Received: 6 May 2022 | Revised: 4 July 2022 | Accepted: 12 July 2022
DOI: 10.1002/rra.4055

RESEARCH ARTICLE

Differences in respiration rates and abrasion losses may muddle attribution of breakdown to macroinvertebrates versus microbes in litterbag experiments

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Funding information
National Science Foundation, Grant/Award Number: DEB-1655789

Abstract

Leaf breakdown is an important process in forested headwater streams. A common method used to quantify the role of macroinvertebrate and microbial communities in leaf litter breakdown involves using paired mesh bags that either allow or exclude macroinvertebrate access to leaves. We examined common assumptions of the paired litterbag method to test (1) whether mesh size alters microbial respiration and (2) whether the effects of abrasive flows (e.g., from water and sediment) differ between coarse- and fine-mesh litterbags. We measured rates of microbial respiration on *Acer rubrum* and *Rhododendron maximum* leaves incubated in coarse- and fine-mesh litterbags. We also measured rates of abrasion using aerated concrete blocks in pairs of coarse- and fine-mesh bags in ten streams across a gradient of discharge. We found that rates of microbial respiration on *Acer rubrum* leaves conditioned in fine-mesh bags were 65% greater than the rates of respiration in paired coarse-mesh bags, but respiration rates on *Rhododendron maximum* were similar in coarse- and fine-mesh bags. Abrasion was, on average, 56% greater in coarse-mesh than paired fine-mesh bags, and these effects were greater in streams with higher discharge. These results suggest that more caution is required when attributing the difference in leaf breakdown between coarse- and fine-mesh bags to macroinvertebrates. Because the effect of mesh size on microbial respiration of *Acer* leaves and abrasion are opposite in direction, the effect that dominates and creates bias likely depends on both environmental context and experimental design.

KEYWORDS
abrasion, coarse-mesh, decomposition, fine-mesh, organic matter, shredders, velocity

1 | INTRODUCTION

Terrestrial leaf litter is a critical resource that is responsible for much of the ecosystem metabolism and production of animal biomass in forested headwater streams (Benstead, Cross, Gulis, & Rosemond, 2020; Wallace, Eggert, Meyer, & Webster, 2015). The biological component of leaf litter breakdown is carried out by bacteria, fungi, and animals, including shredding macroinvertebrates (Marks, 2019). Understanding the relative roles of these organisms in leaf litter breakdown has long been a focus of freshwater ecology (Cuffney, Wallace, & Lugthart, 1990; Cummins & Klug, 1979) and has important implications for how rapidly the carbon and nutrients in leaves are mineralized (Marks, 2019). More recently, research has focused on how global change drivers, such as temperature and pollution, may influence microbes and macroinvertebrates. For example, empirical evidence suggests that the amount of leaf carbon that is routed through animal consumers may be reduced in warmer and altered in highly polluted streams (Boyero et al., 2011; Follstad Shah et al., 2017; Manning et al., 2016; Woodward et al., 2012). Much of the research contrasting the relative roles of microbial and macroinvertebrate communities in leaf breakdown employs a paired litterbag technique in which coarse- and fine-mesh sizes are used to either allow or exclude macroinvertebrate consumers and thus quantify their roles in leaf breakdown (Bärlocher, 2020; Frainer, Bruder, Colas, Ferreira, & McKie, 2021). This technique assumes that the physical conditions in coarse- and fine-mesh bags are similar. However, fine-mesh bags may affect microbial breakdown by limiting the movement of water, gases, and nutrients, and may limit the effects of abrasion on leaf breakdown. While the existence of these effects has been acknowledged (Lecerf, 2017), they have not been sufficiently tested, and are often overlooked in the interpretation of litterbag studies.

Paired litterbag studies assume that microbial breakdown rates are similar in coarse- and fine-mesh litterbags (Gessner & Chauvet, 2002; Lecerf, 2017), though there are some reasons this may not be the case. The exponential model of mass loss is typically used to describe leaf litter breakdown in stream ecosystems (Bärlocher, 2020; Olson, 1963), and implies that mass loss is time-invariant and proportional to the mass remaining. Thus, regardless of the state of breakdown, the mass-specific rate of mass loss due to microbial breakdown should be similar between pairs of coarse- and fine-mesh bags. However, there are several reasons why this may not be the case. First, fine-mesh should reduce water velocity at the leaf pack surface more than coarse-mesh, and slower water velocity has been correlated with lower rates of microbial breakdown, fungal sporulation, and diversity (Bastias et al., 2020; Ferreira & Graça, 2006). In fine-mesh bags, these effects could result from limited access to oxygen and nutrients around the surfaces of leaves that microbes colonize, and may be compounded by increased sediment trapping (Hieber & Gessner, 2002). Alternatively, increased access to nutrients and oxygen in coarse mesh bags may lead to a greater allocation of carbon used for microbial growth opposed to respiration (Manzoni, Taylor, Richter, Porporato, & Ågren, 2012). Additionally, fine-mesh bags may boost microbial breakdown rates by protecting microbes from selective consumption by macroinvertebrates and macroconsumers (Arsuffi & Suberkropp, 1988; Sabetta, Costantini, Maggi, Persiani, & Rossi, 2000).

