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Assessing the effects of mesh enclosures on invertebrates and litter breakdown in a floodplain forest of the Southeastern USA

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Abstract The litter bag method has been used to study litter breakdown for over 50 years but remains a criticized technique. One major criticism is the effect of mesh enclosures, specifically the use of two or more mesh sizes to evaluate the role of arthropods, on litter breakdown. We aimed to evaluate the effectiveness of a new basket-style mesh enclosure in mitigating microclimatic mesh effects while still excluding invertebrates. We evaluated five basket treatments constructed from 300-µm mesh: no basket, closed basket, closed basket with bottom slits, open basket, and open basket with bottom slits, which held invasive Chinese privet (*Ligustrum sinense*) litter on the Oconee-River floodplain, GA, USA. After 134 days, we found that temperature and humidity did not vary among treatments but that litter breakdown rates (k) and invertebrate composition were different among treatments. Litter breakdown was faster in the no basket treatment (the most open treatment) than in closed baskets without slits (the most closed treatment). Microinvertebrates were not effectively excluded from baskets but most macroinvertebrates were excluded from baskets (open and closed) without slits, except for some small predators. Unexpectedly, we found some evidence that using litter bags of two different mesh sizes may have a secondary trophic effect on litter breakdown, further complicating how best to evaluate the impact of arthropods on litter breakdown.

Keywords Litter breakdown · Mesh · Invertebrate · Microclimate · Floodplain · Chinese privet

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Introduction

The litter bag method has been the primary technique for studying leaf litter breakdown for over 50 years (Bocock and Gilbert 1957; Kampichler and Bruckner 2009). This method entails using mesh bags to hold a known mass of leaf litter, which is monitored over time to measure leaf litter breakdown rates. Often, litter bags are used to evaluate the role of arthropods in litter decomposition. This is typically done by excluding arthropods from one set of litter bags in one of two ways: (1) using differing mesh sizes, including one mesh which is sufficiently small to exclude invertebrates, or (2) applying an insecticide to leaves. However, litter decomposition studies and the litter bag method have faced recent criticism (Prescott 2005; Kampichler and Bruckner 2009), especially when evaluating the role of arthropods on litter breakdown rates. Additionally, some studies assert

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that soil arthropods are not important to litter breakdown in their systems and ignore their role altogether (Prescott 2005).

The most popular approach to assess arthropod impacts is the use of varying mesh size. This method includes at least two different mesh sizes in bag construction to either allow or exclude access to arthropods of a certain body size. Use of varying mesh sizes has been employed nearly as long as the litter bag technique itself (Crossley and Hoglund 1962) and continues to be popular. However, there is concern that artifacts of mesh size may create a "mesh effect". Mesh effects may arise from a higher proportion of litter fragments exiting bags with larger mesh in the field, differential handling effects, leaching rates, and/or microclimate conditions for litter enclosed in mesh of differing size (Bradford et al. 2002; Kampichler and Bruckner 2009; Bokhorst and Wardle 2013). In a large meta-analysis of litter bag studies Kampichler and Bruckner (2009) found only one study that attempted to correct for mesh effects. Furthermore, the Kampichler and Bruckner (2009) meta-analysis found that if a mesh effect influenced results by $\geq 7\%$, arthropod effects were nullified. We found only two other studies that directly tested for mesh effects in leaf litter breakdown (but see Stoklosa et al. 2016 & Ulyshen 2014, which evaluate mesh effects on woody substrates), only one of which evaluated arthropods, and the studies yielded conflicting results (Bradford et al. 2002; Bokhorst and Wardle 2013). Potential causes of mesh effects require further examination. We posit that microclimate is likely an important variable relating to mesh effects in litter breakdown studies. However, microclimate is difficult to control under field conditions, and the influences of temperature and moisture on microbes, which are integral in leaf decomposition, are likely crucial (Couteaux et al. 1995).

