**Title:** Litter decomposition in afrotropical streams: effects of land use, home-field advantages, and terrestrial herbivory

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**Abstract**

Land use can strongly affect litter decomposition, a key ecosystem function in low-order streams. Recent evidence suggests that additional drivers of decomposition rates could include 'home-field advantages', when litter decomposes faster at nearby than distant sites, and terrestrial herbivory, whereby inducible plant defenses and changes in leaf geometry caused by herbivore damage could influence subsequent decomposition in streams. To compare the relative importance of these three drivers, we conducted a decomposition experiment in an afrotropical stream system, manipulating land use (farm vs. forest sites), home-field advantage (home vs. away from site of leaf collection), and terrestrial herbivory (using leaves varying in their extent of herbivore damage). We measured decomposition in both fine-mesh and coarse-mesh litter bags to compare drivers of microbial vs. invertebrate-mediated decomposition. Microbial decomposition in fine-mesh bags was unaffected by experimental treatments. In coarse-mesh bags, land use was the only significant (and a strong) driver of decomposition rate, most likely because invertebrate shredders are absent from farm sites. We conclude that home-field advantages and terrestrial herbivory have negligible effects on litter decomposition rates in afrotropical streams, at least relative to a major anthropogenic disturbance such as agricultural land use.

**Introduction**

Leaf litter breakdown is a key ecosystem function in forested headwater streams (Gessner 2010 TREE), where allocthonous detritus is often the main energy source to the stream food web (Vanotte RCC). Factors driving natural variation in rates of litter breakdown include climate, water nutrient concentrations, leaf traits, and biomass and composition of detritivore communities (Webster & Benfield 1986 AREES; Tiggs et al. 2019 Sci Adv; Shah et al. 2016; Boyero 2011 Ecol Lett). Anthropogenic disturbances affecting one or more of these parameters can also influence decomposition rates; for example, agricultural land use often impact litter breakdown considerably via its effect on water nutrient concentrations, silt deposition, and decomposer abundance (Woodward 2012 Science; Encalada 2010 Fw Biol; Sponseller & Benfield 2001). This response of litter breakdown to disturbance has led some authors to argue that decomposition should be employed as a bioindicator of stream ecosystem health (Gessner & Chauvet 2002; Young et al. 2008 JNABS; von Schiller 2017).

Recent experimental evidence has revealed hitherto less-known drivers of litter decomposition, outlining how intraspecific variation in leaves can influence decomposition rates in streams (Jackrel & Wootton 2014, 2015). Distinct genotypes from the same tree species can vary extensively in leaf decomposition rates (Silfver et al. 2007; Lecerf and Chauvet 2007; Whitham et al. 2008), which can impact detritivore growth (Compson 2018 Ecology). Moreover, transplant experiments have shown that leaves can decompose faster at their home site than at distant sites, suggesting local adaptation of decomposer communities to intraspecific variation in trees (Kominowksi 2011; Jackrel & Wootton 2014). This pattern is known as a 'home-field advantage' (HFA) in relation to the greater probability of sports team to win a match in their home venue than abroad (Palozzi & Lindo 2018). Another source of intraspecific variation that could influence decomposition are changes in leaf chemistry and geometry induced by terrestrial herbivores, which could both accelerate and slow leaf decomposition (Choudhury 1988; Burghardt et al. 2018). On the one hand, herbivory often triggers greater investment in chemical defenses and reduced leaf nutrient content, which could slow down decomposition in streams (Jackrel & Wootton 2015). On the other hand, herbivore feeding creates a greater ratio of edge to surface area on the leaf, which could facilitate litter consumption for stream decomposers that feed along edges (Jonsson et al. 2002). Regardless of mechanisms, herbivory on leaves is expected to be spatially hetereogeneous, which could lead to intraspecific variation in decomposition rate in streams.

The roles of HFAs and terrestrial herbivory on litter decomposition have only been examined in a small number of temperate streams. Moreover, no study has quantified the importance of these two drivers relative to other, well-recognized influences on decomposition rates. Here we report a litter decomposition experiment conducted in an afrotropical stream system, in which we compared the effect of land use, a strong driver of decomposition rate, with that of HFAs and terrestrial herbivory. We collected leaves from a single tree species occuring at different sites and quantified their rate of microbial and macroinvertebrate-mediated breakdown in four streams. We assessed whether decomposition rate was affected by stream land use (farm vs. forest sites), HFAs (litterbags deployed at vs. away from site of leaf collection), and terrestrial herbivory (collecting leaves varying in their extent of herbivore damage).

