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Leaf-litter breakdown in urban streams of Central Amazonia: direct and indirect effects of physical, chemical, and biological factors

Renato T. Martins, Adriano S. Melo, José F. Gonçalves Jr, and Neusa Hamada

Abstract: Urbanization alters water physical and chemical variables and may affect leaf-litter breakdown in streams. Higher temperature and nutrient inputs in urban streams can stimulate microbial biomass, which can increase leaf-litter breakdown rates over rates in nonurban streams. On the other hand, urbanization can reduce leaf-litter breakdown rates by eliminating shredders. We evaluated physical, chemical, and biological factors that may directly and indirectly affect leaf-litter breakdown of Coussapoa trinervia and Mabea speciosa in 42 urban streams in Central Amazonia. We used structural equation modeling to assess whether: 1) shredder activity is more important than microbes for leaf-litter breakdown of plant species with softer tissues, 2) microbes (as adenosine triphosphate [ATP] concentration) and fungi (as ergosterol concentration) positively influence leaf-litter breakdown rate, 3) water velocity positively affects leaf-litter breakdown rate, and 4) effects of shredders and microbes, including fungi, on leaf-litter breakdown are mediated by the effects of urbanization. Leaf-litter breakdown of M. speciosa and C. trinervia was fastest in the least urbanized streams. Fungi had a direct positive effect on leaf-litter breakdown of both species, but shredders were the most important factor for leaf-litter breakdown in M. speciosa (softer leaf tissues). Water velocity had a slight indirect effect on leaf-litter breakdown of C. trinervia through its effect on fungi. Microbes were not important for leaf-litter breakdown rates of either species. Urbanization indirectly affected leaf-litter breakdown via negative effects on shredder and fungal biomass. Our study provides evidence for multiple direct and indirect pathways by which urbanization can decrease leaf-litter breakdown rates in tropical streams, mainly through negative effects on the fungal and shredder biomass.

Key words: aquatic invertebrates, fungi, leaf shredders, microorganisms, ecosystem functioning, leaf decomposition

Decomposition of organic matter in streams may be affected by 3 main groups of factors. First, leaf-litter breakdown may depend on intrinsic properties of the organic matter. Previous studies have shown that shredders’ activity and, consequently, leaf-litter breakdown are affected negatively by toughness and the presence of secondary compounds, and are affected positively by the nutrient concentration of tissues (Graça 2001, Onoda et al. 2011, Ferreira et al. 2012). Second, leaf-litter breakdown can be affected by environmental conditions. For instance, high values of water temperature, O2 concentration, velocity, and concentrations of N and P usually have positive influences on the loss of detrital mass (Pascoal and Cássio 2004, Ferreira and Chauvet 2011). Last, fallen organic matter is quickly colonized by microorganisms (principally fungi and bacteria) that start the decay process by producing enzymes that breakdown leaf litter (Fenoglio et al. 2006). Microbial biomass, in turn, favors colonization by invertebrate shredders that consume the coarse particulate organic matter and increase the leaf-litter breakdown rate (Graça and Cressa 2010, Pozo et al. 2011, Boyero et al. 2012).

Urban areas usually show an ‘urban stream syndrome’, which is characterized by increased values of impervious catchment cover, nutrient concentrations, water temperature, stream width and depth in pool areas, and by reduced invertebrate diversity, channel complexity, and stability (Paul

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and Meyer 2001, Walsh et al. 2005). The features of these urban streams can influence the leaf-litter breakdown rate relative to in streams in preserved areas (Chadwick et al. 2006, Imberger et al. 2008, Kominoski and Rosemond 2012). Moreover, high water temperature in urban streams increases leaching of soluble compounds (Chergui and Pattee 1999) and can stimulate fungal activity (Ferreira and Chauvet 2011), thereby positively affecting the leaf-litter breakdown rate. Increased concentrations of N and P caused by domestic effluents typically increase the breakdown rate by inducing an increase in microorganism biomass (Suberkropp et al. 2010, Krauss et al. 2011). On the other hand, the increase in water temperature and nutrients causes high rates of oxidation of dissolved organic matter in urban streams and reduces the availability of O2, decreasing the activity of leaf-associated microorganisms (Pascoal and Cássio 2004, Lecerf and Chauvet 2008, Medeiros et al. 2009), thereby slowing leaf-litter breakdown. Low O2 concentration also can reduce or eliminate sensitive invertebrates, such as Trichoptera, which is perhaps the main group of shredders in the tropics (Couceiro et al. 2007).

