially Lumbricus species, have high calcium demands and strong litter calcium preferences (Reich et al., 2005) that may make this element an important control of their contribution to decomposition. One explanation for Lumbricus species’ high calcium demands is to supply their well-developed calciferous glands, which produce calcium carbonate that could moderate blood CO₂ levels and pH when soil pCO₂ levels are elevated (Robertson, 1936; Crang, Holsen & Hitt, 1968; Canti & Piearce, 2003). Indeed, a recent common-garden study of 14 tree species found that litter lignin was most important for explaining variation in litter decomposition in fine-mesh litterbags, whereas litter calcium was strongly related to forest floor removal rates and biomass of L. terrestris (Hobbie et al., 2006).

Litterbags of different mesh sizes that exclude or permit access by most earthworms, filled with litter of different chemistries, and placed in sites with different degrees of earthworm invasion provide an opportunity to study the relative effects of earthworm invasion on microbially versus earthworm-mediated decomposition. Sugar maple–dominated forests of the western Great Lakes region exhibit different degrees of earthworm invasion, providing suitable sites for such a study. In northern Minnesota and Wisconsin there are active earthworm invasion fronts dividing forest stands into heavily invaded and lightly invaded areas (Hale, Frelich & Reich, 2005). In central Minnesota, earthworm invasion is more extensive than in northern Minnesota, yet not all stands are invaded by the largest-bodied litter-feeding species L. terrestris (Tiuov et al., 2006).

In this study we hypothesized that litter decomposition would depend on the degree of earthworm invasion, litterbag mesh size, and initial litter chemistry. We used mesh size as a tool to broadly measure microbially mediated decomposition in contrast to earthworm-mediated decomposition. Mass loss from fine-mesh bags, which permit access only by the smallest earthworms, was used as a measure of microbially mediated decomposition, while mass loss from coarse-mesh bags, which permit access by Lumbricus (large-bodied litter-feeding earthworms), was used as a measure of earthworm-mediated decomposition. As the fine-mesh also allows access by mesofauna, their contribution to decomposition (Coleman, Crossley & Hendrix, 2004) is included in what we refer to as microbially mediated decomposition. We had 2 predictions: 1) mass loss from coarse-mesh litterbags would be greater in more heavily invaded areas in comparison to mass loss from fine-mesh bags, and 2) mass loss in coarse-mesh bags would be largest for T. americana and least for Q. rubra litter because of differences in litter preferences of Lumbricus earthworms.
**Methods**

We established an experiment using fine- and coarse-mesh litterbags to study decomposition under different degrees of earthworm invasion in 2 different regions that represent different stages of earthworm invasion (Tunov et al., 2006), the Chippewa National Forest (ChipNF) of northern Minnesota and the central region of Minnesota (USA). The ChipNF (47.15° N, 94.23° W) is a region in the early- to mid-stages of earthworm invasion. We chose 4 sites with mature sugar maple–dominated forest on sandy-loam or loam soils that have never been plowed (Table I). The 4 sites each include active earthworm invasion fronts that permit comparisons between heavily and lightly invaded areas of the site with otherwise similar forest conditions. The lightly invaded areas have thick forest floors including a relatively thick (2.1 ± 0.1 cm) well-defined O$_h$/O$_e$ sub-horizon (Brady & Weil, 2002) and a low biomass of earthworms dominated by *Lumbricus rubellus* and *Dendrobaena octaedra* (Table I). The heavily invaded areas are only 150 m away, but have a thin O$_h$/O$_e$ sub-horizon (0.1 ± 0.1 cm) and a three-fold higher earthworm biomass dominated by *Lumbricus juveniles*, *L. terrestris*, and *Aporrectodea* species (Table I). We considered heavily and lightly invaded areas of each stand to be separate sites, making a total of 8 sites in the ChipNF region. See Table I for additional site conditions and the methods used to describe them.

Central Minnesota represents a region at the mid- to late-stages of invasion. There we chose 4 sites that are all included in the Minnesota Scientific and Natural Area system as high-quality remnants of the “Big Woods” ecosystem (Grimm, 1984; DNR, 1999) and have never been plowed. The sites range from 50 to 90 ha and are within 3–100 km of each other in a region centred at 45.31° N, 93.97° W. All sites have mull soils with sandy-loam or loam texture (Table I). The largest litter-feeding earthworm species, *Lumbricus terrestris*, is present and dominant at 2 of the sites that are heavily invaded, but absent at the other 2 sites.

