Limnetica, 34 (1): 29-40 (2015). DOI: 10.23818/limn.34.03
 ISSN: 0213-8409

Macroinvertebrate identity, not diversity, differed across patches differing in substrate particle size and leaf litter packs in low order, tropical Atlantic forest streams

Manuel A. S. Graça[1,*], Wander R. Ferreira[2], Kele Firmiano[2], Juliana França[2] and Marcos Callisto[2]

[1] MARE-Marine and Environmental Sciences Centre. Dep. Life Sciences, University of Coimbra, Portugal.
[2] Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Biologia Geral, Laboratório de Ecologia de Bentos, CP. 486, CEP. 31270-901, Belo Horizonte, MG, Brazil.

[*] Corresponding author: mgraca@ci.uc.pt

Received: 02/07/2014 Accepted: 05/11/2014

ABSTRACT

Macroinvertebrate identity, not diversity, differed across patches differing in substrate particle size and leaf litter packs in low order, tropical Atlantic forest streams

The size of streambed sediment particles is an important determinant of habitat complexity for stream-dwelling invertebrates. In tropical streams we tested if, at the patch scale, sandy substrates were poorer in organisms and taxa when compared with larger grain-size substrates in three experiments: (a) sampling patches with coarse and fine substrates and leaf packs in eight low-order streams, (b) manipulating substrate size at the patch scale in a low-order stream and (c) sampling coarse and fine sediment patches in a larger river. We also investigated the abundance of aquatic hyphomycete conidia in these streams to relate shredder abundance with the presence of fungal decomposers, given that tropical streams are frequently reported as being poor in shredders. In the three experiments, there were consistently no differences in terms of the family richness or total number of invertebrates across the substrate types, but taxa identity differed across substrates. Invertebrate numbers and taxa were positively correlated with current velocity. Macroinvertebrate densities (150-300 individuals/m^2) were lower than those reported in the literature (500->1500 individuals/m^2), but the number of taxa was high (37 families observed, 52 estimated). Shredders comprised 13% of all individuals and 11% of all taxa. The low number of shredders contrasted with the presence of aquatic hyphomycete spores in stream waters and in leaves sampled from the streams. We concluded that the high number of taxa and low number of individuals buffer changes in substrate, allowing the presence of invertebrates even in the less-favorable substrates (sand). Although litter and aquatic hyphomycetes were present in these streams, shredders were poorly represented; this raises the question as to why litter consumers are scarce when resources are abundant in forested tropical streams.

Key words: Sedimentation, hyphomycetes, litter, headwater streams.

RESUMEN

La identidad de los macroinvertebrados, no su diversidad, difirió entre parcelas de sustrato con diferente tamaño de partículas y paquetes de hojarasca en arroyos forestados de orden bajo en la zona tropical Atlántica

La distribución granulométrica del sustrato determina de forma importante la complejidad del hábitat para los invertebrados. En arroyos tropicales comprobamos si, a escala de microhábitat, los sustratos arenosos son más pobres en número de organismos y diversidad de taxones que los sustratos de partículas más grandes, a partir de tres experimentos: (a) muestreo de secciones con sustratos gruesos y finos y paquetes de hojas en ocho ríos de cabecera, (b) manipulación del sustrato a pequeña escala en un arroyo y (c) muestreo de parcelas con sustrato grueso y fino en un río de mayores dimensiones. También se investigó la abundancia de conidios de hifomicetos acuáticos, para relacionar la abundancia de insectos fragmentadores con la presencia de hongos descomponedores, ya que con frecuencia se hace referencia a que los ríos tropicales son pobres en insectos fragmentadores. Los resultados de los tres experimentos fueron coherentes, mostrando la ausencia de diferencias en cuanto a riqueza de familias y número total de invertebrados entre los diferentes tipos de sustrato, pero se encontró que

