Effects of mesh bag enclosure and termites on fine woody debris decomposition in a subtropical forest

Allison M. Stoklosa a,b, Michael D. Ulyshen c,*, Zhaofei Fan a,d, Morgan Varner a,e, Sebastian Seibold f,g, Jörg Müller f,g

a Department of Forestry, Forest & Wildlife Research Center, Box 9681, Mississippi State University, MS 39759, USA
b Academy of Natural Sciences of Drexel University, Philadelphia, PA 19103, USA
c USDA Forest Service, Southern Research Station, Athens, GA 30602, USA
d School of Forestry & Wildlife Sciences, Auburn University, AL 36849, USA
e USDA Forest Service, Pacific Northwest Research Station, Seattle, WA 98103, USA
f Bavarian Forest National Park, Freyunger Str. 2, 94481 Grafenau, Germany
g Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, Center for Food and Life Sciences Weihenstephan, Technische Universität München, Hans-Carl-von-Carlowitz-Platz 2, 85354 Freising, Germany

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Abstract

The role of insects in terrestrial decomposition remains poorly resolved, particularly for infrequently studied substrates like small diameter woody debris. Uncertainty about how mesh bags used to exclude arthropods may affect decomposition rates continues to impede progress in this area. We sought to (1) measure how insects affect the decomposition of small diameter (<2.5 cm) Triadica sebifera L. “twigs” and (2) test for unintended effects of mesh bags on wood decomposition using machined wooden dowels of similar size and specific gravity. In subtropical forests, three twig diameter classes plus dowels were enclosed in two mesh sizes (0.3 or 1 mm openings) or left unenclosed over a 20-month study period. Unenclosed twigs lost significantly more mass than those within fine mesh bags. Because this effect was consistent throughout the study (reaching nearly 80% mass loss), our findings suggest the invertebrate influence does not disappear or attenuate over time. Our dowel data (limited to dowels with no evidence of insect activity) show that fine and coarse mesh bags accelerate the decomposition of enclosed woody material, suggesting insects contributed even more to the decomposition of tallow twigs than our measure of 9 – 10%. Termites exhibited a strong preference for larger diameter twigs, resulting in temporary differences in decomposition rates among diameter classes. Our findings confirm the importance of insects to wood decomposition and highlight the need to incorporate these organisms in models of carbon and nutrient cycling.

Zusammenfassung

Die Rolle der Insekten in Zersetzungsvorgängen an Land ist nur unvollständig geklärt, insbesondere bei selten untersuchten Substraten wie Totholz mit geringem Durchmesser. Die Ungewissheit, wie die Gazebeutel, mit denen Arthropoden ferngehalten werden sollen, die Abbaurationen beeinflussen, behindert den Fortschritt auf diesem Feld. Ziel unserer Studie war es, (1) den Einfluss von Insekten auf den Abbau von dünnen (<2.5 cm) Talbaumzwiegen (Triadica sebifera L.) zu messen und gleichzeitig (2) zu testen, ob unbeabsichtigte Effekte der Gazebeutel auf den Holzabbau eintraten, indem wir maschinell hergestelltes Rundholz von

*Corresponding author. Tel.: +1 706 559 4296.
E-mail address: mulyshen@fs.fed.us (M.D. Ulyshen).