Urban areas present higher percentages of impervious surfaces than nonurban areas, and during storms, the input of urban pollutants into aquatic systems may be amplified by transport of organic compounds and heavy metals into streams (Schueler 1994). Another effect of urbanization is reduction or removal of riparian vegetation, causing a decrease in particulate organic matter input and an increase in light input (Encalada et al. 2010, Pettit et al. 2012, Miller 2013). The low leaf input in urban streams can negatively affect the food available for aquatic organisms in the stretch where the effect occurs and for long distances downstream (England and Rosemond 2004). The high input of light favors increases in temperature and algal biomass (Roy et al. 2005) and may alter the trophic structure of the ecosystem, reducing its dependence on allochthonous sources (England and Rosemond 2004).

We studied leaf-litter breakdown of 2 plant species that are common in riparian zones of Central Amazonia streams. The study was done in 42 streams that differed mainly in the degree of human impact associated with urbanization. We evaluated physical, chemical, and biological factors that may potentially influence leaf-litter breakdown in urban streams. We built models for the 2 plant species with structural equations to disentangle direct and indirect effects of the studied factors. We hypothesized that: 1) the contribution of shredder activity to leaf-litter breakdown should be more important for plant species with soft than with tough tissues; 2) microbes, including fungi, should positively influence leaf-litter breakdown rates directly and indirectly via their positive effects on shredders; 3) water velocity should have a direct positive influence on leaf-litter breakdown rates by enhancing physical fragmentation, and an indirect positive influence by stimulating fungal colonization; and 4) the effects of shredders and microbes, including fungi, on leaf-litter breakdown rates are influenced by the effects of urbanization on stream sites.

**METHODS**

**Experimental design**

We initially selected fifty 1st- and 2nd-order terra firme (upland) streams in the city of Manaus, northern Brazil (Fig. 1). We excluded 8 streams from the study because of loss of samples caused by vandalism or by spates.

We carried out the leaf-litter breakdown experiment between August and September 2010 (dry season) and used leaves of *Coussapoa trinervia* Spruce ex Mildbr. (Cecropiaceae) and *Mabea speciosa* Müller Argoviensis (Euphorbiaceae). *Coussapoa trinervia* has hard leaves that required 348 ± 66 g to be penetrated, whereas *M. speciosa* is softer and required only 154 ± 45 g to be penetrated according to the method suggested by Graça and Zimmer (2005).

We used senescent leaves of *C. trinervia* and fresh leaves of *M. speciosa* because field observations indicated that most leaves of *M. speciosa* found in the studied streams were green, whereas those of *C. trinervia* were always senescent. Kochi and Yanai (2006) used green and senescent leaves of *Acer mono* Maxim. and *Alnus hirsuta* Turcz. and did not observe significant differences in shredder colonization and leaf-litter breakdown between senescent and green leaves. Such results cannot be easily extrapolated to our studied species, but indicate that potential effects of leaf senescence on leaf-litter breakdown in our study might be small.

For each plant species, we built 250 litter bags (10 × 20 cm) with coarse mesh (10 × 10-mm openings) containing 3 ± 0.05 g air-dried leaves. We incubated 5 litter bags of each species in each stream and retrieved them after 30 d, a period necessary to decompose ~50% of the original mass of *M. speciosa* (Landheiro et al. 2010).

**Environmental variables and conservation status of stream sites**

We measured the pH (model PH90; WTW, Weilheim, Germany), electrical conductivity (µS/cm; model LF90; WTW), dissolved O2 (mg/L; model 55; Yellow Springs Instruments, Yellow Springs, Ohio), and water temperature (°C) on the day the experimental leaves were installed in the streams and during their removal. We estimated total P (mg/L) and total N (mg/L) concentrations using the method of Valderama (1981). We measured water velocity with a flow meter (model 2030R; General Oceaneics, Miami, Florida).