diferentes taxones colonizaban diferentes sustratos. El número de invertebrados y taxones se correlacionaron positivamente con la velocidad de la corriente. Las densidades de macroinvertebrados (150-300 individuos/m^2) fueron inferiores a los reportados en la literatura (500->1500 individuos/m^2), pero la riqueza de taxones fue alta (37 familias observadas, 52 estimadas). Los fragmentadores comprendían el 13% de la abundancia total y el 11% de todos los taxones. El bajo número de fragmentadores contrasta con la presencia de conidios de hifomicetos acuáticos en el agua de los arroyos y en las hojas analizadas. Se concluye que el elevado número de taxones y el bajo número de individuos permite la presencia de invertebrados en todos los sustratos, incluso en los menos favorables (arena). A pesar de la presencia de hojarasca e hifomicetos acuáticos en estos arroyos, los fragmentadores estaban poco representados, lo que suscita la pregunta del porqué de su baja abundancia cuando los recursos no son escasos en estos arroyos.

Palabras clave: *Sedimentación, hifomicetos, hojarasca, ríos de cabecera.*

INTRODUCTION

Low-order streams are numerically abundant in watersheds and very important in terms of allochthonous organic matter processing (Abelho, 2001; Downing *et al*., 2012) conducted by specific biota, with a predominance of shredder detritivores, consumers of fine particulate organic matter (Benfield & Webster, 1985; Graça *et al*., 2001) and aquatic hyphomycetes (Bärlocher, 1992; Graça *et al*., 2001). Invertebrate and fungal consumers promote the energy transference from leaf litter into the biotic compartments, including other aquatic invertebrates, fish, and terrestrial consumers feeding on emergent insects.

Given the ecological importance of low-order streams, it is important to understand factors controlling local community structure and functional processes. One of the important factors in this context is substrate particle size (Allan & Castillo, 2007). At the patch scale, coarse inorganic sediments are spatially more complex than fine sediments (Barnes *et al*., 2013), retain more coarse particulate organic matter (CPOM) (Flores *et al*., 2013; Larrañaga *et al*., 2003) and are colonized by a larger biomass of microalgae (Allan & Castillo, 2007); both CPOM and microalgae can be used as food resources by macroinvertebrates (Graça *et al*., 2004; Jun *et al*., 2011). Therefore, litter packs in coarse sediments are expected to house higher numbers of macroinvertebrates than homogeneous substrates such as sand and gravel (e.g., Buendia *et al*., 2011). Large numbers of macroinvertebrates in coarser substrates are also likely to result in high diversity because of sampling effects due to the positive relationship between the number of individuals and the number of taxa (Graça *et al*., 2004). Additionally, macroinvertebrate species differ in their substrate preferences and different species are known to colonize different habitats (e.g., Sarr *et al*., 2013; Vasconcelos & Melo, 2008).

Several human practices such as agriculture and forestry, as well as the construction of pastures, may result in erosion and the consequent accumulation of fine sediments in low-order streams (Larsen *et al*., 2009; Von Bertrab *et al*., 2013; Wood & Armitage, 1997). The biological consequences of fine sediment accumulation include reduced invertebrate density and diversity (Jones *et al*., 2012; Larsen *et al*., 2011) and reduced retentiveness of coarse particles of organic matter (Larrañaga *et al*., 2003). Low retentiveness may result in changes in the energy used by the biota: with less coarse particulate organic matter and biofilm on coarse substrates, the biota is expected to shift from shredder-scraper-dominated assemblages to collector-gatherer assemblages.

The first aim of our study was to relate invertebrate community structure in tropical secondary forest headwater streams with substrate grain size. Our first question was to test if, at the patch scale, sandy substrates were poorer in organisms and taxa when compared with larger grain size substrates. This hypothesis was tested with three experiments: (a) comparison of macroinvertebrate abundance and diversity in natural sandy and stony substrate reaches in eight streams; (b) substrate manipulation at the patch

scale in one stream (creation of patches with fine and coarse inorganic substrate particles); and (c) comparison in macroinvertebrate abundance and diversity in sandy and stony substrates in a larger river dominated by sand.