The paired litterbag technique also assumes that the difference in breakdown rate between coarse- and fine-mesh bags is attributable primarily to macroinvertebrate consumption. This assumption ignores other mechanisms of mass loss that may be greater in coarse- than in fine-mesh bags, such as physical abrasion (Ferreira, Graça, De Lima, & Gomes, 2006; Heard, Schultz, Ogden, & Griesel, 1999; Hubai et al., 2017). Abrasion effects on leaves may be greater in coarse-mesh bags, as the wider mesh openings may lead leaves to experience higher water velocities (Moulton, Magalhães-Fraga, Brito, & Barbosa, 2010) and greater physical fragmentation caused by suspended sediment relative to fine-mesh bags (Spänhoff, Augspurger, & Küsel, 2007). This may lead to an overestimation of macroinvertebrate contributions to leaf litter breakdown, particularly when water velocity and/or suspended sediment loads are high (Canton & Martinson, 1990). Furthermore, if abrasion varies across flow gradients, it may also confound empirical estimates of the contribution of shredding macroinvertebrates to leaf breakdown across those gradients.

Here, we investigate whether there are differences in rates of mass loss between coarse- and fine-mesh litterbags due to microbial respiration and abrasive flows. We hypothesize that coarse-mesh bags will have higher rates of microbial respiration than fine-mesh bags, potentially due to greater access to dissolved nutrients and oxygen (H1). Alternatively, we hypothesize that coarse-mesh bags may have lower rates of respiration than fine-mesh bags, potentially due to the selective consumption of microbial biomass by shredding macroinvertebrates (H1a). We further hypothesize that coarse-mesh bags experience more physical abrasion than fine-mesh bags (H2), and that this difference in abrasion between coarse- and fine-mesh bags will increase at higher discharge conditions (H3). We test these hypotheses by measuring respiration on leaves incubated in coarse- and fine-mesh bags, and by measuring abrasive forces in both coarse- and fine-mesh bags across a gradient of stream sizes and discharge.

2 | METHODS

2.1 | Study site

This study was conducted at the United States Department of Agriculture, Forest Service (USDA FS) Southern Research Station, Coweeta Hydrologic Laboratory (Coweeta) in the southern Appalachian Mountains, Macon County, North Carolina, USA (see Swank and Crossley, 1988 for site information). To test hypothesis H1 we incubated leaves in streams in watersheds (WS) 55 and 5a at Coweeta, which we used to make respiration measurements. These two streams are similar in size, though the stream in watershed 5a was experimentally warmed at the time of the study (additional physical and chemical information in Table S1). To test hypotheses H2 and H3 we used an additional 10 streams across Coweeta that varied in size (Table 1). In WS55 and WS5a, we estimated stage height using a pressure transducer located in the flume at the downstream terminus of our sample reach. We converted stage height to discharge using a stage-discharge rating curve. We characterized discharge in all other sites based on mean daily discharge measured at weirs provided by the USDA FS. We measured widths and depths in each stream at least four different times over the year of each study across at least four transects near to where litterbags were deployed.

TABLE 1 Physical characteristics of streams included in this analysis and the study that that they were used in

Stream	Study	Watershed area (hectare)	Average discharge (L s^{-1})	Average width (m)	Average depth (cm)
WS5a	Respiration	8.28	1.8	1.2	3.2
WS55	Respiration	8.01	1.4	1.6	2.2
WS01	Abrasion	16	3.3	1.37	3.1
WS02	Abrasion	12	4.5	1.1	2.3
WS07	Abrasion	59	24.0	1.9	5.5
WS08	Abrasion	760	393.8	5.1	17.4
WS13	Abrasion	19	5.9	2.5	2.5
WS14	Abrasion	61	27.2	2.6	4.8
WS27	Abrasion	39	32.0	2.3	6.0
WS31	Abrasion	34	20.9	2.6	4.9
WS32	Abrasion	41	28.6	2.4	7.0
WS36	Abrasion	49	38.6	2.7	19.0