We calculated the percentage of deforested and total impervious area (TIA) around each stream from 2010 Landsat satellite images classified into forested and deforested areas. We delimited a circle with radius = 300 m around each collection site and quantified the deforested area and the areas with primary and secondary forests (Couceiro et al. 2007). We opted to estimate the deforested areas within a circle...
because adult insects of some groups (e.g., Trichoptera) have enough flight ability to colonize sampling areas from downstream reaches (Petersen et al. 2004). We classified deforested areas as urban, exposed soils, or agricultural land. To reduce possible underestimation of TIA, we followed the method of Chadwick et al. (2006) and pooled the exposed soil fraction (usually small) and urban cover together as impervious surface.

**Sample processing**

We removed samples from streams and placed them in plastic bags for transport (~3 h) to the laboratory in coolers with ice. We stored the samples in a refrigerator (4°C) until processing. We selected 5 leaves from each litter bag and cut 15 disks (16 mm diameter). We divided them into 3 sets of 5 disks, and used the sets to measure ash-free dry mass (AFDM), ergosterol concentration (to estimate fungal biomass), and adenosine triphosphate (ATP) concentration (to estimate microbial biomass). To obtain AFDM, leaf disks were oven-dried at 60°C for 72 h and subsequently combusted at 500°C for 4 h.

**Fungal and total microbial biomass**

We estimated fungal biomass on decomposing leaves by quantifying the ergosterol concentration in 1 set of disks. The leaf disks were frozen at ~20°C until ergosterol extraction. We carried out the extraction at 80°C for 30 min in methanol and potassium hydroxide. We purified the extract by passing it through solid phase extraction (SPE) cartridges (model Sep-Pak Vac-RC-500 mg; Waters, Milford, Massachusetts). The ergosterol retained on the column was eluted with isopropanol and quantified by high-performance liquid chromatography (HPLC) (model 625 LC System; Waters). The mobile phase was 100% methanol and the flow rate was set to 1.4 mL/min. We calculated final ergosterol concentrations on the basis of g AFDM of the disks (Gessner 2005).

We quantified total microbial biomass in terms of ATP concentration in the detritus. We stored the disks at ~20°C until extraction. We ground and centrifuged the disks in a solution of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sulfuric acid, and oxalic acid. We filtered the samples (0.22-μm pore size), neutralized them, and recorded their volume. We measured ATP in a luminometer (model TD-20/20; Turner Designs, Sunnyvale, California) via light emission by luciferase. We obtained concentrations by measuring relative changes in light peak values before and after the addition of ATP solutions. We corrected final ATP concentrations for extraction efficiency determined by adding a known amount of ATP to an extra disk set. Final ATP concentrations were calculated on the basis of g AFDM of the disks (Abelho 2005).
Invertebrates

We washed the material remaining in the litter bags in running water and passed it through a 0.12-mm-mesh sieve to retain detritus and invertebrates. We preserved invertebrates in 80% alcohol and sorted them under a stereomicroscope. We identified organisms to family or genus with the aid of keys provided by Pes et al. (2005) and Hamada and Ferreira-Keppler (2012).

We obtained shredder biomass as dry mass (60°C for 48 h; balance accurate to 10 μg). Because of the poor state of knowledge of the functional feeding groups of invertebrates in the Neotropics, we followed the conservative decision of Landeiro et al. (2010) and considered only 2 caddisfly genera as shredders: Tripletides (Leptoceridae) and Phylloicus (Calamoceratidae).

Leaf mass loss

After removing 15 disks from each litter bag, we oven-dried the remaining leaves at 60°C for 72 h and weighed them to the nearest 0.001 g to measure dry mass remaining. We estimated the dry mass of the 15 removed disks based on the dry mass of the 5 disks used to obtain AFDM. We obtained the final dry mass by summing of the oven-dried mass of the remaining leaves and the estimated dry mass of the removed disks.