Our second question addresses the presence and abundance of aquatic hyphomycetes in the same low-order streams and its relationship with shredders abundance. Fungi are facilitators of leaf litter ingestion by shredders and therefore an important element in energy transference from the litter pool to the biotic compartment (Gessner & Chauvet, 1994; Pérez *et al*., 2011). Some reports suggest that a number of tropical streams are poor in both aquatic hyphomycetes and shredders (Jabiol *et al*., 2013; Wantzen *et al*., 2008). The absence or paucity of aquatic hyphomycetes may result in low resource quality for shredders and therefore low abundance and diversity of shredders and slow decomposition.

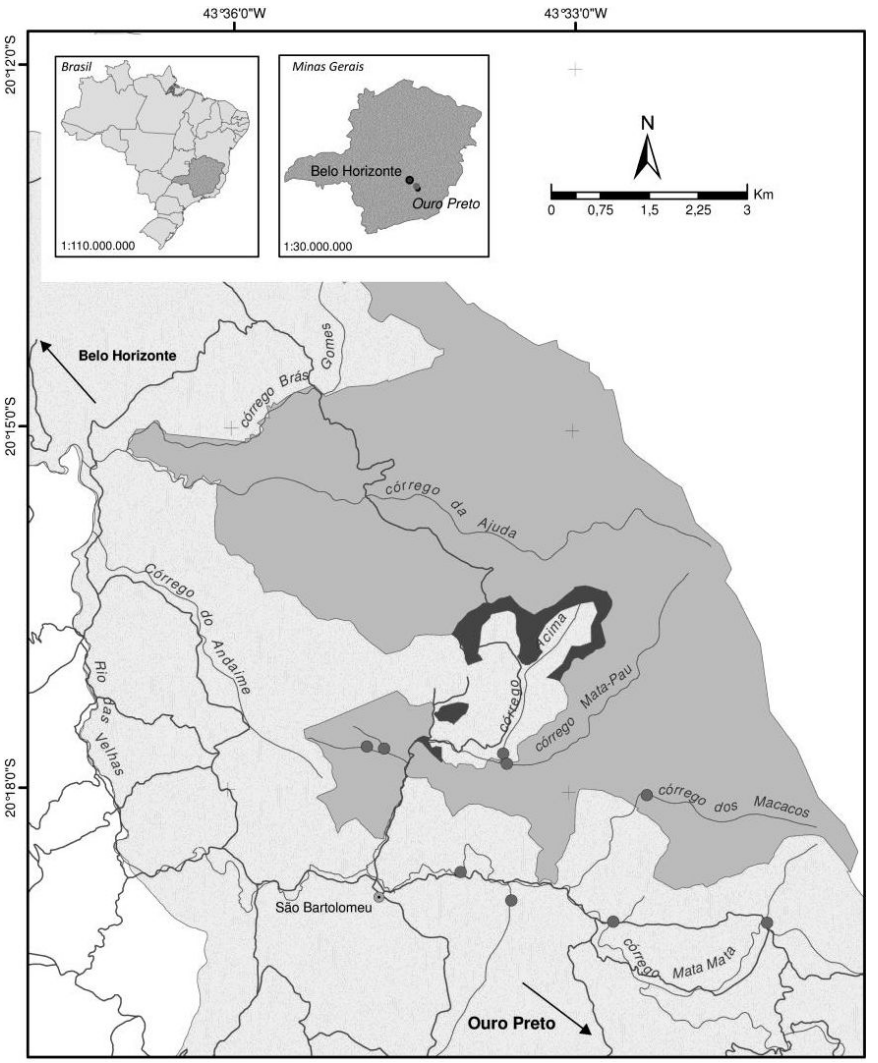

Figure 1. Location of sampling sites (dark dots) within the conservation unit. Light grey, APA Estadual Cachoeira das Andorinhas; medium grey, Uaimii Estadual Forest; dark grey, Particular forest of Natural Patrimony RPPN. *Lugares de muestreo (puntos negros) en la unidad de conservación. Gris claro, APA Estadual Cachoeira das Andorinhas; gris medio, Floresta Estadual Uaimii; gris oscuro, Floresta Particular Património Natural RPPN.*

We therefore quantified aquatic hyphomycete spore abundance in stream waters and sporulation rates in a mixture of naturally occurring leaves in the eight streams.