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ähnlicher Größe und spezifischer Dichte einsetzten. In subtropischen Waldstandorten wurden drei Zweigdickenklassen und ein Rundholz in Gazebeuteln (Maschenweiten: 0.3 und 1.0 mm) bzw. offen für 20 Monate ausgelegt. Die offen ausgelegten Zweige verloren signifikant mehr Masse als die in engmaschigen Beuteln. Da dieser Effekt über die gesamte Untersuchungsdauer hinweg anhielt (mit fast 80% Massenverlust), legen unsere Befunde nahe, dass der Einfluss der Invertebraten nicht mit der Zeit abnimmt oder verschwindet. Unser Experiment mit Holzstäben, bei dem nur die Stäbe ohne erkennbare Insektenaktivität berücksichtigt wurden, zeigt, dass feine und gröbere Gaze den Abbau des eingeschlossenen Holzmateriales beschleunigt, was schließen lässt, dass Insekten sogar mehr zum Abbau der Talbaumzweige beitragen als unser Messergebnis von 9 – 10%. Termiten zeigten eine deutliche Präferenz für dickere Zweige, was zeitweilige Unterschiede bei den Abbauratoren der einzelnen Dickeklassen zur Folge hatte. Unsere Ergebnisse bestätigen die Bedeutung von Insekten bei der Holzzersetzung und unterstreichen die Notwendigkeit, diese Organismen in Modellen der Kohlenstoff- und Stickstoffkreisläufe zu berücksichtigen.

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Introduction

Although less important than fungi in most environments, invertebrates play an important role in the decomposition of both plant litter (Seastedt 1984) and woody debris (Ulyshen 2016). Major mechanisms by which invertebrates influence wood decomposition include consumption and digestion, substrate alteration (e.g., tunneling and fragmentation) and interactions with decay fungi and other organisms (Ulyshen 2016). Wood-dwelling insects also have the potential to influence nitrogen dynamics in decomposing wood by accelerating the release of nutrients immobilized in fungal tissues and by promoting nitrogen fixation (Ulyshen 2015). Most studies of wood decomposition, including those aimed at measuring the contributions of invertebrates to the process, have focused on coarse woody debris (i.e., material >10 cm in diameter) whereas few efforts have considered the decomposition of fine woody debris. The smallest fine woody debris, e.g., small twig and branch material, remain almost entirely unstudied. Moreover, while a number of studies have shown that decomposition rates increase with decreasing wood diameter (Abbott & Crossley, 1982; Mackensen, Baulhus, & Webber, 2003; Müller-Using & Bartsch, 2009), nothing is known about how these patterns may be influenced by invertebrate activity. Termites in particular are known to discriminate based on resource size. Many studies have shown a preference for larger resources (Abe 1980), for instance, although some species are known to preferentially attack smaller resources, presumably to avoid competitive interactions with other taxa (Evans et al., 2005). In terms of wood volume consumed, subtropical termites belonging to at least five species of Reticulitermes (Rhinotermitidae) (Lim & Forschler, 2012) are the dominant wood-feeding insects in the southeastern United States (Ulyshen, Wagner, & Mulrooney, 2014). These organisms can cause considerable damage to wooden structures throughout the region but much remains unknown about their role in forested environments, including patterns of fine woody debris utilization.

Methods used to assess the contributions of invertebrates to terrestrial decomposition remain controversial (Kampichler & Bruckner, 2009; Ulyshen & Wagner, 2013). Mesh bags, for instance, are the most widely used method for excluding invertebrates in decomposition studies but these devices are known to alter microclimate (e.g., higher humidity) relative to unenclosed substrates (Louisier & Parkinson, 1976). Such effects may, in turn, inadvertently promote fungal activity within mesh bags, potentially obscuring reductions in decomposition caused by the exclusion of invertebrates. While such unintended effects, or “hidden treatments” (Huston 1997), clearly confound efforts to isolate the contributions of invertebrates to decomposition, this complication has received little attention from researchers. According to a recent review by Kampichler and Bruckner (2009), this uncertainty renders the findings from forty years of litterbag studies inconclusive. Very few studies have sought to determine whether decay rates are affected by mesh-bag effects but the findings from these efforts are mixed, suggesting that mesh bags may (Bradford, Tordoff, Eggers, Jones, & Newington, 2002; Siedentop 1995) or may not (Bokhorst & Wardle, 2013; Ulyshen 2014) significantly alter decomposition rates. Therefore, additional research is needed to establish methods for assessing the effects of mesh bags in future decomposition experiments and to finally determine how insects affect decomposition while accounting for mesh bag effects.