2.2 | Respiration rates

To test our first hypothesis we deployed 12 pairs of coarse- and fine-mesh litterbags in both WS55 and WS5a randomly along 120-m reaches ($n = 6$ per mesh size per stream). We filled half of the coarse-mesh bags with senesced red maple (*Acer rubrum)* leaves (5.0 ± 0.1 g) and half with rosebay rhododendron (*Rhododendron maximum*) leaves (10.0 ± 0.1 g). We filled fine-mesh bags with 1.5 ± 0.1 g of leaf litter. We made coarse-mesh bags of 5-mm plastic mesh pecan bags (22 × 40 cm, Cady Bag, Incorporated, Pearson, Georgia, USA), and fine-mesh bags with 250-μm nylon mesh (*Acer* 14 × 12 cm, *Rhododendron*: 6 × 23 cm, Industrial Netting, Maple Grove, MN, USA). We deployed litterbags on November 30, 2020 and retrieved them after 133 and 154 days of incubation for *Acer* and *Rhododendron* respectively. We brought the coarse- and fine-mesh litterbags back to the lab, gently rinsed sediment from the leaves, cut fragments from the leaves, and measured respiration on leaf fragments. To measure respiration, we placed leaf fragments in 30-mL glass chambers filled with stream water at the ambient stream temperature. We measured the oxygen concentration (YSI™ 5,100 Dissolved Oxygen Meter; Yellow Springs, Ohio) in the stream water immediately after adding the leaf fragments and then sealed the incubation vials. After approximately two hours, we recorded the time and measured the oxygen concentration again. Dissolved oxygen concentration at the conclusion of the trial were never lower than 8.0 mg L^{-1}, so we expect kinetic limitation of respiration rates caused by depletion of dissolved oxygen was not a major issue. We then freeze-dried and weighed the leaf fragments. We calculated respiration as the mass of oxygen consumed divided by the incubation time and the mass of leaves in the vial (mg O_2 g dry mass^{-1} h^{-1}).

2.3 | Measuring abrasion

To test for differences in abrasion between coarse- and fine-mesh bags, we used lightweight aerated concrete blocks (dimensions 5 × 1 × 2.5 cm), hereafter referred to as "blocks", that have been used in previous studies as an index of abrasive flow (Xella Aircrete North America Inc., Atlanta, Georgia, USA, Webb, Downes, Lake, & Glaister, 2006). We dried and weighed the blocks before placing them into either a coarse- or fine-mesh bag. We attached pairs of coarse- and fine-mesh bags with zip-ties and deployed them in the field on December 11, 2017. We deployed four pairs of bags in each of 10 different streams varying in watershed size and discharge (Table 1). We collected pairs of bags after 87–284 days. Bags deployed in smaller streams had longer incubation times to ensure a measurable change in block mass. After incubation, we rinsed, dried, and reweighed the blocks. We discarded observations where the blocks had visual evidence of crumbling or cracking to ensure that only physical abrasion of the surfaces was considered as an effect in this study. As a result, we discarded 13% percent of our observations from the analysis.

We calculated the first-order decay rates of the abrasion blocks as the negative of the natural log of the final mass (M_t) divided by the initial mass (M_0) divided by the incubation time in days (t, Equation 1). We calculated coarse-mesh (k_c) and fine-mesh (k_f) breakdown rates separately.

$$k = -\ln\left(\frac{M_t}{M_0}\right)/t \tag{1}$$

Because we expected there to be no biological loss from the blocks in the fine- or coarse-mesh bags, both k_c and k_f are estimates of abrasion. We estimated how much faster the breakdown of blocks occurred in coarse- compared to fine-mesh bags as the fragmentation rate (λF, Lecerf, 2017).

$$\lambda F = k_c - \frac{k_f - k_c}{\ln(k_f) - \ln(k_c)} \tag{2}$$

λF is calculated as k_c minus the difference in breakdown between the fine and coarse bags (k_c -k_f), divided by the difference of the $\log_e$

transformed breakdown rates (Equation 2). Other metrics have also been used to partition breakdown effects based on mesh size. For instance, the ratio of breakdown in coarse- and fine-mesh bags or the differences in breakdown rates between bag types (Gessner & Chauvet, 2002; Woodward et al., 2012). λF overcomes some mathematical issues with these approaches (Lecerf, 2017), but we also report the ratio of decay rates (k_c:k_f) to provide a more intuitive effect size for our estimates of abrasion (Gessner & Chauvet, 2002).