The leaves used in the experiment were air-dried, and we filled bags based on air-dried mass (ADM). We estimated oven-dried initial mass of leaves based on a correction factor (CF) obtained from the oven-dried mass (ODM) (60°C, 72 h) of leaves in 15 litter bags that were not used in the experiment. We calculated the CF as mean ODM/mean ADM. The CF values were 0.918 and 0.442 for C. trinervia and M. speciosa, respectively. We calculated the leaf-litter break-down coefficient (k) according to the negative exponential model:

\[ k = -\ln(\text{final mass/initial mass})/\text{time}. \]  

(Eq. 1)

Mass was expressed in grams and time in days (Petersen and Cummins 1974).

Statistical analyses

Principal Components Analysis (PCA). We used PCA of environmental variables to synthesize stream conditions along the urbanization gradient. The environmental variables were water velocity, dissolved O₂ concentration (DO), temperature, pH, electrical conductivity, total dissolved N (TDN), total dissolved P (TDP), % deforested area, and TIA. Prior to the analysis, we standardized all variables to unit variance ([observed – mean]/standard deviation).

Structural Equation Modeling (SEM). We used SEM to model the relationship between the leaf-litter breakdown rates of both species and the explanatory (exogenous) variables. This analysis enables estimation of the direct and indirect effects of exogenous variables on a response variable. The indirect effect is the contribution of an exogenous variable to the response variable through the influence of a mediator variable (Grace 2006). SEM also allows inclusion of latent variables, which are variables that are difficult or impossible to measure directly but can be defined from several measurable variables. In addition, SEM allows inference of causal factors (Shipley 2004, Grace 2006).

We constructed a model that represented the 4 hypotheses raised in our study. First, a latent variable was created to represent the urbanization factor. We used the following variables to define urbanization: water temperature, pH, DO, electrical conductivity, TDN, TDP, % deforested area, and TIA. The regression part of the model included the direct effects of microbial, fungal, and shredder biomass and water velocity on leaf-litter breakdown. Indirect effects of water velocity on the leaf-litter breakdown rate were included through mediated factors: fungal and shredder biomass. We also included indirect effects of fungal and microbial biomass through their effects on shredder biomass. Last, we included the effects of the latent variable (urbanization) on microbial, fungal, and shredder biomass.

We ran the SEM analysis using the lavaan package in R (version 0.4; R Project for Statistical Computing, Vienna, Austria; Rosseel 2012). In cases of poor fit of the model to the data, we removed variables to obtain a model that represented the data. This procedure was done carefully (Grace 2006), by first removing defining variables from the latent urban variable. Model fits were evaluated by the χ² test and its associated p-value. The χ² represents the difference between the observed data and the hypothesized model. Thus, an adequate model should have a low χ² statistic and a non-significant p-value. We also evaluated the fit of the models with the comparative fit index (CFI). This test compares each proposed model with an alternative baseline model. A common rule-of-thumb is that good models should have values >0.95 (maximum = 1) (Shipley 2004). The response and explanatory variables for each stream site are available in Appendix S1.

RESULTS

Environmental variables and the conservation status of stream sites

The 1st axis of the PCA based on environmental variables explained 70.76% of the total variation and separated streams along an urbanization gradient with the most-impacted streams scoring at the right of the ordination (Fig. 2A, B). Axis 1 was positively associated with high values of pH (r = 0.897), TDN (r = 0.874), TDP (r = 0.792), water temperature (r = 0.898), electrical conductivity (r = 0.946), % deforested area (r = 0.916), and TIA (r = 0.908). On the other hand, the least-impacted sites scored to the left of the ordination (Fig. 2A, B) and were associated with high DO (r = −0.930). Water velocity was associated with axis 2 (r = −0.960).
Leaf-litter breakdown
The leaf-litter breakdown rates in the studied streams ranged from 0.0086 to 0.0243/d in *C. trinervia* (tough leaves) and from 0.0071/d to 0.0386/d in *M. speciosa* (soft leaves) (Appendix S1). Leaf-litter breakdown of the 2 plant species tended to decrease with urbanization, although at different rates (analysis of covariance [ANCOVA]; leaf species × urbanization interaction: \( F_{1,78} = 21.30, p < 0.001 \)). The leaf-litter breakdown rates for *C. trinervia* (tough leaves) were lower than those for *M. speciosa* (soft leaves) in the least urbanized streams, but this pattern tended to reverse in the most urbanized streams (Fig. 3).