This paper presents the results from a study aimed at testing the hypothesis that invertebrates play a significant role in the decomposition of fine woody debris. In addition, a novel method for testing for unintended effects of mesh exclusion bags on decomposition is described. The following hypotheses were tested: (1) mesh bags will accelerate the decomposition of enclosed woody substrates compared to unenclosed substrates that remain similarly unaffected by insect activity, i.e., mesh exclusion bags introduce a “hidden treatment” when used in decomposition experiments; (2) invertebrates contribute significantly to decomposition, especially after accounting for the effects of mesh bags; and (3) invertebrates more strongly influence the decomposition
of larger-diameter woody substrates compared to smaller-diameter substrates. These results will have implications for future research methodology on fine wood decomposition and will more broadly advance knowledge about the role invertebrates play in wood decomposition.

Materials and methods

Study area

The study took place in mature mixed hardwood/pine forests on the Noxubee National Wildlife Refuge and John W. Starr Memorial Forest in Oktibbeha, Noxubee and Winston counties, Mississippi, U.S.A. The climate of the region is classified as humid subtropical with annual precipitation and temperature averaging 141 cm and 16.9 °C, respectively (usclimatedata.com, accessed 11.06.15). Five locations were selected within the study area and two linear transects, separated by at least 100 m, were established at each location (Fig. 1). Each transect began at least 20 m from the forest edge and ran perpendicular to the edge for 40 m. Five ~1 m² plots were established along each transect, separated by 10 m.

Twig and dowel preparation

Twigs were cut from branches of Chinese tallow (Triadica sebifera L.), an invasive tree species established throughout much of the southeastern United States (Miller, Manning, & Enloe, 2010). The trees were felled for this purpose at a single location in Pearl River County, Mississippi in September 2012. The branches were cut into 20 cm lengths and only the straightest and most uniform pieces were retained for the experiment. One 5 cm-long subsample was removed from the end of each 20 cm section. For each subsample, wet (i.e., as collected from the field) bark and wood weights were measured separately. The wet volumes of the subsamples (without bark) were measured using the water displacement technique. Then the bark and wood from each subsample were oven-dried at 102 °C for 24 h. Specific gravity of the samples was measured using the oven-dried wood weight divided by the wet wood volume. The initial dry wood weights of the remaining 15 cm sections, without bark, hereafter referred to as “twigs”, were estimated based on their initial wet weights and the information collected from the subsamples. In total, 150 twigs belonging to each of the following diameter classes were used in this study; 0.2 – 0.4 cm (“Small”), 0.8 – 1.1 cm (“Medium”) and 1.5 – 2.5 cm (“Large”). Twigs were then randomly assigned to transects, plots and treatments as described in the following section.

To test whether the mesh bags affected decomposition beyond the exclusion of insects, e.g., by changing the micro-climate relative to unenclosed twigs, we included a wooden dowel with each grouping of tallow twigs. In contrast to natural woody substrates, the mechanically formed surface of dowels can be easily examined for signs of insect activity. Thus, decomposition rates of dowels unaffected by insects can be compared between enclosed and unenclosed treatments to evaluate mesh-bag effects (as long as a sufficient number of unenclosed dowels remain free from insect damage). The dowels were made from tulip-poplar (Liriodendron tulipifera L.), measured 0.7 cm in diameter (i.e., within the diameter range of the tallow twigs) and were cut into the same 15 cm lengths. Dowels from this species were specifically selected from among the commercially available options as they most closely matched the specific gravity of our tallow twigs (~0.45 and ~0.50, respectively). The dowels were dried at 102 °C for 24 h before determination of their initial dry weights and were then randomly assigned to locations, plots and treatments.