2.4 | Analysis

To test our hypothesis that respiration would differ between leaves in coarse- and fine-mesh litterbags (H1), we analyzed the respiration data in two ways. First, we used linear mixed-effects models with interacting effects of bag type and leaf mass along with interacting effects of bag type and leaf species as explanatory variables and a random effect to account for the pairing of coarse- and fine-mesh bags (i.e., respiration rate ~ bag type * leaf mass + bag type * leaf species [1|bag ID]). Because we noticed a relatively strong negative relationship between the mass of leaves incubated and the mass-specific respiration rates ($R^2 > .5$ for fine-mesh *Acer* bags, weaker in other subsets of the data), we include this as a covariate in our models. We centered leaf masses by subtracting the mean value so that the effect of bag type could be interpreted as the respiration rate at the mean leaf mass. We ran mixed-effects models with the *lme4* package and *p*-values were calculated using *lmerTest* (Bates, Maechler, Bolker, & Walker, 2014; Kuznetsova, Brockhof, & Christensen, 2017). In our second analysis, to confirm that our results were not a consequence of statistically controlling for leaf fragment mass we used a paired *t*-test to test for differences in mean respiration rate between the two mesh sizes. We used separate *t*-tests for the *Acer* and *Rhododendron* leaves.

To test whether objects in coarse-mesh bags experience more abrasion than objects in fine-mesh bags (H2), we used a one-sample *t*-test to test whether λF was different than zero and whether the block k_c:k_f was different than one. A positive λF that is significantly different than zero would indicate more abrasion in coarse-mesh bags than in fine-mesh bags, as would an average k_c:k_f greater than one. We evaluated the effect of discharge on k_c and k_f using linear mixed-effects models accounting for the repeated measurements within a stream by including a random effect for stream. To test whether the difference in abrasion between pairs of coarse- and fine-mesh bags changed with discharge (H3), we evaluated the effects of average stream discharge on the magnitude of λF again accounting for repeated measurements within each stream. Because these relationships appeared to be leveraged on the points from the largest stream, we report result with and without the largest stream included in the statistical models. We calculated the portion of variance explained by the fixed effects (marginal R^2) and by the fixed and random effects together (conditional R^2) using the *MuMIn* package. All analyses were conducted using R version 3.6.2 (R Core Team, 2018). Data and code are available at https://github.com/nathantomczyk/litter-bag-effects.

3 | RESULTS

When we controlled for the effects of leaf fragment mass we found that *Acer* leaves in the coarse-mesh bags had mean respiration rates of 0.07 mg O_2 g^{-1} h^{-1}, while *Acer* leaves in fine mesh bags had respiration rates 0.12 mg O_2 g^{-1} h^{-1}. This represented a difference in respiration rates of roughly 66% (mean difference = 0.046 mg O_2 g^{-1} h^{-1}, SE = 0.018, $t_{25.1} = 2.39$, $p = 0.025$). Microbial respiration rates on *Rhododendron* leaves were similar between coarse- and fine-mesh bags (mean difference = 0.00 mg O_2 g^{-1} h^{-1}, SE = -0.018–0.019, $t_{24.3} = -1.24$, $p = 0.24$). When we analyzed these data using paired *t*-tests we found similar results; mass-specific microbial respiration rates on *Acer* leaves in fine-mesh bags were about twice as fast as microbial respiration rates in paired coarse-mesh bags (mean difference = 0.045 mg O_2 g^{-1} h^{-1}, 95% CI range = 0.003–0.086, $t_{10} = -2.43$, $p = 0.035$, Figure 1). Similar to our findings from the more complex model, the t-test also demonstrated that microbial respiration rates on *Rhododendron* leaves were similar between coarse- and fine-mesh bags (mean difference = 0.01 mg O_2 g^{-1} h^{-1}, 95% CI range = −0.04–0.019, $t_{1,11} = 0.77$, $p = 0.46$).

We found that the rate of abrasion was, on average, 56% higher in coarse-mesh bags than in paired fine-mesh bags ($t_{30} = 9.3$, $p < 0.0001$, Figure 2) and that λF_B was significantly different from zero ($t_{30} = 6.25$, $p < 0.0001$). We found that both k_c and k_f increased with average discharge, though k_c increased more with discharge than k_f (Figure 3a,b, Table 2), which meant that λF_B also increased with average discharge (Figure 3c, Table 2). These relationships with discharge were steeper and stronger when the highest discharge site was included in the analysis, but each relationship still explained >20% of the variation in the response when the largest site was excluded from the analysis (Table 2).

4 | DISCUSSION

We found that in some circumstances different mesh sizes may lead to experimental artifacts in measuring litter breakdown, but the effects of mesh size on respiration and abrasive forces were, in some cases, in opposing directions. Microbial respiration on *Acer* leaves was higher in fine-mesh bags than in coarse-mesh bags, but there was no difference between respiration rates of *Rhododendron* leaves in litterbags of different mesh sizes. Higher respiration rates in fine-mesh *Acer* bags challenges our ability to infer microbial losses in coarse-mesh bags based on breakdown rates in fine-mesh bags. We also found that abrasion rates were significantly higher in coarse-mesh bags than in fine-mesh bags, and this difference was greater where there was higher discharge. This implies that, in settings where abrasion contributes significantly to leaf breakdown, the effect of shredding macroinvertebrates may be overestimated if their contribution is calculated as the difference in breakdown rate between coarse- and fine-mesh bags.