Invertebrate colonization
We collected 15,683 invertebrates in all litter bags of both plant species (33.4% were in *C. trinervia* bags). The number of invertebrate families ranged from 0 to 15 in *C. trinervia* and from 0 to 24 in *M. speciosa* bags. The relationships between urbanization and the number of invertebrate families in *C. trinervia* (\( R^2 = 0.52, F_{1,40} = 44.83, p < 0.001 \)) and *M. speciosa* (\( R^2 = 0.65, F_{1,3}= 73.74, p < 0.001 \)) were negative. Shredder biomass was negatively associated with the urbanization gradient in *C. trinervia* (\( R^2 = 0.07, F_{1,40} = 4.04, p = 0.051 \)) and *M. speciosa* (\( R^2 = 0.36, F_{1,39} = 23.30, p < 0.001 \)). We did not record shredders (*Triplectides* and *Phylloicus*) in the most urbanized streams. Instead, the invertebrate fauna in these streams were dominated by oligochaetes and dipterans (collector-gatherers).

Microbial colonization
Fungal biomass ranged from 83 to 392 µg/g AFDM in *C. trinervia*, and from 22 to 374 µg/g AFDM in *M. speciosa*. Fungal biomass was negatively associated with the urbanization gradient in *C. trinervia* (\( R^2 = 0.42, F_{1,40} = 30.01, p < 0.001 \)) and *M. speciosa* (\( R^2 = 0.24, F_{1,39} = 13.93, p = 0.001 \)). Microbial biomass ranged from 13 to 115 nmol/g AFDM in *C. trinervia* and was negatively associated with urbanization (\( R^2 = 0.12, F_{1,40} = 6.41, p = 0.016 \)). Microbial biomass ranged from 16 to 118 nmol/g AFDM in *M. speciosa* and was not associated with the urbanization gradient (\( R^2 = 0.02, F_{1,39} = 0.09, p = 0.769 \)).

Structural equation modeling
The original SEM was unable to properly describe the hypothesized causal relationships for both plant species (\( \chi^2 \) test, \( p < 0.05 \)). We simplified the model by dropping...
single environmental variables defining the latent urban variable. For *C. trinervia*, we obtained an adequate model fit ($\chi^2 = 43.39, \text{df} = 38, p = 0.252, \text{CFI} = 0.98$; Fig. 4) by dropping electrical conductivity and % deforested area. In the case of *M. speciosa*, a good model fit was obtained only after dropping electrical conductivity and % deforested area from the latent urban variable and removing microbial biomass ($\chi^2 = 39.83, \text{df} = 31, p = 0.133, \text{CFI} = 0.97$; Fig. 5).

The SEM for *C. trinervia* indicated that fungal biomass was the exogenous variable with the greatest direct effect on leaf-litter breakdown rate (path coefficient = 0.65, $p < 0.001$; Fig. 4, Table 1). Water velocity affected leaf-litter breakdown rate negatively by its indirect effect on fungal biomass (indirect effect = –0.19, $p = 0.030$). Shredder and microbial biomass did not affect leaf-litter breakdown rate of *C. trinervia*. The latent urban variable had a strong effect on fungal biomass (path coefficient = –0.62, $p < 0.001$), indirectly affecting leaf-litter breakdown rate (sum of indirect effects = –0.49, $p = 0.001$; Table 1).

The SEM that described the causal relationships for *M. speciosa* indicated that shredders (path coefficient = 0.47, $p < 0.001$) and fungal biomass (path coefficient = 0.33, $p = 0.008$) were the only direct effects on leaf-litter breakdown rate (Fig. 5, Table 1). Water velocity did not have a direct (path coefficient = –0.02; $p = 0.884$) or indirect (path coefficient = –0.09, $p = 0.220$) significant effect on leaf-litter breakdown rate of *M. speciosa*. The urban latent variable negatively affected shredders (path coefficient = –0.58, $p < 0.001$) and fungal biomass (path coefficient = –0.52, $p < 0.001$), resulting in a net negative effect on leaf-litter breakdown rate (sum of indirect effects = –0.52, $p < 0.001$; Table 1).