We tested the application of the open-pan design described by Ulyshen et al. (2016), addressing wood decomposition, to litter bags. This design attempts to exclude macroinvertebrates without completely enclosing the substrate within mesh bags in order to minimize mesh effects on microclimate. We created baskets out of fine mesh within which we placed litter bags. We sought to test whether basket walls could prevent colonization of macroinvertebrates while avoiding unwanted effects that traditional use of differing mesh sizes may have on microclimate. Rather than using two mesh sizes, we created baskets of the same fine mesh size and used slit openings in the bottom of baskets to mediate arthropod access. We hypothesized that baskets with the same mesh size, despite having slits or not, would have similar temperature and humidity conditions. Additionally, we assessed whether having a lid, or having the top open to the environment, could still be used to control soil invertebrate access, while maintaining a largely natural microclimate. We hypothesized that litter placed in baskets with bottom slits, providing access to soil arthropods, would breakdown faster than those without slits. Further, the study was conducted under floodplain forest conditions, a macrohabitat that has received scant attention in terms of assessing litter breakdown.

Methods

Study sites

Four study sites were selected on the Chinese privet (*Ligustrum sinense*)-invaded Oconee River floodplain in the Georgia Piedmont region (Athens-Clarke and Greene Counties). The four sites were the privet-invaded reference plots in a long-term privet eradication study, see Hanula et al. (2009) for descriptions of the sites.

Experimental design

Basket treatments consisted of two parts: an inner litter bag and an outer exclusion basket. A) Litter bags served as the base (an inner bag) for all 300-µm exclusion basket treatments to prevent loss of privet leaves through bottom slits or out of open-topped bags, and to inhibit entry of ambient privet leaf-fall. Thus, 5-mm mesh litter bags (this mesh size was the largest that would still contain the majority of privet leaves while also allowing free access to large invertebrates) containing 10-g air-dried privet leaves were placed inside each 300-µm basket before sealing and secured with fishing line or secured alone on the soil surface for the no basket control treatment. As privet leaves can be quite small (some < 5 mm) leaves were first sifted through 5-mm mesh before weighing so as not to unnecessarily lose leaves through the mesh which could cause an over estimate of mass-loss. B)

Exclusion baskets constructed with 300-µm nylon-mesh in a box-shaped design 25-cm (L) X 25-cm (W) X 24.4-cm (H). All baskets also had a 0.6-cm folded over "lip" that protruded outward from each top edge intended to further impede invertebrates from crawling into the bags over the sides. In preliminary testing, 24.4 cm was estimated to be an adequate height to exclude most soil dwelling invertebrates from entry despite some treatments having an open top. 300-µm nylon-mesh was selected because it was the smallest mesh size that we estimated would exclude macroinvertebrates while still allowing regular air flow and movement of microbes.

To evaluate different basket designs, one basket each of five basket treatments were placed at two subplots at each site (10 bags per site, n = 40; Fig. 1). Treatments included: (1) The *no basket treatment* served as a control and consisted only of a 5-mm mesh leaf bag. (2) *Closed-top baskets* were fully intact on all sides with all seams sealed, intended to exclude invertebrates. (3) *Closed-top baskets with slits* were identical to closed-top bags except for three parallel slits (approximately 18.4 cm long, 8.9 cm apart, and 5 cm from the bag edge on all sides) on the bottom surface, intended to allow entry of soil-dwelling invertebrates. (4) *Open-top baskets* were also fully intact on all sides with seams sealed other than an absence of the top panel, intended to allow a more natural microclimate than closed-top bags. (5) *Open-top baskets with slits* were identical to the open-top treatment except for the addition of three slits in the bottom surface (identical in size and placement to the close-topped bags with slits) to allow access to soil dwelling invertebrates. All five treatments were placed underneath 1-m^2, 1-mm mesh canopies each standing 1-m high, to further inhibit ambient privet litter from entering open-top baskets.

Each bag contained one Hygrochron ibutton (Maxim Integrated, San Jose CA, USA) data logger that recorded temperature and humidity every 2 h. Each logger was hung slightly above the ground inside an over-turned plastic cup and secured to the inside of the litter basket, for rain protection (loggers were not waterproof). For no basket bags, data logger cups were hung immediately next to the bag from a tent stake.