Differences in microbially mediated mass loss between coarse- and fine-mesh bags may depend on leaf type and stage of breakdown,

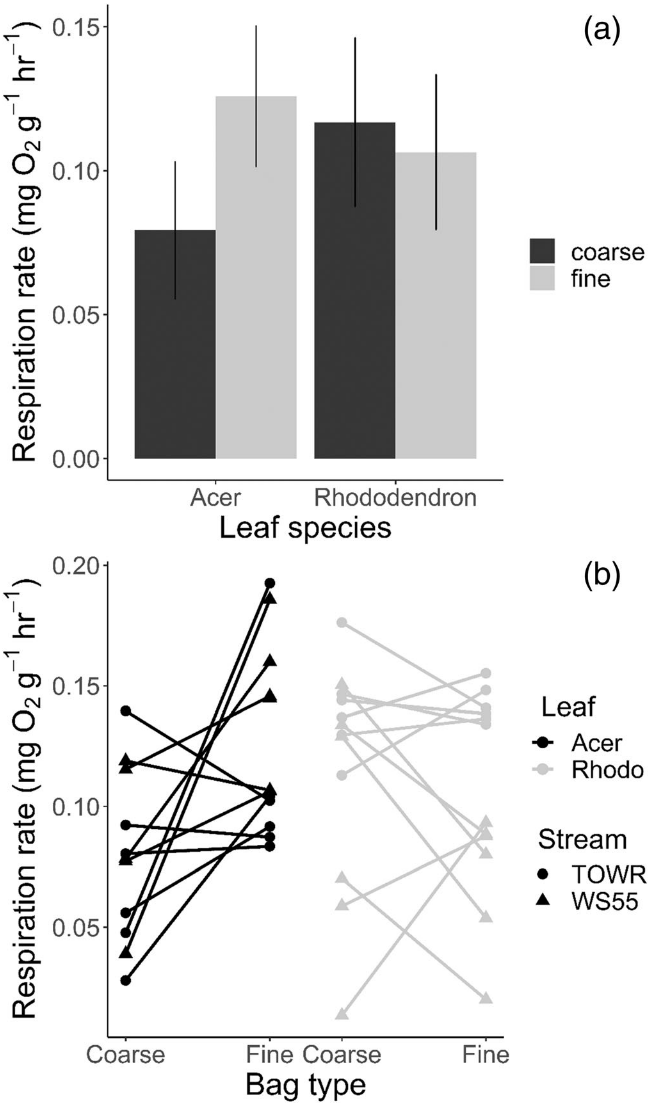

FIGURE 1 Respiration rates of leaf litter incubated in coarse- and fine-mesh litterbags. Average rates of respiration for *Acer rubrum* and *Rhododendron maximum* are presented with their 95% confidence intervals (a) and the rates from individual bags, with lines connecting pairs of coarse- and fine-mesh bags (b)