**DISCUSSION**

**Urbanization and leaf-litter breakdown**

We observed lower rates of leaf-litter breakdown of *M. speciosa* and *C. trinervia* in highly urbanized streams. In these streams, litter bags did not harbor shredders, whereas *Phylloicus* and *Triplectides* occurred in less urbanized streams. Thus, our results indicate that these caddisflies are sensitive to urban impacts (high concentrations of P and N, low availability of $O_2$, high water temperature, high TIA, and removal of riparian vegetation) (Schueler 1994, Couceiro et al. 2007). Increases in P and N resulting from environmental degradation usually are accompanied by a reduction in $O_2$ and increases in other variables (e.g., conductivity, pesticides; Woodward et al. 2012). These changes can reduce shredder abundance and concomitantly slow leaf-litter breakdown (Woodward et al. 2012). In our most urbanized streams, invertebrate communities were composed mostly of chironomids (preponderantly *Chironomus* spp.) and oligochaetes, which usually are present in high densities in the detritus. However, these organisms do not directly affect leaf-litter breakdown be-
Table 1. Correlation coefficient ($r$), and standardized path coefficients for direct (d), indirect (i) and total (e) effects for the structural models of Coussapoa trinervia and Mabea speciosa leaf-litter breakdown rate ($k$) in 42 streams in Manaus, Central Amazonia. * indicates significant $p$-value ($p < 0.05$). In $M$. speciosa, only the correlation coefficient for microbial biomass is shown because this variable was not included in the structural model.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Explanatory variable</th>
<th>$r$</th>
<th>d</th>
<th>i</th>
<th>$e = d + i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C$. trinervia $k$</td>
<td>Fungal biomass</td>
<td>0.58</td>
<td>0.65*</td>
<td>&lt;0.01</td>
<td>0.67*</td>
</tr>
<tr>
<td></td>
<td>Shredder biomass</td>
<td>0.07</td>
<td>-0.05</td>
<td>-</td>
<td>-0.05</td>
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<tr>
<td></td>
<td>Microbial biomass</td>
<td>0.11</td>
<td>0.07</td>
<td>&lt;0.01</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Water velocity</td>
<td>-0.13</td>
<td>0.22</td>
<td>-0.19*</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Urbanization</td>
<td>-</td>
<td>-</td>
<td>-0.49*</td>
<td>-0.49*</td>
</tr>
<tr>
<td>$M$. speciosa $k$</td>
<td>Fungal biomass</td>
<td>0.50</td>
<td>0.33*</td>
<td>0.03</td>
<td>0.36*</td>
</tr>
<tr>
<td></td>
<td>Shredder biomass</td>
<td>0.59</td>
<td>0.47*</td>
<td>-</td>
<td>0.47*</td>
</tr>
<tr>
<td></td>
<td>Microbial biomass</td>
<td>-0.11</td>
<td>-</td>
<td>-0.11</td>
<td></td>
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<tr>
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<tr>
<td></td>
<td>Urbanization</td>
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<td>-</td>
<td>-0.52*</td>
<td>-0.52*</td>
</tr>
</tbody>
</table>

cause they feed mainly on fine particulate organic matter (Walsh et al. 2005). Landeiro et al. (2008) observed that free-living chironomids were not associated with leaf breakdown of $M$. speciosa. However, leaf-mining chironomids were positively associated with leaf breakdown (Landeiro et al. 2008). We did not find chironomids inside the leaf matrix in the most urbanized streams, reinforcing the low importance of these organisms in leaf-litter breakdown in urban streams.

Despite a general negative effect of urbanization on leaf-litter breakdown, our results indicate that the mechanisms were not identical for the 2 plant species studied. For $C$. trinervia (tough leaves), the negative effects of urbanization on leaf-litter breakdown were mediated by decreased fungal biomass. This mechanism also was important for $M$. speciosa. However, for $M$. speciosa, urbanization also affected leaf-litter breakdown by reducing shredder biomass. Thus, in the least urbanized streams, leaf-litter breakdown of $M$. speciosa was mediated by both fungi and shredders, resulting in faster leaf-litter breakdown than observed for $C$. trinervia. However, in the most urbanized streams, the lack of shredders caused the 2 litter species to have more similar breakdown rates.