Exclusion baskets and associated litter bags were deployed in the field on 13 May 2015 to approximate the spring leaf fall of privet and capture maximal seasonal arthropod activity. Five additional baskets (one of each type) were also deployed in the field but were immediately retrieved to account for handling loss. All baskets were collected from the field on 24 September 2015 after 134 days.

Inner litter bags were carefully removed from outer baskets (if applicable), placed in paper bags and sealed for transport back to the laboratory, and immediately placed in Berlese funnels (BioQuip Products, Rancho Dominguez, CA) to extract invertebrates for ca. 48 h.

Fig. 1 Example of litter basket treatments. Letters denote treatments as follows: **a** no basket, **b** open-top, **c** open-top with slits, **d** closed-top, and **e** closed-top with slits

Following Berlese extraction, leaves were dried at 55 °C for 24 h and then weighed, ashed, and reweighed to determine ash free dry mass (AFDM) remaining. Extracted invertebrates were counted and identified to the lowest practical taxonomic level and then categorized into trophic groups.

Statistical analysis

Temperature, humidity, and arthropod abundance differences among treatments were evaluated using a linear mixed-effects model (LME) using the nlme package (Pinheiro et al. 2017) in R (R Core Team 2017) with treatment (basket type) as a fixed effect and site as a random effect. Temperature and humidity values were averaged across the entire incubation period prior to analysis.

Litter breakdown (g AFDM litter remaining) was compared among treatments via an analysis of covariance (ANCOVA) using a linear mixed-effects model (LME) using the nlme package for each site with days of exposure as the co-variate. In addition, the litter breakdown coefficient (k) was calculated for each treatment using a linear regression of ln-transformed AFDM values versus days of exposure. All statistical analyses were conducted in R version 3.3.3 (R Core Team 2017). Data were Log (x + 1) transformed where necessary to meet statistical assumptions.

Results

Mean temperature in litter baskets ranged from 20.4 to 25.4 °C, seasonally, and mean relative humidity in litter baskets ranged from 83.9 to 114.4%, seasonally (Fig. 2). Neither temperature ($F_{4,29}$ = 0.17, P = 0.95) nor humidity ($F_{4,29}$ = 1.03, P = 0.41) were significantly different among basket types.

Leaf litter breakdown rates ranged from 0.0064 to 0.0240 (k, d^{-1}; Table 1) and ash-free dry mass

Table 1 Leaf breakdown rates (k) expressed per day for each treatment (basket-type)

Treatment	k (day^{-1})	R^2
Loose bag	0.0159	0.6509
Open-top + slits	0.0143	0.5500
Open-top	0.0093	0.8731
Closed-top + slits	0.0113	0.7428
Closed-top	0.0074	0.8546