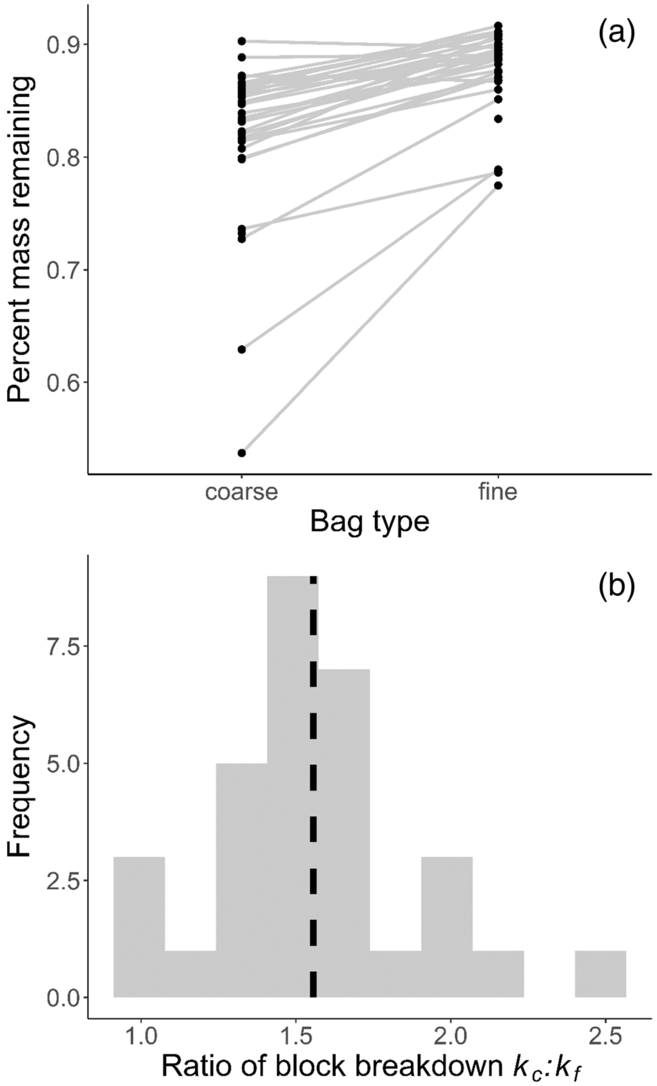

FIGURE 2 The percent mass of aerated concrete blocks in coarse- and fine-mesh bags remaining after incubation (a) and the frequency distribution of the ratio of coarse-mesh to fine-mesh breakdown rates (k_c:k_f) (b). Dashed line indicates mean value in panel b

or may just be highly variable. If the higher rate of microbial respiration on *Acer* leaves in fine-mesh bags is persistent during the decomposition process, it implies that we may underestimate the role of shredding macroinvertebrates in the breakdown of *Acer* leaves. This could result from the consumption of microbial biomass by shredding macroinvertebrates, which have been shown to affect fungal communities when shredders have access to leaves (Bärlocher, 1980; Sabetta et al., 2000). The effects we observed for *Acer* leaves may have resulted from the relatively advanced stage of decomposition in the coarse-mesh bags (mean ~ 13% mass remaining) compared to the fine-mesh bags (mean ~ 34% mass remaining, Table S2). This difference in decomposition stage could imply that the only carbon substrates left in the coarse mesh bags were more recalcitrant structural components. However, we found no effect of mesh size on the respiration rates on *Rhododendron* leaves, which were less broken down when we analyzed them (roughly 65% and 75% of initial mass remaining in coarse- and fine-mesh bags respectively, Table S2). Microbial biomass and rates of respiration vary throughout the decomposition process (Gulis & Suberkropp, 2003; Hieber & Gessner, 2002), and differences between coarse- and fine-mesh bags may also change over time. Other studies also did not detect find an effect of bag type on microbially mediated mass loss. For instance, a study with *Liriodendron tulipifera* leaves found no differences in respiration or microbial biomass between coarse- and fine-mesh bags (Howe & Suberkropp, 1994). Further, a study in artificial channels that excluded macroinvertebrates found similar rates of breakdown of *Alnus glutinosa* litter in coarse- and fine-mesh bags (Ferreira et al., 2006). However, a study of *Phragmites australis* breakdown in the littoral zone of a volcanic lake found higher rates of respiration in