In autumn 2001 we collected recently fallen leaf litter of *Acer saccharum*, *Quercus rubra*, and *Tilia americana* at the nearest heavily invaded site of the central region for use in all 12 study sites. *Acer saccharum*, *T. americana*, and *Q. rubra* were chosen because they are among the most common tree species in hardwood forests of the upper Midwest and represent a range of litter chemistry in terms of carbon, nitrogen, calcium, and lignin (Table II). We used litter from only 1 site to minimize differences in initial litter chemistry that could confound site effects. Litter was air-dried and stored over the winter for placement in litterbags in the late spring.

We placed ~3.3 g of each of the 3 litter types into 480 fine-and coarse-mesh litterbags (20- × 20-cm) for a total of 5 replicates and 4 harvests at 12 sites. Fine-mesh bags were constructed of fibreglass window screen with 1.5-mm openings. Coarse-mesh bags were constructed of polyester fishnet material with 6-mm openings on the top side of the litterbag to allow access by meso- and macro-fauna and 1.5-mm mesh on the bottom to minimize loss of small litter bag contents.

### Table I. Earthworm body mass and soil conditions (± SE) by invasion degree in the Chippewa National Forest and central Minnesota (USA) study regions.

<table>
<thead>
<tr>
<th>Earthworms 1 (g·m⁻²)</th>
<th>Chippewa NF</th>
<th>Heavily invaded (n = 4)</th>
<th>L. terrestris absent (n = 2)</th>
<th>L. terrestris present (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total 2</td>
<td>1.91 (0.35)</td>
<td>6.35 (0.88) b</td>
<td>4.54 (0.54) a</td>
<td>12.85 (2.60) b</td>
</tr>
<tr>
<td>Dendrobaena</td>
<td>0.49 (0.09)</td>
<td>0.09 (0.02) b</td>
<td>0.74 (0.19) a</td>
<td>0.00 (0.00) b</td>
</tr>
<tr>
<td>Lumbricus juveniles</td>
<td>0.63 (0.17)</td>
<td>2.64 (0.36) b</td>
<td>0.65 (0.16) a</td>
<td>6.80 (1.13) a</td>
</tr>
<tr>
<td>Lumbricus rubellus adults</td>
<td>0.50 (0.21)</td>
<td>0.37 (0.13) a</td>
<td>0.24 (0.10) a</td>
<td>0.06 (0.06) a</td>
</tr>
<tr>
<td>Lumbricus terrestris adults</td>
<td>0.00 (0.00)</td>
<td>1.36 (0.71) a</td>
<td>0.00 (0.00) a</td>
<td>4.15 (2.16) b</td>
</tr>
<tr>
<td>Aporrectodea</td>
<td>0.06 (0.04)</td>
<td>1.84 (0.31) b</td>
<td>2.75 (0.49) a</td>
<td>1.79 (0.27) a</td>
</tr>
<tr>
<td>Octolastion</td>
<td>0.22 (0.10)</td>
<td>0.07 (0.04) a</td>
<td>0.15 (0.09) a</td>
<td>0.05 (0.02) a</td>
</tr>
<tr>
<td><strong>Soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O$_h$/O$_e$ thickness (cm)</td>
<td>2.1 (0.3)</td>
<td>0.1 (0.1)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>54.3 (4.0)</td>
<td>55.8 (4.0)</td>
<td>54.0 (6.3)</td>
<td>59.2 (6.3)</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>35.7 (4.0)</td>
<td>34.2 (4.0)</td>
<td>32.0 (4.2)</td>
<td>27.5 (4.2)</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>10.0 (0.0)</td>
<td>10.0 (0.0)</td>
<td>14.1 (2.2)</td>
<td>13.4 (2.2)</td>
</tr>
<tr>
<td>pH</td>
<td>6.6 (0.3)</td>
<td>6.5 (0.3)</td>
<td>6.3 (0.2)</td>
<td>6.5 (0.2)</td>
</tr>
<tr>
<td>Total carbon (%)</td>
<td>3.3 (0.4)</td>
<td>3.4 (0.4)</td>
<td>2.8 (0.2)</td>
<td>2.7 (0.2)</td>
</tr>
<tr>
<td>Total nitrogen (%)</td>
<td>0.3 (0.0)</td>
<td>0.3 (0.0)</td>
<td>0.2 (0.0)</td>
<td>0.2 (0.0)</td>
</tr>
</tbody>
</table>

**CLIMATE**

| Precipitation (cm) | 57 | 79–85 |
| Max. temp. (°C)    | 10.7 | 11.7–13.0 |
| Min. temp. (°C)    | –0.7 | 1.2–2.1 |

1 Earthworm data are ash-free dry mass and are pooled across sites by invasion degree. Genera listed include the following species: *Dendrobaena octaedra*, *Aporrectodea caliginosa* (sensu Sims & Gerard, 1999), and *Octolasion tyrtaeum*.