MATERIALS AND METHODS

Macroinvertebrate abundance and diversity in relation to substrate particle size

To test the hypothesis that sandy substrates in headwater streams are species poor when compared with coarse substrates, we sampled eight headwaters in the Environmental Protection Area of Andorinhas and State Forest UAMII forests (Velhas watershed, MG, Brazil; Fig. 1; Table 1); no human settlements were located upstream of the sampling zones (experiment 1). The streams were located at 20°(17′39″ – 19′05″)S, 43°(31′15″ – 34′47″)W. We selected streams with widths ranging between 1 and 2 meters and uniform depths that were mostly between 10 and 20 cm. In each stream, we sampled invertebrates in three discrete patches: fine substrates (< 2 mm diameter), stony substrates (> 30 mm diameter) and leaf packs (generally accumulated over shallow stony substrates); one sample of each type in each stream. A patch was defined as a uniform area covered by a Surber samples. Macroinvertebrates were sampled with a Surber net (30 × 30 cm; 0.25 mm mesh size). Samples were transported in an ice chest to the field station, and invertebrates were sorted alive several hours later, identified to the family level and preserved in 70% alcohol. Leaf packs collected in the Surber sampler were air dried in the field station and oven dried in the laboratory (60 °C) for 72 hours and weighed to the nearest 0.01 g. Grain size of substrates (dominant and accessory) was measured by placing handful samples of substrates over a plasticized graph paper in the location where invertebrates were sampled. Current velocity (current meter) was also measured in the place where Surber samples were collected. At each site, we also measured pH, conductivity and total suspended solids (YSI field probes). On the same day, we also determined oxygen (Winkler method) and total alkalinity (titration with H_2SO_4, 0.01 N to pH 4.5). Water samples (250 ml each) were collected, filtered (45 μm glass fiber filter) preserved in 1 ml of sulfuric acid (only P samples) and frozen for later analysis. Total nitrogen (N) was determined by the Kjedahl method, whereas total phosphorus (P) was determined by the ascorbic acid method (APHA, 2005).

The hypothesis that fine inorganic substrates are species poor in comparison with coarser substrates was also tested with substrate manipulation in one stream (stream 7, Mata Pau; Experiment 2). Sandy substrates were taken from deposition areas of a nearby larger river (Velhas) given the scarcity of large areas with this substrate in the Mata Pau stream. The fine substrates were passed through a 15 mm pore size sieve to eliminate large particles and the resulting material (0.5 to 12 mm in diameter), transported to the experimental stream and washed at the site to eliminate invertebrates and clay.

Table 1. Environmental parameters of eight low-order streams and the Velhas River in Minas Gerais, Brazil. *Parámetros ambientales de ocho arroyos y del río Velhas en Minas Gerais, Brasil.*

Stream	1	2	3	4	5	6	7	8	Velhas
Temperature (°C)	12.8	12.5	13.0	13.2	13.8	15.9	13.8	14.8	14.4
pH	7.1	7.0	7.2	7.3	7.7	7.7	7.8	7.4	7.8
Oxygen (mg/l)	8.1	8.9	9.0	8.7	9.3	9.5	9.1	9.4	9.8
Conductivity (μS/cm)	72.7	62.6	42.6	87.6	70.1	33.3	27.3	40.0	23.8
TDS (mg/l)	29.7	26.1	4.6	37.0	28.5	10.6	7.4	10.4	5.5
Alkalinity (μEq/l)	46.2	30.4	35.5	99.1	94.9	32.1	27.6	111.3	32.5
Total N (mg/l)	0.077	0.042	0.042	0.035	0.042	0.049	0.077	0.070	0.049
Total P (μg/l)	8.93	6.58	8.15	12.85	90.43	11.28	27.75	8.15	5.79