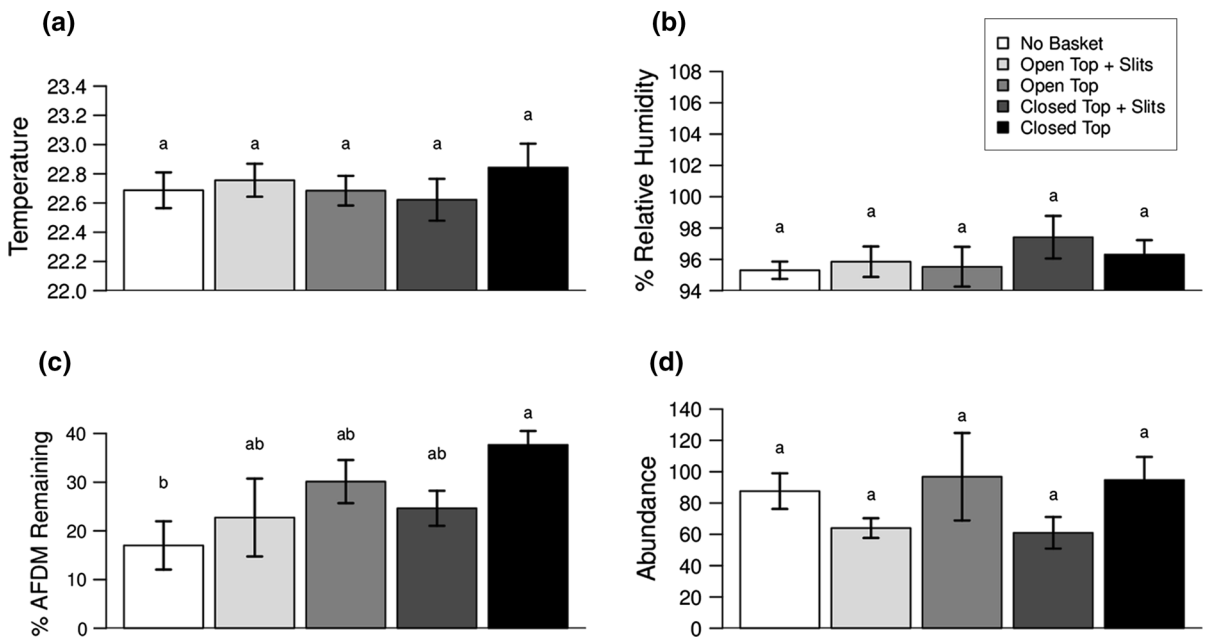

Fig. 2 Mean (± SE) **a** temperature (°C), **b** percent relative humidity, **c** percent AFDM remaining and, **d** total invertebrate abundance per sample for all basket types. Bars indicated by the same letter, within each graph, are not significantly different

(AFDM) lost over the course of the study ranged from approximately 54–96%. Leaf litter breakdown rates were significantly different among treatments ($F_{4,44} = 3.065$, $P = 0.026$). A post hoc Tukey HSD test showed that the no basket treatment had a significantly faster breakdown rate than closed-top baskets without slits (Table 1).

As expected, we were unable to completely exclude invertebrates from any of the exclusion basket treatments as many small invertebrates (e.g., collembolans and oribatid mites) were able to pass through the holes in the mesh (Fig. 2d). Baskets with slits allowed access, as expected, to medium and large sized invertebrates. Closed baskets without slits were able to exclude most macroinvertebrates except for several very small predators (< 2 mm) in some baskets. Open baskets without slits were successful in excluding large detritivores like millipedes, however large detritivores were rare in all treatments. Open baskets without slits were not very effective at excluding spiders (Fig. 3c), though most spiders found in these baskets were very small (< 2 mm) and were presumably capable of passing through the mesh openings. Detritivores, dominated by collembolans and oribatid mites, were the most abundant trophic group in all bag types comprising 40–98% of invertebrate abundance per bag. Predators, dominated by Araneae (spiders), ranged from 1 to 70% of invertebrate abundance per bag.

Total invertebrate abundance per sample was not significantly different among treatments (Fig. 2d; Table 2). Invertebrate abundance of trophic groups important to litter breakdown (detritivores and predators) were significantly different among treatments, however (Table 2). Tukey tests showed that detritivores were significantly more abundant in closed baskets without slits than in closed baskets with slits (Fig. 3a). Predatory invertebrates were significantly less abundant in closed baskets without slits than in all other bag types (Fig. 3a). Based upon overall abundance Collembola, Oribatida, and Araneae had the greatest potential to play important roles in the trophic dynamics associated with litter breakdown, so these individual taxa were also analyzed. Collembola abundance was significantly different among treatments (Table 3), with Tukey tests indicating that abundance was higher in open and closed baskets without slits than in all other treatments (Fig. 3b). Oribatida abundance was not significantly different among basket types (Table 3). Araneae abundance was significantly different among treatments (Table 3) and a Tukey test revealed that spiders were significantly less abundant in closed baskets without slits than in all other basket types (Fig. 3c).