2 Data with different superscript letters represent a significant difference (P < 0.05) (Kruskal–Wallis tests) between different invasion degrees within each region.

3 Soil data are from a soil core of the top 10 cm of mineral soil adjacent to each litterbag plot. Samples were composited by site and analyzed at the University of Minnesota’s Research Analytical Laboratory, USA.

4 Climate data are for the 2001–2003 study period and collected from the nearest weather stations (12 km in the Chippewa NF and < 10 km in central Minnesota) (Climatology Working Group, http://climate.umn.edu).
fragments. Mixed-species litterbags were used to better represent decomposition of naturally occurring mixed-species litter. As litter was weighed, sub-samples were collected to estimate air-oven dry (65 °C) mass conversions and for measurements of litter chemistry (see below). We also constructed traveler bags to estimate loss of mass during transport to each site and empty coarse-mesh bags to account for inputs of fine litter at each site.

We deployed litterbags in June 2002. We placed litterbags at the interface of the mineral soil and the organic horizon because in the case of the heavily invaded sites fresh litter typically falls on exposed mineral soil (Hale et al., 2005). Five replicates of each mesh size were harvested from each site in August and October 2002 and June and September 2003. After each harvest, litter was removed from the bags and sorted by litter type based on colour, venation pattern, and texture. Unrecognizable litter fragments were also sorted but only accounted for a small portion (median < 2%) of total harvest mass. Weighed litter of each litterbag was combined, ground, and ashed (4 h at 500 °C) to calculate ash-free dry mass (AFDM) of each sample. We assumed that the ash proportion of sorted *A. saccharum*, *T. americana*, and *Q. rubra* was equal to the proportion of their ashed composite. A composite of the 14 sub-samples of initial litter was also ground and ashed to calculate AFDM. Another portion of the initial litter composite was analyzed for carbon and nitrogen using a Costech ECS4010 Element Analyzer (COSTECH Analytical, Valencia, California, USA) at the University of Nebraska, Lincoln, USA. Calcium was measured using inductively coupled argon plasma emissions spectroscopy (Applied Research Laboratory ICP 3560) after digestion in 10% HCl at the University of Minnesota Research Analytical Laboratory, St. Paul, Minnesota. While other elements besides calcium were measured using ICP, we present only calcium because it is strongly correlated with earthworm biomass and litter decomposition (Reich et al., 2005) and was better correlated with litter disappearance in a related study (Holdsworth, 2006). Lignin (plus other recalcitrants measured on an ash-free dry mass basis) was analyzed using an ANKOM Fiber Analyzer (Ankom Technology, Macedon, New York, USA).

We sampled earthworms in July 2004 using a liquid mustard solution (Lawrence & Bowers, 2002; Hale, Frelich & Reich, 2005) in one 0.12-m² plot 1 m from each of the 5 replicate litterbag plots. All earthworms were identified to species for adult specimens and to genus for juveniles, measured for ash-free dry biomass (AFDM) (Hale, Frelich & Reich, 2004), and placed in the same analytical groups as Hale et al. (2005) and Holdsworth et al. (2007). The July 2004 estimates of biomass are comparable to previous estimates in the same sites made by Hale et al. (2005), Holdsworth et al. (2007), and A. R. Holdsworth (unpubl. data).

The effects of invasion degree, mesh size, and time on the percent of initial litter mass remaining were analyzed using repeated measures ANOVA with litterbag replicate within site as the whole plot and harvest as the repeated factor as implemented in SAS PROC Mixed (Littell et al., 1996). Percent remaining of the 3 litter types and total litter mass remaining were analyzed separately. Percent litter mass remaining was arcsine square-root transformed. A compound symmetry correlation structure was applied for the repeated harvest factor (time) to each plot within each site. This structure has a constant correlation for any 2 time points (Littell et al., 1996) and is appropriate for the relatively small number of time points in this study (S. Weisberg, pers. comm.). The Chippewa National Forest and central Minnesota sites were analyzed separately because they are in different stages of earthworm invasion, as demonstrated by differences in earthworm community composition and forest floor composition (Table I and Holdsworth, 2006). Homogeneity of variances was verified for each model.