Study design

At each of the 50 plots, twigs and dowels were randomly assigned to one of three treatments. Those enclosed within fine (0.3 mm) nylon mesh bags (“fine mesh”) were protected from all macro-fauna (>2 mm in body size), all but the smallest of meso-fauna (0.1 – 2 mm) but not from any of the micro-fauna (<0.1 mm) (Bradford et al., 2002). Meanwhile, a greater range of meso-fauna (including termites, see Results) had access to twigs and dowels enclosed within coarse (1 mm) nylon mesh bags (“coarse mesh”) (Fig. 2). Both mesh sizes were obtained through Normesh Limited, Oldham, Lancashire, UK. Substrates assigned to the third “no mesh” treatment were not enclosed within mesh bags and were unprotected from all invertebrates (Fig. 2).

The fine and coarse mesh bags measured 25 × 25 cm² and were stitched closed with nylon thread. The twigs and dowels assigned to the no mesh treatment were connected around
each dowel referred to Wood Termite their 466 (i.e., these cleaned mites by twigs of In the three figures, termite termites, termites were sampled given invertebrates in our study region (Ulyshen et al., 2014). These insects rapidly locate and feed upon woody substrates but the number of actively feeding termites at any given time varies greatly. Indeed, it is not uncommon for termites to abandon a piece of wood after only partially consuming it. Moreover, the amount of damage caused by termites can only be measured early in the decay process when the wood remains relatively intact (Ulyshen et al., 2014). For these reasons, no effort was made to record the number of termites found within a given twig or the volume consumed by termites, recording instead only the presence or absence of termite damage, hereafter referred to as termite incidence. In figures, termite incidence is presented as the percentage of twigs with visible termite damage.

**Wood measurements**

Every four months the collected wood samples were transported to the laboratory. Each tallow twig and dowel was inspected for evidence of termite activity (presence/absence), cleaned of any external debris or soil and weighed. All dowels with visible damage from termites or other invertebrates were discarded from the further investigation of mesh effect. Bark was carefully removed from all twigs before obtaining oven-dried (102 °C for 24 h) weights for bark and wood separately. To isolate soil brought into the twigs by termites, twigs exhibiting termite damage were burned in ceramic crucibles using a propane burner, following the method described by Ulyshen and Wagner (2013) until only ash and soil remained. The samples were then mixed with water and all floating ash or char was discarded. The remaining mineral samples were oven-dried at 102 °C for 48 h and weighed, allowing us to correct final dry wood weights (i.e., by subtracting soil weights). Finally, to better understand how mesh bags affect microclimate, the water content (final wet weight — final dry weight)/final wet weight) of each dowel was measured at collection for comparison among treatments (note that only dowels with no evidence of insect activity were included in this analysis).

**Statistical analysis**

To compare dowel mass loss and dowel water content among treatments, the non-parametric Friedman’s chi-square (Q) test was applied to mean values, with month as the blocking factor, using SAS (Cary, NC). The mixed procedure of SAS was used to compare tallow twig mass loss among mesh treatments, diameters and sampling times. Site and site × mesh × month were treated as random effects because twigs belonging to each of the three diameter classes were grouped together within individual mesh treatments. No data transformations were necessary to satisfy model assumptions. The complete initial model was reduced through backward selection by successively dropping the least significant term until only significant effects remained. Initial wood specific gravity was included in the complete initial model as a potential covariate but was found to be an insignificant source of variation and therefore dropped. Finally, contrasts were used to compare pairings of mesh treatments and diameter classes.

As termites were unable to enter fine mesh bags, binomial termite incidence data (i.e., presence or absence of feeding termites or visible damage) were limited to the coarse mesh and no mesh treatments. A generalized linear mixed model was fitted with a binomial distribution using the function ‘glmer’ in R 3.0.2 (R Development Core Team 2008). Diameter, month and mesh were treated as fixed effects whereas site and site × mesh × month were treated as random effects. A further model included interactions between significant variables but found no significant interactions and is therefore not shown.