We also observed a negative relationship between fungal and microbial biomass with urbanization. Several authors have observed increased microbial biomass and high breakdown rates in environments enriched with inorganic nutrients, especially N and P (Pascoal et al. 2001, 2003, 2005, Gulis and Suberkropp 2003). However, we found the lowest leaf-litter breakdown rates in the most urbanized streams, where nutrient concentrations were high. This finding indicates that urbanization can impair the functioning of ecosystems by altering microbial and invertebrate community structures. Our results should be interpreted by taking into account variables associated with general stream condition.

For instance, the high values of nutrients (P and N) in the most urbanized streams were associated with low DO values ($r = -0.78$). Low O$_2$ availability inhibits the positive stimulus of N on microbial activity and biomass and can exclude some species of aquatic hyphomycetes (Pascoal and Cássio 2004, Medeiros et al. 2009, Krauss et al. 2011). Moreover, a potential additional negative influence of urban waters on leaf-litter breakdown rates is the deleterious effects of NH$_4^+$ on invertebrate taxa (Lecerf et al. 2006, Woodward et al. 2012).

**Shredder effects on leaf-litter breakdown**

Shredders were not important for leaf-litter breakdown of $C$. trinervia. This species has hard leaves that may have prevented colonization by shredders. Previous investigators of temperate and tropical streams have found weak effects of shredders on tough leaves (Rincón and Martínez 2006, Li and Dudgeon 2008, Graça and Cressa 2010). Tough leaves usually require long periods of conditioning by microorganisms before they become attractive to shredders (Gonçalves et al. 2006). In contrast, shredders exerted a strong direct effect on $M$. speciosa leaf-litter breakdown despite the low abundance and biomass of shredders. In previous studies of leaf-litter breakdown of $M$. speciosa in the same region, this species was colonized rapidly by shredders and had rapid leaf-litter breakdown (Landeiro et al. 2008, 2010). We observed that leaf toughness influenced the effects of shredders on leaf-litter breakdown of the 2 species. On the other hand, Treplin and Zimmer (2012) studied leaf-litter breakdown in aquatic and terrestrial environments and found that detritus quality (according to breakdown rates and nutritional value) was not important for the breakdown process in the presence of decomposers (shredders and grazers). Moreover, in the absence of decomposers, leaf quality tended to be less important in aquatic than in terrestrial environ-
ments because of leaching of soluble components. We found that shredders were rare on the tough leaves and that the breakdown rates of such leaves were low. A possible explanation is that the duration of the experiment (30 d) was not sufficient to condition C. trinervia. In a study by Ligeiro et al. (2010), the duration of exposure was the principal factor explaining invertebrate colonization during the leaf-breakdown process.

**Fungal and microbial effects on leaf-litter breakdown**

We observed that fungal biomass tended to increase the breakdown rate of the soft leaves of M. speciosa, but the magnitude of the effect was lower than that exerted by shredders. However, for the tough leaves of C. trinervia, shredders had no effect, and fungi carried out most of the leaf-litter breakdown. Previous investigators have shown that fungi play a dominant role in the breakdown of detritus and can represent up to 96% of total microbial biomass during this process (Baldy et al. 1995, Findlay et al. 2002, Das et al. 2007). In fact, most investigators who quantified fungal biomass recorded positive relationships with leaf-litter breakdown rates (Pascoal and Cássio 2004, Imberger et al. 2008, Lecerf and Chauvet 2008). In tropical streams, some data indicate that shredders are poorly diversified and not abundant and that leaf-litter breakdown in these streams is carried out preponderantly by fungi, organisms capable of degrading complex polysaccharides (Irons et al. 1994, Dobson et al. 2002). Our study was restricted to 2 species, but our results for C. trinervia support the view that fungi can compensate for a low abundance or absence of shredders during leaf-litter breakdown.

Microbial biomass was not important in the leaf-litter breakdown of either plant species. ATP values can represent the biomass of various microorganisms, including fungi. Several investigators have used the relationship between ATP and ergosterol to obtain fungal participation in microbial biomass (Suberkropp et al. 1993, Ruzicka et al. 2000, Abelho 2009). We recorded a low association between ATP and ergosterol concentrations (r < 0.1) for both plant species. Accordingly, fungi were not the principal group of microorganism in the total microbial biomass (measured as ATP) in our study. After 46 d of leaf submersion, Abelho (2009) found a weak relationship (r = 0.04) between ergosterol and ATP and attributed this result to greater importance of bacteria and other nonfungal microorganisms in the microbial biomass.