### Results

In both regions degree of earthworm invasion alone did not significantly affect remaining litter mass (Tables III, IV). There was a significant effect of time in the ChipNF and central Minnesota sites for all litter types (Tables III, IV). There was a significant effect of time in the ChipNF and central Minnesota sites for all litter types (Tables III, IV).
The effect of time varied by mesh size, invasion degree, litter type (chemistry), and region (Tables III, IV). In the ChipNF there was a significant interaction between mesh size and invasion intensity for total mass. Total mass remaining in coarse mesh bags in heavily invaded plots was a modest 7 percentage points less than in fine-mesh bags (55% versus 62%, respectively), but it was only 2 points lower in lightly invaded plots (58% versus 60%, respectively) (Table III, Figure 1a). In central Minnesota, there was not a significant interaction between mesh size and invasion intensity for total mass, but the results showed the same pattern (Table IV, Figure 2a). Total mass remaining in coarse mesh bags in _L. terrestris_-invaded plots was 7 percentage points less than in fine mesh bags (45% versus 52%, respectively), whereas it was 2 points lower in lightly invaded plots (47% versus 49%, respectively).

In both regions there was a significant interaction between mesh size and invasion intensity for _T. americana_ but not for _Q. rubra_ (Tables III, IV). For instance, in the ChipNF overall mean _T. americana_ mass remaining in

<table>
<thead>
<tr>
<th>Table IV. ANOVA table of F-values for the responses of total % litter mass remaining, % <em>Tilia americana</em>, % <em>Acer saccharum</em>, and % <em>Quercus rubra</em> litter remaining to invasion degree ( <em>L. terrestris</em> present or absent), mesh size (1.5 or 6 mm), and time at the 4 central Minnesota sites (USA).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect</td>
</tr>
<tr>
<td>Invasion degree (I)</td>
</tr>
<tr>
<td>Mesh size (M)</td>
</tr>
<tr>
<td>Time (T)</td>
</tr>
<tr>
<td>I × M</td>
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<tr>
<td>I × T</td>
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<tr>
<td>M × T a</td>
</tr>
<tr>
<td>I × M × T a</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01; ***P < 0.001.

Denominator df are different across the separate tests of total mass and the 3 constituent litter types because of random loss of 1 of the 3 litter types during processing of 3 different litterbags.

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![Figure 1](https://example.com/figure1.png)

**Figure 1.** Percent of initial litter mass remaining in fine- and coarse-mesh litterbags placed in heavily and lightly invaded sites of the Chippewa National Forest (USA). Data are means ± SE of a) Total mass, b) _Tilia americana_, c) _Acer saccharum_, d) _Quercus rubra_.

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coarse-mesh bags in heavily invaded plots was 11 percentage points less than in fine-mesh bags (36% versus 47%, respectively), whereas it was only 3 points lower in lightly invaded plots (42% versus 45%, respectively) (Figure 1b). However, for Q. rubra the respective differences due to mesh bag size were much smaller (Figure 1d). Similarly, in the central region T. americana mass remaining in coarse-mesh bags in L. terrestris-invaded sites was 15 percentage points lower than in fine-mesh bags (17 versus 32%), whereas it was only 7 points lower in plots without L. terrestris (26% versus 33%) (Figure 2b). For Q. rubra the respective differences were only 1 and 2 percentage points (Figure 2d). In the central region, there was also a significant interaction between mesh size and invasion intensity for A. saccharum. Overall mean A. saccharum mass remaining in coarse-mesh bags in L. terrestris-invaded sites was 15 percentage points lower than in fine-mesh bags (31% versus 46%), whereas it was only 5 points lower in plots without L. terrestris (34% versus 39%) (Figure 2c).

During the latter half of the study in the ChipNF, remaining T. americana and A. saccharum mass in fine-mesh bags was 5–8 percentage points higher in the heavily invaded sites than in the lightly invaded sites (Figure 1b,c). Similarly, by the last litter harvest A. saccharum mass in fine-mesh bags was 12 percentage points higher where L. terrestris was present in the central Minnesota sites (Figure 2c).