**Results**

**Mesh effect**

Mass loss from dowels exhibiting no evidence of insect activity varied among mesh treatments (Q = 5.2, p = 0.07),
insect activity. The water content of dowels exhibiting no evidence of insect activity also varied among mesh treatments ($Q = 5.2, p = 0.07$), with dowels enclosed within fine or coarse mesh having higher water contents compared to unenclosed dowels (Fig. 3B). The 12-month collection period was an exception to this pattern, likely because it rained heavily the night before these samples were collected (AMS, pers. obs.). For this reason, the 12-month data were excluded from the final analysis of water content ($Q = 6.0, p = 0.05$).

**Mass loss**

Twig mass loss increased over time and ranged between 60% and 80% by the end of the 20-month study (Fig. 4A – B). Mass loss varied among mesh treatments. In support of hypothesis 2, twigs protected within fine mesh lost significantly less mass than those completely exposed to attack from insects (Table 1, Fig. 4A). Mass loss from twigs enclosed within coarse mesh was intermediate, more variable and did not differ significantly from either of the other treatments (Table 1, Fig. 4A). Mass loss varied significantly among twig diameters and there was a significant interaction between diameter and time (Table 1, Fig. 4B). During the first year, the largest diameter class lost more mass than the smallest, with the middle diameter class exhibiting intermediate mass loss. These differences diminished with time, however, and disappeared by 16 months (Fig. 4B). Because there was no significant interaction between diameter and treatment, the prediction (hypothesis 3) that invertebrates more strongly influence the decomposition of larger diameter twig material...
Table 1. ANOVA table showing results from the final backward-selected model of the effects of mesh (coarse, fine, no mesh), wood diameter (small, medium and large twigs) and sampling date on fine wood decomposition in the southeastern United States.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Contrast</th>
<th>$F$</th>
<th>df</th>
<th>$p$</th>
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<td>Coarse vs. fine</td>
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<td>2, 116</td>
<td>&lt;0.01</td>
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<tr>
<td></td>
<td>Coarse vs. none</td>
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<td>1, 116</td>
<td>0.08</td>
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<tr>
<td></td>
<td>Fine vs. none</td>
<td>2.15</td>
<td>1, 116</td>
<td>0.14</td>
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<tr>
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<td>1, 239</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Small vs. large</td>
<td>15.88</td>
<td>1, 239</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Medium vs. large</td>
<td>3.42</td>
<td>1, 239</td>
<td>0.07</td>
</tr>
<tr>
<td>Month</td>
<td></td>
<td>155.27</td>
<td>4, 116</td>
<td>&lt;0.001</td>
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<tr>
<td>Diameter $\times$ Month</td>
<td></td>
<td>5.03</td>
<td>8, 239</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

was not supported. Termites did exhibit a strong preference for larger twigs, however (see below).

**Termite incidence**

As intended, no signs of termite activity were observed in fine mesh bags. Beginning at 12 months, termite incidence was lower in twigs enclosed within coarse mesh compared to those completely open to attack (Fig. 4C) but this difference was insignificant ($z = 1.74, p = 0.08$). Incidence varied significantly among diameter classes ($z = 5.99, p < 0.001$), increasing with increasing twig diameter (Fig. 4D). After 8 months, for example, termites had attacked 65% of the largest twigs but only 5% of the smallest twigs (Fig. 4D).

**Discussion**

The dowel method described herein was effective in revealing the accelerative effects of both fine and coarse mesh bags on fine woody debris decomposition. The mesh bags were also shown to increase the water content of enclosed dowels, possibly explaining differences in dowel mass loss observed among the treatments. These results confirm that significant unintended effects are probable in decomposition studies involving the use of mesh bags for excluding invertebrates. Our findings suggest that mesh bags are likely to underestimate, rather than exaggerate, the effects of invertebrates on decomposition although additional research will be needed to determine how consistent this pattern is across study systems and mesh sizes. Tallow twigs completely unprotected from attack by termites and other insects lost significantly more mass than those enclosed within fine mesh bags. Beginning at 8 months, there was a consistent difference in mass loss between these treatments of 9 – 10%. This likely underestimates the true contribution of invertebrates, however, judging from the nature of the mesh bag effect reflected in our dowel data. While these data clearly indicate the direction of the mesh effect, the magnitude of this influence on tallow twig mass loss cannot be determined given differences in the decay rates and characteristics (e.g., bark coverage) between the two substrates. Despite this limitation, it is possible to conclude, with an unprecedented level of certainty, that the contribution of insects to fine woody debris decomposition, as measured in this study, is at least 9 – 10%.