Ten circular aluminum trays (19 cm diameter, 4 cm high) were implanted into the streambed and filled with sand. Ten additional trays were filled with cobbles (diameter range 40-65 mm) taken from the same stream (washed to eliminate invertebrates). A full hand of sand was added to the cobble trays to fill the interstitial spaces among cobbles. After three days, the trays were recovered in a Surber sampler placed downstream of the tray. Samples were transported to the field station, sorted and identified as previously described. According with the literature, maximum invertebrate numbers in substrates incubated in streams are already attained by day 3 (Hofer & Richardson, 2007) day 4 (Mathooko, 1995; Olomukoro & Okologume, 2008) or day 5 (Miyake *et al*., 2003).

Because sandy substrate occupied < 10% of the area of the small streams, we also sampled a larger river (Velhas; Table 1; Experiment 3). Here, sandy substrate occupies nearly 75% of the river bottom. The wet channel was 5-10 m wide and maximum depth ~ 1 m. The river was mostly canopy covered. We collected 5 Surber samples in each of the sandy and stony substrate types at depths similar to the smaller streams, i.e., < 0.3 m. The samples and the invertebrates were treated as stated above. Water samples were also retrieved for chemical analysis as indicated in experiment 1.

Aquatic hyphomycetes survey

To evaluate the presence of aquatic hyphomycetes, water and litter samples were taken from the same eight streams referred to in experiment 1. At each site, we took 5 liters of water; the water was immediately transported to the field station and filtered (45 μm pore size). Given the high amount of suspended solids, only 500 ml was filtered to avoid clogging. Filters were preserved with lactophenol blue, and spores were counted under a microscope at 400 ×.

From each site, 20 leaves were randomly collected from the streambed and transported to the field station in plastic bags. From each leaf, a 10 mm diameter disc was cut with a core borer. Groups of 10 discs were transferred to 125 ml Erlenmeyer flasks with 40 ml of stream-filtered water. The Erlenmeyer flasks were placed on an orbital shaker at 110 r.p.m. At this rotation, the wave inside the Erlenmeyer flask was ~ 2 cm high. After 48 hours, the liquid was filtered, the filters were preserved, and the spores were counted as above. Finally, pieces from the same leaves were placed in 9 cm diameter petri dishes with stream water and maintained at the environmental temperature and photoperiod. After 24 hours and 48 hours, petri dishes were inspected for fungal spores.

Data analysis

Comparisons of the total number of invertebrates and total number of taxa among the three substrates (leaf packs, coarse and fine substrates; experiment 1) were performed by one-way ANOVA. To determine if the substrates differed in terms of invertebrate assemblages, we performed a Bray & Curtis similarity analysis among the 24 samples, followed by a multi-dimensional scale analysis (MDS) using Primer 6 (version 6.1.13) and Permanova+ (version 1.0.3) software (PRIMER-E Ltd; Lutton, UK). Differences among groups were tested by ANOSIM (Clarke & Warwick, 2001).

As an estimator of taxa richness, family richness per substrate (sand, pebbles and leaf packs) in the 8 samples streams was expressed in terms of collector curves in which samples were randomized (n = 1000), and the cumulative number of taxa estimated (Sobs rarified Mau Tau) in each run using EstimateS software, version 8.2 (Colwell, 2006). Indicator value analysis (IndVal) was performed to identify representative taxa for each treatment (Dufrene & Legendre, 1997) using R software, package labdsv (Roberts, 2013). The analysis used the frequency and relative abundance of taxa in each treatment. The IndVal values obtained in each treatment were tested for statistical significance using a Monte Carlo procedure (10 000 permutations). Correlations between the number of invertebrates and number of families and between the number of invertebrates and current and dominant particle size were performed with Spearman Rank correlation.