**Methods**

*Study system*

Fieldwork was conducted in and around Kibale National Park, a 795 km2 mid-altitude (1100-1600 m) rainforest located in southwestern Uganda (0°13' – 0°41' N, 30°19' – 30°32' E; Struhsaker book). We selected one forested and one agricultural (farm) stream from each of two main watersheds draining the park (the Mpanga River and Dura River watersheds, both of which are subwatersheds of the Nile Basin). All sites are located within 3 km of the Makerere University Biological Field Station in the northwestern part of Kibale. The two study streams inside the park have a fully forested and protected watershed. The two study streams outside of the park have a watershed dominated by intensive agriculture of food and cash crops (e.g., bananas, cassava, tea, and coffee), pastures for goats and cows (< 10 animals/ha), and sparse exotic trees planted for timber (pine and eucalyptus trees). All sites are small first-order streams (<1.5 m mean wetted width; < 10 cm mean depth) with a similar geomorphology and hydrology, but they vary in water chemistry and community composition based on land use; these differences have been described extensively elsewhere (Fugere FW biol). Briefly, the two farm sites have a much lower canopy cover than the two forest sites, as well as higher water temperature, higher turbidity, lower specific conductance, lower nitrogen and phosphorus concentrations, and a much lower richness and biomass of benthic invertebrates. Invertebrate shredders dominate the composition of forested sites but are largely absent from farm sites (Fugere ecosphere), leading to slower litter breakdown rates at farm sites (Fugere Fw biol).

We delineated a 100-m study reach in each stream to conduct a litterbag experiment (Graça, Bärlocher, & Gessner, 2005). This decomposition experiment focused on the tree species *Neoboutonia macrocalyx* Pax (Euphorbiaceae), the only species of tree occuring within < 5 m of all four sites. This tree, abundant in riparian habitats within the park (Chapman et al. 1999), is also kept for shade outside of the park in pastures that are otherwise entirely cleared. *N. macrocalyx* leaves are readily consumed by both terrestrial herbivores and by macroinvertebrate shredders in streams (Fugere 2018 FW Biol; Savilaasko 2009 D & D; Masenene & Roininen 1999). Other than *N. macrocalyx*, vegetation in the riparian zone of agricultural sites was composed exclusively of grasses and emergent macrophytes, while the riparian zone of forested sites included a high diversity of trees, shrubs and ferns, with a canopy height generally > 20 m.

*Transplant experiment*

We conducted our decomposition experiment in June-August 2011. Twenty leaves varying in their extent of herbivore damage were collected from 3-5 trees around each stream (total = 80 leaves from 17 trees). Leaves were divided into four fragments by cutting two halves along the main stem, and then folding and cutting each half into two additional fragments. This cutting procedure was used to assess whether a given leaf decomposes faster at its home site vs. at a distant site with different land use. All fragments (n = 320) were individually-marked, pressed in-between two glass plates, and then photographed against a white background and from a constant distance using a digital camera mounted on a tripod. Leaf fragments were then air-dried for 48 hours in a food dehydrator and weighed individually. Air-dried mass was converted to leaching-adjusted ash-free dry mass (Graça, Bärlocher, & Gessner, 2005) using an air-dried to oven-dried mass conversion equation, a leaching correction factor, and a mean ash content calculated for this tree species (Fugere 2018 FW Biol). Air-dried leaf fragments were randomly assigned to litter bags constructed of 0.5 or 10 mm mesh (fine and coarse-mesh bags, respectively). Decomposition in fine-mesh bags occurs via microbial breakdown only, whereas decomposition in coarse-mesh bags is the product of microbial breakdown, macroinvertebrate consumption, and (greater) physical fragmentation.

Bags were randomly assigned to arrays of 5 fine-mesh and 5 coarse-mesh bags attached at regular intervals along a 1 m circular metal line. Eight such circular arrays were randomly assigned to each study stream, leading to 80 litter bags (40 bags of each mesh type) being deployed at each site. We transplanted leaf fragments across streams (and land use conditions) but always within watersheds. For example, leaves collected at the forest site from the Dura watershed could be deployed at either the Dura forest site (home site) or the Dura farm site ('away' site), but not at sites from the Mpanga watershed. Thus, circular arrays of 10 bags included leaf fragments from the same watershed only. Arrays were anchored to the stream bottom at 10 m intervals using stones and twist ties. Every 7 days over 28 days, two arrays (20 bags; 10 of each mesh type) were pulled from each site. The litter content of retrieved bags was dried to constant mass for 48 hours at 60 C, weighed, and combusted at 550 C for 4 hours to calculate AFDM of litter remaining in each bag. The proportion of leaf mass that decomposed in the stream is given by: 1-(AFDM after in-stream deployment / initial AFDM). In addition to this proportion-the variable analyzed in statistical models described below-we also calculated standardized decomposition rates (*k*/day) using the exponential decay model: Mt = M0\*e^-kt, where Mt is the proportion of litter AFDM remaining at time t, M0 is the % mass remaining at time 0 (100%), k is the exponential decay coefficient and t is the time in days. We used the nls() function in R to fit this exponential function to the leaching-unadjusted AFDM data, fitting a separate function for fine-mesh and coarse-mesh bags and, when of interest, for separate factor levels (e.g. *k*/day for farm vs. forest streams).