We are aware of only four previous studies that specifically sought to determine how mesh bags affected the decomposition of litter (Bokhorst & Wardle, 2013; Bradford et al., 2002; Siedentop 1995) or wood (Ulyschen 2014). The results from those efforts were mixed and Siedentop (1995) in particular noted significant differences in mesh effect depending on the types of substrates and soils involved. Given this variability, it is recommended that future research tests for the direction and magnitude of a mesh effect as part of every study until predictable patterns emerge. The dowel method described herein, or some variation of it, may have utility in studies focused on wood decomposition.

Patterns of termite incidence showed these insects were largely unimpeded by the coarse mesh bags (with 1 mm openings) but were unable to colonize twigs enclosed within fine mesh bags. As only twigs placed in fine mesh bags showed significantly lower rates of mass loss than unenclosed twigs, our results indicate that the reduction of decomposition rates was largely caused by the exclusion of termites and possibly other invertebrates. At the end of the study, after 20 months in the field, twigs assigned to the fine mesh and no mesh treatments had average mass losses of 68.4% and 77.6%, respectively. No previous study examining the effects of invertebrates on wood loss has extended this far into the decomposition process, largely due to the historical focus on coarse woody debris which decomposes much more slowly. The difference in mass loss (~9 – 10%) attributable to invertebrates was consistent over the course of our study, which encompassed most of the tallow twig decomposition process. These findings suggest the invertebrate influence
does not disappear or attenuate over time, at least for small-diameter twig material, underscoring the important role these organisms play in terrestrial decomposition processes.

While wood decomposition rates commonly increase with decreasing diameter (Abbott & Crossley, 1982; Mackensen et al., 2003; Müller-Using & Bartsch, 2009), large diameter twigs were found to decompose faster than small diameter twigs at the beginning of this study. This probably reflects the preference of termites for larger-diameter twigs evident in the incidence data. Indeed, when mass loss data from the fine mesh treatment (i.e., twigs protected from termites) were analyzed separately, we found no significant differences among diameter classes and no significant interaction between diameter and time (results not shown). This is not the first study to show termites preferentially attack larger resources. Abe (1980) found that small branches in Malaysia were attacked much less readily by termites than large branches, for instance. Moreover, past field surveys conducted in Mississippi showed Reticulitermes to strongly prefer larger-diameter resources, with material less than 2.9 cm in diameter attacked much less readily than material >3 cm in diameter (Wang, Powell, & Scheffrahn, 2003). Although differences in termite incidence among diameter classes were consistent over the entire study, differences in mass loss among diameter classes disappeared after 12 months. These patterns may have been caused by termites responding quickly to new inputs of wood and temporarily driving observed differences in decomposition patterns and may have been offset by fungi over time. Because all twigs used in this study were <2.5 cm in diameter and Wang et al. (2003) found wood <2.9 cm to be less readily utilized by Reticulitermes than larger diameter material, studies including a wider range of diameters may be needed to better test the hypothesis that the contribution of termites to wood decomposition increases with increasing diameter.

It can be concluded that invertebrates, and particularly termites, contribute significantly to the decomposition of fine woody debris, accounting for at least ~9 – 10% of mass loss in our study area. This confirms the importance of these organisms to wood decomposition and highlights the need to consider invertebrates when modeling carbon and nutrient cycles.

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