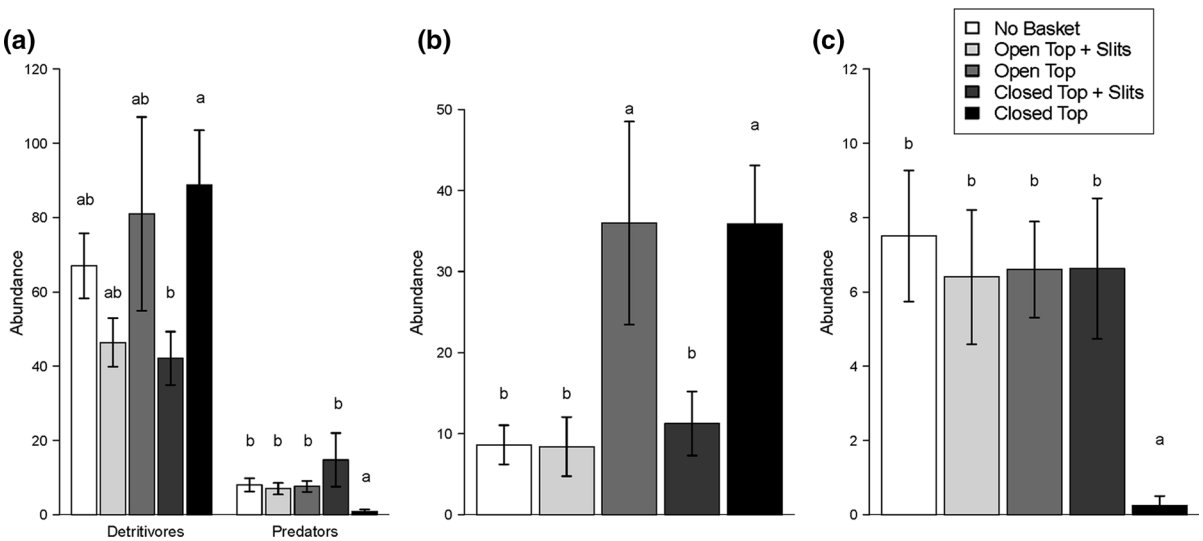

Fig. 3 Invertebrate abundances (mean ± SE) for all basket types: **a** by trophic group, **b** for Collembola, and **c** Araneae. Bars indicated by the same letter, within each trophic group, are not significantly different

Table 2 Summary of mixed-effects models (LME) testing for the effects of treatment (basket-type) on invertebrate abundances (per sample) for detritivores, predators, and total invertebrate abundance

Trophic Group	Numerator Df	Denominator Df	F	P
Detritivores	**4**	**25**	**3.393**	**0.024**
Predators	**4**	**25**	**9.148**	**< 0.001**
Total Abundance	4	25	1.703	0.181

Significant results ($P \leq 0.05$) are indicated in bold type

Table 3 Summary of mixed-effects models (LME) testing for the effects of treatment (basket-type) for Collembola, Oribatida, and Araneae

Taxa	Numerator Df	Denominator Df	F	P
Collembola	**4**	**25**	**4.818**	**0.005**
Oribatida	4	25	1.904	0.141
Araneae	**4**	**25**	**13.941**	**< 0.001**

Significant results ($P \leq 0.05$) are indicated in bold type

Discussion

We found no differences in temperature or humidity among basket types. While we predicted baskets with the same mesh size would have similar microclimates, it was unexpected that open and closed-top baskets and the no basket control treatment also had similar microclimates. These results suggest that in our study, basket design has little influence on temperature or humidity. These results are similar to Bokhorst and Wardle (2013) who found that fine mesh bags were slightly warmer (0.7 °C) in the morning but otherwise did not find differences in temperature, water entry, or evaporation rates among mesh sizes.