*Terrestrial herbivory*

Leaf photographs were used to quantify damage by terrestrial herbivores, defined as the relative area of a leaf consumed prior to leaf collection. The software Image J version 1.46 (available at https://imagej.nih.gov) was used to measure total and consumed leaf area. An outline of the leaf fragment was first traced assuming no herbivore damage (Fig. 1). This selected area was then binarized using the treshold function in Image J, leading to pixels forming part of the leaf being assigned a brightness value of 1 (white) and pixels contained within holes being assigned a brightness value of 0 (black). The % of original (undamaged) leaf area consumed by terrestrial herbivores is given by: black pixels / (black + white pixels). Herbivore damage was calculated at the fragment scale, at the leaf scale (taking the average of the four fragments), and at the tree scale (taking the average of all leaves/fragments from a given tree). We ran all analyses described below at each of these 3 scales; results remained qualitatively similar (i.e. no effects of herbivore damage; see Results section). Thus, we only present results of analyses at the fragment scale, the same scale at which decomposition was measured and reported.

*Statistical analyses*

R version 3.5.0 (R core team, 2018) was used for all analyses. The response variable for all statistical models is the proportion of leaf litter that decomposed in the stream, ranging from 0 (no loss of AFDM) to 1 (complete loss of AFDM). We analyzed decomposition data with generalized linear mixed models (GLMMs) with a beta error distribution and a logit link function. This family of GLMM was chosen because our proportion data has a highly bimodal and bounded distribution. Proportions were first transformed with the following equation: (p\*(n-1)+0.5)/n, where p is the proportion of AFDM lost and n is the total number of samples in the dataset (n = 313, as 7 litter bags were lost during the experiment). This transformation shrinks the distribution of the response variable to [0.005, 0.995], removing 0s and 1s and permitting beta regression, while minimally affecting the shape of the distribution (Smithson & Verkuilen 2006).

Two GLMMs were fitted separately for each type of litter bags (fine vs. coarse mesh), using the R package 'glmmTMB' (Brooks et al. 2017). The first GLMM incorportated the temporal dimension of decomposition and included the following fixed effects: weeks in stream (1-4), stream watershed (Mpanga vs. Dura), area of leaf fragment damaged by herbivore (0-20 %), home vs. away of site of leaf collection (i.e, transplanted or not), stream land use (farm vs. forest), and land use at site of leaf collection (farm vs. forest; note that this is different from stream land use when leaves are transplanted), as well as all two-way interactions between weeks and all other fixed effects. These two-way interactions were the parameters of interest in this analysis, as we aimed to determine what factors influenced decomposition over time, not the factors determining AFDM on the first time point of the experiment. The model also included a number of random effects: 'stream site' (4 groups), and 'leaf' (80 groups) nested within 'tree' (17 groups) nested within 'site of leaf collection' (4 groups). Both random intercepts and slopes (for the effect of weeks) were included. Multicollinearity among predictors was determined with variance inflation factors (Zuur 2007), which were all < 2.5. The two continuous variables (weeks and leaf damage) were also re-scaled from 0 to 1 before the analysis, to ensure that all variables had the same scale (including factors). We report the results of this model in Table 1.

The second model focused on the last time point of the experiment, using data from day 28 only, and did not include a time effect. This second model was used because the dynamics of decomposition from day 7 to day 28 could be non-linear, leading some variables to have statistically-significant effects only because they affect litter mass loss temporarily at an intermediate time point. The second model included the following fixed effects: herbivore damage, stream watershed, home. vs. away, stream land use, and land use at site of leaf collection. 'Stream site' and 'leaf' nested within 'tree' nested within 'site of leaf collection' were included as random intercepts. This second model effectively asks: by the end of the experiment, which variable predicted how much leaf litter remained in the litter bags? We report the results of this model in Figure 3. All data and analysis code are available at https://github.com/VFugere/decompProj.