In a nutrient-enriched river with low values of DO and high sedimentation, Pascoal et al. (2005) observed low fungal activity and increased bacterial production. We did not observe a positive relationship between microbial biomass and stream urbanization. However, in the most urbanized streams, substitution of fungi by bacteria in the microbial communities may have occurred, as indicated by our low relationship between ergosterol and ATP. These results are similar to those of Quintão et al. (2013) who found low fungal biomass and an increase in facultative anaerobic bacteria biomass in eutrophic waters. Thus, increased importance of bacteria in urbanized streams (Paul and Meyer 2001) may indicate changes in ecological processes in these ecosystems. Moreover, we think that the relationship between ergosterol and ATP can be an important tool in assessing health of tropical stream ecosystems.

In contrast to our hypothesis, fungal and microbial biomass did not affect leaf-litter breakdown indirectly through positive effects on shredders. Fungi and bacteria can degrade leaf detritus and immobilize dissolved N and P from the water column, making leaf litter more palatable to shredders (Gessner et al. 1999). Plausible explanations for the absence of these relationships may be the nature of the leaf species studied. Cossapoa trinervia is a plant species with tough leaves that few shredders were able to colonize. Thus, the potential increase in palatability apparently was not enough to enable high-level colonization of the species by shredders. On the other hand, the higher densities of shredders colonizing the soft leaves of M. speciosa (~3x more than C. trinervia) may have occurred independently of fungal colonization. This speculation is based on the finding of Landeiro et al. (2008) that this species was colonized by shredders 1 d after submersion. In addition, these authors observed that leaves of this species are used intensively by Phylloicus to construct their leaf cases.

**Water velocity effects on leaf-litter breakdown**

Water velocity, in general, has a positive effect on leaf-litter breakdown by increasing physical fragmentation (Paul et al. 2006, Dewson et al. 2007). However, in our study, its direct effect was weak for C. trinervia and absent for M. speciosa. Our results should be interpreted cautiously because detritus in the most urbanized streams was buried by fine sediment and, thus, not subjected to water flow over the substrate surface (Sponseller and Benfield 2001, Navel et al. 2010). Moreover, the detritus burial may in part have been a result of the method used because under natural conditions, a portion of the detritus would be carried downstream and deposited at the surface of the substrate.

Water velocity also can affect leaf-litter breakdown by its indirect effect on organisms. For instance, increased water velocity can increase oxygenation and turbulence, which positively affect the production of conidia, fungal biomass, and the number of fungal species, and in turn, may increase leaf-litter breakdown (Ferreira and Graça 2006, Schlief and Mutz 2009). However, we found a negative effect of water velocity on fungal biomass for C. trinervia and unimportant effects for M. speciosa. Again, these unexpected effects probably are a result of burying of detritus as a consequence of urbanization.

A 2nd indirect effect of water velocity is on the biomass of shredders. We did detect weak negative effects of water velocity on shredders. These negative effects probably are related to behavior of 2 shredder genera from the study.
region. Generally, species of *Phylloicus* and *Triplectides* are found in both riffles and pools, but they occur at high densities only in pools (Prather 2003, Landeiro et al. 2008, 2010).

**Conclusions**

We conclude that, in urban streams, the positive effect of shredders on leaf-litter breakdown may depend on the plant species. On the other hand, fungal biomass showed positive effects on the leaf-litter breakdown of both plant species. However, the effects of shredders and fungi were negatively modulated by the effects of urbanization, which reduced physical abrasion by burying detritus in the impacted streams. Last, urbanization of urban streams, which reduced physical abrasion by burying detritus in the impacted streams. Last, urbanization of urban streams, which reduced physical abrasion by burying detritus in the impacted streams. Last, urbanization exerted strong negative effects on leaf-litter breakdown by reducing the biomass of shredders and fungi in the Amazonian streams.

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