We did, however, find faster breakdown in the no basket treatment (the most open treatment) than in closed baskets with no slits (the most enclosed treatment). It is difficult to compare our breakdown rates to other mesh effects studies because we were unable to restrict very small arthropods access and we employed unique bag designs. However, in a laboratory microcosm study of defaunated litter bags, Bokhorst and Wardle (2013) did not find a difference in decomposition rates among bags with different mesh sizes which is contrary to our findings. Yet, in a field mesocosm experiment where arthropods were present, Bradford et al. (2002) found that litter breakdown rates increased with mesh size. While we did not find significantly different breakdown rates in intermediary basket types, we did find a difference between the treatment most restrictive to arthropods and isolated from the environment (closed baskets with no slits) and those that were most open to arthropods and the environment (no basket), similarly to Bradford et al. (2002). While we cannot know for certain, we hypothesize that differences in breakdown rates that we saw are likely related to differences in access of arthropods to litter and/or differences in fragmentation between treatments. Our privet litter breakdown rates were within the range of those found by Lobe et al. (2012) who also used 100% privet leaves, but somewhat faster rates than those found by Mitchell et al. (2011), using 50% privet litter in mixed bags.

Bag design affected invertebrate composition in our study. Total detritivore abundance, consisting mainly of Collembola and oribatid mites (30% and 69%, respectively), was higher in closed baskets without slits than in closed baskets with slits (Fig. 3a). By contrast, spider abundance was lower in closed baskets without slits than in closed baskets with slits (Fig. 3c), suggesting predation may explain the detritivore pattern. This interpretation is complicated, however, by the fact that Collembola abundance was just as high in open baskets without slits as in closed baskets without slits (Fig. 3b) even though spider abundance

in the former treatment did not differ from the no basket treatment (Fig. 3c). Another possibility is that the mesh bottom of baskets without slits reduced the egression rates of collembolans that had colonized litter in these treatments, resulting in elevated numbers of these invertebrates. (Figure 3). While litter breakdown studies employing different mesh sizes usually aim to exclude larger detritivores, we found that a completely enclosed fine mesh treatment (closed-top baskets without slits) also significantly reduced predator abundance. Potentially, differences found in breakdown rates among mesh sizes in other studies, may have been mediated by the effects of both predators and large detritivores. We hypothesize that in our system, large detritivores were so rare (3 total across all treatments) that if invertebrates did influence differences in breakdown rates, that predator effects likely played a much larger role than those of large detritivores. We have designed supplemental studies to directly assess the possible importance of trophic interactions on leaf breakdown.

Importantly we were unable to exclude all invertebrates from any of our treatments, despite using a very fine mesh. Other studies assessing mesh effects on arthropods suggest that exclusion requires $\leq$ 100-µm mesh (Bradford et al. 2002; Bokhorst and Wardle 2013). Such a fine mesh is very likely to create unrealistic breakdown conditions. Existing mesh-effect studies have not examined microclimate and arthropods concurrently, nor evaluated the impact of extremely fine mesh on the microbial community. Studies examining the interacting effects of extremely fine mesh ($\leq$ 100-µm), arthropods, microbes, and microclimate should be conducted. We were able to exclude most, but not all, macroinvertebrates from open and closed baskets without slits. However, those that were able to access these treatments were nearly exclusively very small predators, which may impact trophic interactions, albeit to a possibly small degree, but should not affect breakdown directly. Additionally, we found evidence which suggests that using two different mesh sizes may cause an unintended trophic effect on litter breakdown. Large mesh bags seem to create natural microclimate conditions and permit a natural arthropod community to develop, and thus may best reflect natural breakdown rates. The large mesh litter bag approach is already widely used in terrestrial forests (Coleman et al. 2004), and aquatic systems (Benfield 2007), and our work suggests the technique is similarly useful under floodplain conditions. Large mesh bags do not, however, allow for direct investigation of arthropod impacts on litter breakdown.

Overall, basket design did not influence microclimate in our study, but did influence litter breakdown rates and the relative abundances of certain arthropod groups like spiders and springtails. This does not, however, indicate that microclimate should not be a concern when considering litter bag design, despite minimal importance in our floodplain system. The strong effect of complete mesh enclosure on spider abundance was unexpected and may provide an opportunity to test how predators may indirectly affect decomposition rates by altering detritivore abundance. Future studies of the microclimate within different bag designs across different regions and ecosystems remain necessary.

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