**Results**

On average, *N. macrocalyx* leaves in fine-mesh bags lost 52.1 +/- 3.19 % of their ash-free dry mass (AFDM) after 4 weeks of stream exposure. When adding mass loss due to leaching and fitting an exponential decay model, this is equivalent to a mean decomposition rate (*k*/day) of 0.034 +/- 0.001. Watershed significantly influenced both the origin and the slope of the time-decomposition relationship in fine-mesh bags, with the proportion of AFDM lost being both higher at the onset of the experiment and increasing faster over time at the two Mpanga sites relative to the two Dura sites (Table 1). Leaf damage and land use at the site of leaf collection also had siginificant effects on % leaf mass lost at the beginning of the experiment, but not on leaf mass loss over time (Table 1). None of the three variables of interest (stream land use, decomposition at or away from site of leaf collection, and leaf damage by terrestrial herbivores) had a significant effect on decomposition rates in fine-mesh bags (Table 1, Figure 2a). By the end of the experiment, none of the variables that we quantified or manipulated had a significant effect on how much leaf AFDM remained in litterbags (Figure 3a). This suggests that the significant watershed effect on decomposition rate in fine-mesh bags identified in the time series model (Table 1) had vanished by the end of the experiment, on day 28 (Figure 3a).

Decomposition was faster in coarse-mesh bags than in fine-mesh bags, with leaves loosing on average 67.9 +/- 5.57 % AFDM over 28 days (*k*/day = 0.046 +/- 0.003). Land use had a strong influence on decomposition rate in coarse-mesh bags (Table 1). While leaves had almost entirely decomposed at forest sites by the end of the experiment (mean AFDM lost by day 28 = 97.7 +/- 0.93 %; *k*/day = 0.077 +/- 0.005), decomposition was three times slower at the two farm sites (*k*/day = 0.025 +/- 0.002), proceeding in fact at a similar rate than decomposition in fine-mesh bags (Figure 2b). The effect of land use on % AFDM remaining in litterbags at the end of the experiment was highly-significant (Figure 3b). In contrast, terrestrial herbivory and transplanting leaves away from the site of leaf collection had no effect on decomposition rates in coarse-mesh bags (Table 1, Figure 2b, Figure 3b).

**Discussion**

Our transplant experiment quantifying the effects of land use, home-field advantage (HFA), and terrestrial herbivory on litter decomposition in afrotropical streams revealed that microbial breakdown was unaffected by these three variables, whilst decomposition rates in coarse-mesh bags was strongly affected by land use but not by the other two variables. Overall, *N. macrocalyx* leaves were characterized by rapid decomposition rates, with a mean *k*/day of 0.04 when pooling all treatments. This rate is in the upper range of what has been reported in reviews (e.g., Webster & Benfield 1986), but is comparable to some other tree species decomposing in tropical streams (Mathuriau & Chauvet 2002). Litter decomposition in streams usually proceeds much faster in tropical wet areas relative to other biomes, even when using standardized substrates (Tiegs et al. 2019). Our experiment confirms the high palatability of *N. macrocalyx* to macroinvertebrate shredders in Kibale streams. This tree species is also a common resource of terrestrial invertebrate herbivores in the region (Heimonen 2013; Kasenene & Roininen 1999; Savilaakso et al 2009).

We had already reported the effects of land use on litter breakdown in this system, and attributed the lower decomposition rates at farm sites to the almost-complete absence of macroinvertebrate shredders at these sites (Fugere FW Biol 2018). Decomposition rate in coarse-mesh bags is influenced by microbial breakdown, macroinvertebrate consumption, and physical fragmentation; since microbial breakdown does not differ between farm and forest sites, and since stream discharge is similar at all sites (and thus so is physical fragmentation, presumably), reduced macroinvertebrate consumption is the most likely explanation for the much slower decomposition rate at farm sites. Key shredder taxa that are very abundant in Kibale streams include *Calamoceratidae*, *Lepidostomatidae*, *Pisuliidae*, and *Tipulidae*; these four taxa were found to be very rare or completely absent at 23 agricultural streams outside of Kibale (Fugere 2018 FW Biol; Fugere 2016 Ecosphere). Negative impacts of forest conversion on shredder abundance, and thus on litter breakdown rates, have also been reported for other tropical sites (Masese 2014; Encalada 2010).