After 15 months, the order of proportional litter mass remaining was Q. rubra > A. saccharum > T. americana regardless of invasion intensity, mesh size, and region (Figures 1 and 2). The relationship between litter mass remaining and measures of litter chemistry varied for the 3 litter types. Decomposition (assessed by litter mass remaining) was positively related to litter calcium concentration (Figure 3a) and negatively related to lignin:nitrogen ratio (Figure 3b) but had no clear relationship to litter lignin, nitrogen, or carbon concentrations (Figure 3c–e).

**Discussion**

The effect of earthworm invasion degree on litter decomposition varied by initial litter chemistry, mesh size, and time of litter collection. Our results supported our prediction that mass loss in coarse-mesh bags in heavily invaded areas (including areas where L. terrestris is present) would be largest for T. americana and least for Q. rubra litter (Tables III, IV). Recent studies have found that litter calcium is the best predictor of litter mass loss when the litter is accessible to Lumbricus earthworm species (Holdsworth, 2006). The mass of the litter with the lowest initial calcium concentrations (and highest lignin:nitrogen), Q. rubra, was not significantly influenced by earthworm invasion degree. In contrast, for both regions the litter with the highest calcium
concentrations (and lowest lignin:nitrogen), T. americana, lost the most mass in coarse-mesh litterbags (where large-bodied earthworms had the most access), especially in the most heavily invaded sites. Furthermore, the majority of T. americana mass loss occurred within 4 months (Oct. 2002), especially in sites with greater invasion degree (Figures 1b, 2b). The litter with moderate calcium and lignin:nitrogen content, A. saccharum, was affected by invasion degree only in central Minnesota sites, where it lost the most mass in coarse-mesh bags where L. terrestris was present (Table IV). This difference between the regions is likely due to the three-fold greater biomass of L. terrestris in the central Minnesota sites (Table I) and L. terrestris' order of preference for litter burial being T. americana > A. saccharum > Q. rubra (Holdsworth, 2006). In the central Minnesota sites the larger population of L. terrestris initially reduced the supply of high calcium (low lignin:nitrogen) T. americana litter more quickly (Figure 2b) than occurred in the ChipNF (Figure 1b) and then started removing A. saccharum from coarse-mesh litterbags earlier (Figure 2c versus 2b), which resulted in significant two-way interactions of invasion degree with mesh size and with time (Table IV).

As we predicted, in heavily invaded areas, mass loss was greater where earthworms had access (Tables III, IV and Figures 1a, 2a). The controls on invasive earthworms’ effect on decomposition may be different than those of microbes. Hobbie et al. (2006) found that litter calcium was the best predictor of forest floor turnover rates, which include the effect of earthworms, while litter lignin was the best predictor of decomposition rates in fine-mesh litterbags, which largely exclude earthworms. Working in a subset of the current study’s sites, Holdsworth (2006) also found litter calcium was the best predictor of litter disappearance when earthworms had full access. In the current study we could not tease apart the effects of calcium and lignin:nitrogen on litter loss (Figure 3). Although earthworms were found in litterbags and there was evidence of leaves being partially removed from bags, even the coarse-mesh bags likely still excluded substantial earthworm activity. Furthermore, with only 3 litter types, we had limited ability to tease apart the effects of calcium versus lignin:nitrogen.

One of the major impacts of earthworms on litter decomposition is the comminution of litter that facilitates microbial processing of the organic matter into CO$_2$ (Edwards & Bohlen, 1996). In the case of this study, the fine-mesh (1.5 mm) litterbags provide an estimate of the decomposition effects of microbes, mesofauna, and other members of the decomposer community that can access the litter, including small-bodied earthworm species (e.g., Dendrobaena octaedra) and juvenile Lumbricus species. In contrast, coarse-mesh (6 mm) litterbags provide an estimate of the effects of the decomposers mentioned above plus litter translocation out of the bags. We frequently observed partially skeletonized T. americana and A. saccharum litter partially removed from coarse-mesh litterbags in heavily invaded and L. terrestris-invaded plots, suggesting the activities of such litter-feeding species as L. terrestris. Given this, the degree to which mass loss from the coarse-mesh bags represents decomposition is unknown. This highlights the need to study litter decomposition processes in both the organic horizon and in the mineral soil into which much of the highest-quality litter is being moved, especially in sites with L. terrestris (Raw, 1962; Knollenberg, Merritt & Lawson, 1985; Bohlen et al., 2004b; Hobbie et al., 2006; Holdsworth, 2006; Suárez et al., 2006).