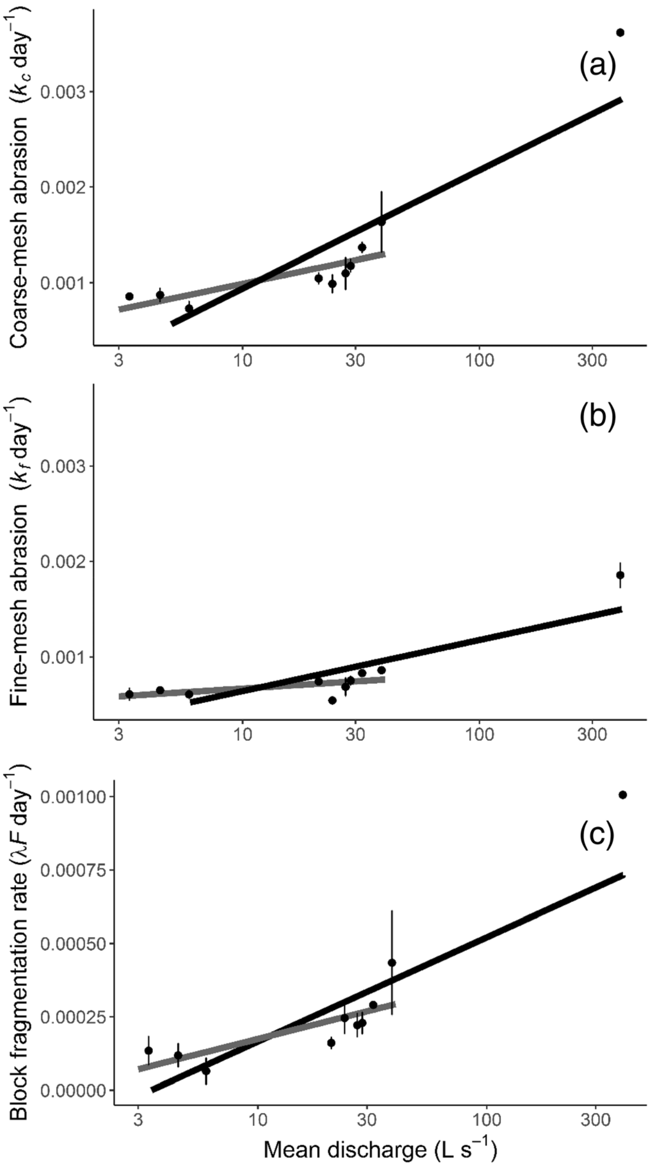

FIGURE 3 Relationship between average discharge and abrasion in coarse-mesh (k_c, a), abrasion in fine-mesh (k_f,b), and the fragmentation rate (λF, c). The fragmentation rate represents how much more rapidly breakdown is occurring in the coarse-mesh bags and is calculated based on the breakdown rates in coarse- and fine-mesh bags. The axis for mean discharge is $\log_{10}$ scaled and the y-axis scales differ among figures. Black lines represent model fits over the whole data range, and gray lines represent model fits excluding the highest discharge site. Parameters of lines of best fit are given in Table 2

coarse-mesh bags on two of the three measurement dates (Sabetta et al., 2000). Taken together, these results suggest that differences in microbial activity between mesh sizes may not be common, and may depend on leaf type and decomposition stage.

When abrasion by water and sediments contributes significantly to leaf mass loss, the role of macroinvertebrates in leaf breakdown may be overestimated by paired mesh litterbag experiments. The role of abrasion in leaf litter breakdown depends on both environmental and leaf properties. Abrasive flows should have the biggest influence when sediment loads and water velocities are high (Hubai et al., 2017), and softer leaves may be more susceptible to mechanical abrasion (dos Santos Fonseca, Bianchini, Pimenta, Soares, & Mangiavacchi, 2013). Ferreira et al. (2006) incubated leaves in artificial channels without macroinvertebrates or sediment and found no effect of velocity, with treatments as high as 2.35 m s^{-1}. However, they found that in natural streams, litterbags in higher velocity areas broke down significantly faster over a smaller velocity gradient (max = 1.29 m s^{-1}), but only during autumn when sediment loads were high, though other factors such as shredder biomass may have varied seasonally in their study. Abrasion has also been evaluated by placing stones into coarse-mesh bags; one study found that stones increased leaf fragmentation by 45–93% over bags without stones (Heard et al., 1999). Another approach involved comparing leaf breakdown from coarse-mesh bags between times when macroinvertebrates had been extirpated due to a chemical spill, and a time when macroinvertebrates were present (Hubai et al., 2017). This study estimated that abrasion and drifting of fine particles from bags contributed between 5–47% of leaf mass loss (Hubai et al., 2017). The different effect of abrasion on coarse- and fine-mesh bags is important to consider when experimental gradients overlap with potential gradients in abrasion, such as when evaluating organic matter dynamics across streams of different sizes or when comparing across time periods with drastically different flows (Graça, Ferreira, & Coimbra, 2001; Webster, 2007). When breakdown is measured across gradients of abrasion, the effects of abrasion may be misattributed to the effects of macroinvertebrates. However, this may only matter when the gradient in abrasive forces is large. We found that there was a positive relationship between abrasion rates that we measured, and rates of leaf fragmentation measured in the same streams (Supplemental Materials). However, this relationship between leaf fragmentation and abrasion was highly leveraged on our largest site (mean discharge = 393.8 L s^{-1}), and the relationship was not significant when we analyzed the relationship across the smaller streams (range in mean discharge = 1.8–38.6 L s^{-1}). In circumstances where abrasive flows may contribute substantially but are not the primary focus of a study, abrasion could be accounted for statistically by deploying abrasion blocks simultaneously with litterbags and using the independent measurements of abrasion as a covariate in statistical models. While the aerated concrete blocks we use in this study have meaningfully different properties than leaves (e.g., blocks are less flexible and lack potentially protective biofilms), which may influence rates of abrasion – use of some type of standardized substrate that is not subject to biological degradation is required to independently measure abrasive forces. If abrasion cannot be measured directly, covariates of abrasion, such as velocity, discharge, or sediment loads could also be used to statistically account for the effects of abrasive flows. While we did not use this approach in the present study, including statistical controls for abrasion should improve the precision of estimates of biological effects on leaf breakdown.