We had hypothesized that terrestrial herbivory would influence in-stream decomposition via its effect on leaf chemistry (e.g. inducible defences and reduced nutrient content) and geometry (e.g. increased edge-to-area ratio) but instead found no association between leaf damage and decomposition rate. We calculated leaf damage at the leaf fragment scale because this is the scale at which decomposition was measured, and because any geometry effect would only be relevant at this scale. However, although herbivore damage can induce changes in leaf chemistry in damaged leaves only, it can also trigger changes across the whole tree, including undamaged leaves (Oleksyn et al. 1998; Jackrel & Wootton 2015). We thus calculated leaf damage at the whole-leaf and whole-tree scale as well and repeated all analyses, but still found no effect of mean leaf damage on mean decomposition rate. We consider two reasons why herbivory might have failed to influence decomposition in this system. First, some macroinvertebrate taxa hypothesized to benefit from a greater edge-to-area ratio in litter (e.g. amphipods; Jonsson et al. 2002) are not present in Kibale. Second, it is possible that herbivory did not influence leaf chemistry (e.g., C:N ratio) significantly–or at least not enough to constrain decomposer feeding given the high basal palatability of this tree species. Impacts of herbivory on leaf chemistry have not been examined in *N. macrocalyx*, but cutting entire branches do not influence the density of insect herbivores occuring on an individual tree, suggesting modest investment in herbivore deterance following tree damage (Savilaakso et al. 2011). In any case, effects of terrestrial herbivory on litter decomposition remain unclear and are likely complex and context-dependent; for example, both Jackrel & Wootton (2015) and Burghardt et al. (2018) found a negative effect of herbivory on decomposition, but only when paired with a plant fertilization treatment.

We also did not find a significant HFA for *N. macrocalyx* decomposition; litter mass loss was similar at home vs. distant sites. In coarse-mesh bags, both farm and forest leaves decomposed faster at forest sites, while for fine-mesh bags transplanting leaves across sites and land use categories had no effect on decomposition. This result contrasts with some previous studies that have found strong HFAs (Kominowksi et al. 2011; Jackrel and Wootton 2014). However, results from two recent transplant experiments conducted in streams also found no support for the HFA hypothesis (Stoker 2017; Fenoy 2016). Studies conducted in other types of aquatic ecosystems have sometimes reported HFAs (Franzitta 2015; Luai 2019; Leroy 2017), sometimes the opposite pattern of a home-field disadvantage (Luai 2019), and sometimes–as in our experiment–no pattern at all (Leroy 2017). It is possible that a significant HFA would have been detected had we included more replicate litter bags; models fitted to the data from the last time point found *p* values around 0.1 for the home vs. away treatment, with mean decomposition being qualitativelyy faster at home sites (Figures 2 and 3). Thus, instead of concluding that HFAs do no exist in this study system, we warrant the more cautious interpretation that if such effects do exist, they are weak relative to effects of land use on breakdown.

We speculate that HFAs could generally be weaker in tropical than temperate systems, as the species composition of litter inputs from tropical riparian forests could be more temporally-variable and species-rich than litter inputs in temperate forests, making it harder for decomposer communities to specialize on any particular resource. Moreover, the amount of intraspecific trait variation in plants decreases with species richness of local communities (Siefert 2015). This could limit the potential for adaption in decomposer communities to tree intraspecific variation if such variation is low in species-rich tropical forests. Systems in which strong HFAs have been found were indeed characterized by relatively homeogenous forests composed of one or a few tree species that dominated litter inputs to streams (e.g. Jackrel & Wootton 2014). Future work should address the conditions under which intraspecific variation in trees, and microgeographic adaptation of decomposer communities to such variation, can indeed play an important role in litter decomposition rates in streams.

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**Figure legends**

*Figure 1*. Measurement of herbivore damage. The photograph shows a *N. macrocalyx* leaf fragment with some herbivore damage. The solid black line depicts leaf area assuming no damage while dashed lines encircle areas eaten by herbivores. Leaf damage was quantified as the total number of pixels inside dashed polygons divided by the number of pixels within the solid polygon.

*Figure 2*. Decomposition rates in fine-mesh (a) and coarse-mesh (b) bags as a function of land use (left panels), home-field advantage (middle panels), and terrestrial herbivory (right panels). Lines and shaded polygons indicate means +/- 95% confidence intervals of the mean. For terrestrial herbivory, data are shown for the final time point of the experiment.

*Figure 3*. Results of GLMMs quantifying the influence of five variables on the proportion of leaf litter decomposed by the end of the experiment. Separate models were fitted for fine-mesh bags (a) and coarse-mesh bags (b). Symbols and error bars indicate parameter estimates +/- 95 % confidence intervals; statistically-significant effects with confidence intervals that do not overlap zero are shown in solid colour, while non-significant effects are shown in transparent colour. Models were fitted using data from the final time point of the experiment.