The relative patterns of mass loss and the accelerating effect of invasion intensity on T. americana and A. saccharum litter mass loss in the coarse-mesh bags are similar to a study using the same litter mixture and monocultures of the litters in mesocosms that allowed full access to litter by soil macro-fauna (Holdsworth, 2006). However, the rate of total litter mass loss in mesocosms was 37–44% greater in a single season compared to the 15 months of decomposition in litterbags. While Staaf (1987) did not work in the context of

**Figure 3.** Percent of initial litter mass remaining versus 5 measures of initial litter chemistry after 15 months for all litterbags combined in the Chippewa National Forest (USA). Data are means ± SE of a) Calcium, b) Lignin:nitrogen, c) Lignin, d) Nitrogen, e) Carbon concentrations. Patterns were similar in the central Minnesota sites regardless of invasion intensity and mesh size.
non-native earthworm communities, he also found increased mass loss from coarse-mesh bags in *L. terrestris*-dominated beech forests of Sweden.

Towards the latter part of the study litter mass loss of the highest-quality litters in the fine-mesh bags started to exhibit different responses to earthworm invasion degree. In contrast to the lightly invaded plots, remaining mass of *T. americana* and/or *A. saccharum* in fine-mesh bags was 5–12 percentage points higher in the heavily invaded plots of the ChipNF and *L. terrestris*-invaded plots of central Minnesota sites (Figures 1b, 1c, 2c). Bradford et al. (2002) also found reduced decomposition due to the indirect effects of meso- and macro-fauna. In our study there are several possible indirect effects of earthworm invasion on decomposition in the litter layer, including changes in earthworm community composition, microbial biomass, and micro-environment. Lightly invaded areas have significantly higher biomass of the litter-dwelling earthworm *Dendrobaena octaedra* (Table I). As invasion of the larger-bodied litter-feeding species progresses, *D. octaedra* biomass declines since it is uncommon in *L. terrestris*-dominated areas (Table I) (Hale, Frelich & Reich, 2005). As *D. octaedra* was small enough to enter the fine-mesh litterbags, its decline during the invasion by larger-bodied earthworms likely reduced its influence in heavily invaded areas. Indirect effects of invasion also likely include earthworm invasion’s effect on the distribution of microbial biomass. In studies of earthworm invasion in sugar maple forests of New York State (USA), Groffman et al. (2004) found that earthworm invasion decreased microbial C and N (on a g m$^{-2}$ basis) in the organic horizon while increasing it in the mineral soil. This shift in biomass could explain why mass loss was lower in our fine-mesh bags in the more heavily invaded sites. Changes in mesofaunal and microbial community composition and functioning also could have contributed (see Eisenhauer et al., 2007; Scheu et al., 2002). Finally, reduced decomposition in heavily invaded areas could also be due to earthworm invasion’s effect on the forest floor micro-environment. In contrast to heavily invaded areas, higher moisture conditions would likely be maintained with the thicker O$_{h}$ and O$_{e}$ organic sub-horizons of the lightly invaded plots of the ChipNF (Table I) and the thicker O$_{h}$ sub-horizon of the *L. terrestris*-free plots of central Minnesota sites (Holdsworth, 2006).

Earthworm invasion is occurring in many ecosystems (Hendrix & Bohlen, 2002; James & Hendrix, 2004; Hendrix, 2006) and can have significant effects on decomposition rates (Liu & Zou, 2002; Pouyat & Carreiro, 2003). In the several million km$^{2}$ of temperate and boreal forest of North America previously devoid of earthworms, the addition of invasive earthworms creates a major change in the decomposer community. This study and other recent research shows that the effect of earthworm invasion on decomposition rates depends on earthworm species composition and tree species composition, especially through its effect on litter chemistry (Holdsworth, 2006; Suárez et al., 2006). Litter calcium, which varies widely among tree species of northern temperate and boreal forests (J. Oleksyn, unpubl. data), is likely an important predictor of litter decomposition in areas invaded by Lumbricid earthworms and could be a good predictor of forest susceptibility to invasion by Lumbricid earthworms (Holdsworth, 2006).

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**Literature cited**