Methods other than paired coarse- and fine-mesh litterbags may better quantify the relative roles of macroinvertebrates, microbes, and

TABLE 2 Results of linear mixed-effects models of the effect of average discharge on rates of abrasion in fine-mesh bags (k_f) coarse-mesh bags (k_c), and the difference between abrasion in fine- and coarse-mesh bags (λF)

Variable	Portion of data	Slope (se)	Marginal R^2	Conditional R^2
k_c	Full range	0.0005 (0.0001)	.67	.93
k_c	Low discharge	0.0002 (0.00007)	.4	.56
k_f	Full range	0.0002 (0.00005)	.62	.93
k_f	Low discharge	0.00007 (0.00003)	.23	.59
λF	Full range	0.00015 (0.00003)	.55	.69
λF	Low discharge	0.00009 (0.00003)	.29	.31

Notes: We report slopes and standard errors (se) associated with discharge, the R^2 associated with only the fixed effects (marginal R^2), the R^2 associated with the whole model (conditional R^2). For each parameter we report values for the models fit to all the data, and a subset of the data that excluded the highest discharge site.

physical processes in leaf breakdown. The effects of macroinvertebrate on litter breakdown and transformation of organic matter have been quantified from litterbag to whole-stream scales with the addition of insecticide (Cuffney et al., 1990; Wallace et al., 1991; Wallace, Vogel, & Cuffney, 1986). Chemical agents that kill macroinvertebrates can be used to quantify the effects of abrasion (Hubai et al., 2017), and insecticide treatments manage to exclude macroinvertebrates and maintain similar physical conditions between the access and exclusion treatments. While application of insecticides is not appropriate in all settings and raises ethical concerns, insecticide treatments should yield better estimates of the contribution of macroinvertebrates to leaf breakdown. The effects of larger consumers (e.g., crayfish, fish, and shrimp) have been quantified with electrical exclusion (March, Benstead, Pringle, & Ruebel, 2001; Rosemond, Pringle, & Ramírez, 1998; Schofield, Pringle, Meyer, & Sutherland, 2001), often in conjunction with tethered leaf packs (Aguiar, Neres-Lima, & Moulton, 2018; Andrade, Neres-Lima, & Moulton, 2017; Usio, 2000). This design also maintains environments with similar physical forces between access and exclusion treatments. Alternatively, some studies have minimized differences between coarse- and fine-mesh bags by constructing bags with the same fine-mesh, but with a circular "window" with variable mesh size facing downstream, reducing differences in physical abrasion between mesh treatments (Moulton et al., 2010; Moulton, Andrade, & Neres-Lima, 2019). Our study employed fine-mesh with relatively small openings (250 μm), and thus, effects of mesh size may be less important when fine-mesh with larger openings is used. Additionally, measuring parameters such as microbial assimilation and macroinvertebrate consumption rates should help to understand their contributions to breakdown (Benfield, Fritz, & Tiegs, 2017; Hieber & Gessner, 2002).

The comparison of coarse- and fine-mesh bags is an imperfect tool to understand the contributions of microbes and shredding macroinvertebrates to leaf litter breakdown. Microbial parameters can differ between coarse- and fine-mesh bags (Bärlocher, 1980; Sabetta et al., 2000), and abrasion can contribute meaningfully to leaf breakdown (dos Santos Fonseca et al., 2013; Heard et al., 1999). However, these insights have not been fully integrated into the interpretation of litterbag experiments (e.g., Wiederkehr et al., 2020; Woodward et al., 2012). Recent books on the subject of leaf litter breakdown do not discuss differences in abrasion or microbially mediated mass loss between coarse- and fine-mesh bags, and encourage the use of different mesh sizes to separate the effects of macroinvertebrate shredding and microbial activity (Bärlocher, 2020; Frainer et al., 2021). While several mathematical techniques have been used to quantify the role of shredding macroinvertebrates relative to microbes in litterbag experiments (Gessner & Chauvet, 2002; Lecerf, 2017; Woodward et al., 2012), none of these methods solve the underlying issue of physical differences between coarse- and fine-mesh bags. Our data suggest that using paired litterbags to attribute leaf breakdown to shredding macroinvertebrates and microbes should be done with (1) additional data collection on respiration rates at multiple points during the decomposition process, (2) collection of covariates that describe abrasive losses, and (3) careful interpretation of results that accounts for environmental conditions.

ACKNOWLEDGEMENTS

We thank the USDA FS personnel for their support and for providing the discharge data used in this experiment. We thank Charles T. Bond for helping to prepare the abrasion blocks for deployment in the field. We thank the other members of the Rosemond lab, Caitlin Conn and Laura Naslund for their input on this study. Finally, we thank Jonathan Benstead for a friendly review of this manuscript.

CONFLICT OF INTERESTS

The authors have no competing interests to declare that are relevant to the content of this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in github at https://github.com/nathantomczyk/litter-bag-effects.

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How to cite this article: Tomczyk, N. J., Rosemond, A. D., Bumpers, P. M., Cummins, C. S., Yang, C., & Wenger, S. J. (2022). Differences in respiration rates and abrasion losses may muddle attribution of breakdown to macroinvertebrates versus microbes in litterbag experiments. *River Research and Applications*, *38*(10), 1721–1729. https://doi.org/10.1002/rra.4055