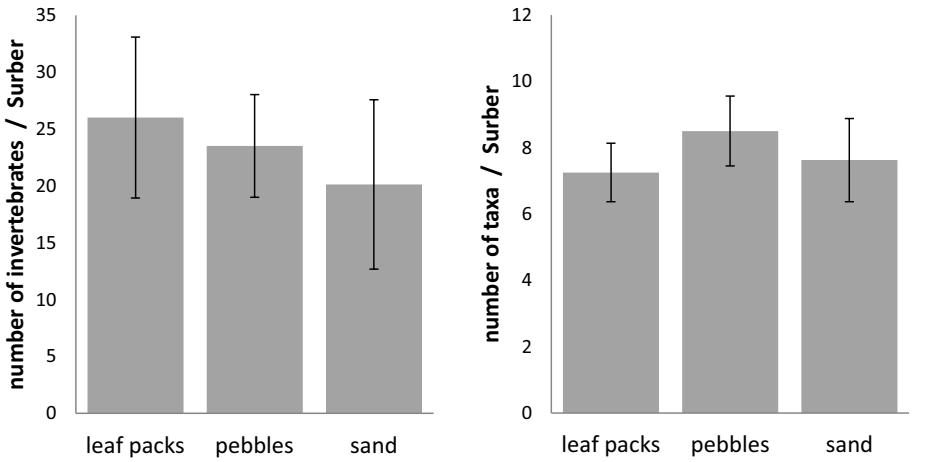

Figure 2. Number of invertebrates and number of taxa in three substrate types in eight low-order streams in São Bartolomeu, MG, Brazil (mean ± standard error). *Número de macroinvertebrados y de taxones, en tres tipos de sustratos, en ocho ríos de bajo orden en São Bartolomeu, MG, Brasil (medias ± error standard).*

Comparisons of taxa and invertebrate numbers between sand and stony substrates in experiments two and three were performed using a t-test. Comparison of proportions of functional feeding groups in the three substrates across the eight streams (experiment 1) was done by one way ANOVA or Kruskal-Wallis ANOVA when the data were not normally distributed (data was arc-sin transformed; chironomidae were excluded because of feeding uncertainty). Comparinson of proportion of functional feeding groups between the two substrates in the Velhas river (experiment 3) was done by t-test (or Mann-Whitney when data were not normally distributed). Here again data was arc-sin transformed. Aquatic hyphomycetes were not subjected to statistical treatments because most samples had zero counts.

RESULTS

Streams sampled in the experiment 1 were circumneutral (pH 7.0-7.8), with low conductivity (23.8-87.6 µS/cm), full canopy cover and shallow (depths < 15 cm; Table 1). The diameter of the dominant particles in the stony substrates across streams ranged from 20 to 80 mm, whereas it was < 2 mm in the fine sediment areas. Litter samples (experiment 1) contained 27 ± 3 g of leaves (SE)/Surber sample (approx. 300 ± 36 g/m^2). Water conductivity, alkalinity, nitrogen and phosphorus were all very low across all sampled streams. In the same way, oxygen levels were also near saturation.

Macroinvertebrate abundance and diversity in relation to substrate particle size

In the eight tested streams, we collected 557 invertebrates classified in 37 families. This corresponded to a density of 258 individuals/m^2. The most abundant taxa were Perlidae (15%), Leptophlebiidae (13%), Elmidae (12%), Leptohyphidae (7%), Tipulidae (7%) and Chironomidae (6%). The mean invertebrate abundance in leaf packs, coarse substrates and fine substrates was 26, 24 and 20 /sampling unit (Surber 0.09 m^2), respectively, with no differences among substrates (F = 2.035; D.F. = 2; p = 0.362; Fig. 2). Taxa richness in leaf packs, coarse substrates and fine substrates were 7, 9 and 8, respectively, with no differences among substrates (F = 0.352; D.F. = 2; p = 0.704; Fig. 2). Taxa richness and invertebrate abundance were correlated (Pearson, r^2 = 0.71, n = 24; p < 0.001); the number of invertebrates was positively correlated with the current velocity (r^2 = 0.526, p = 0.008; n = 24; range 0.05-0.72 m/s). Densities of macroinvertebrates were relatively low, but the number of

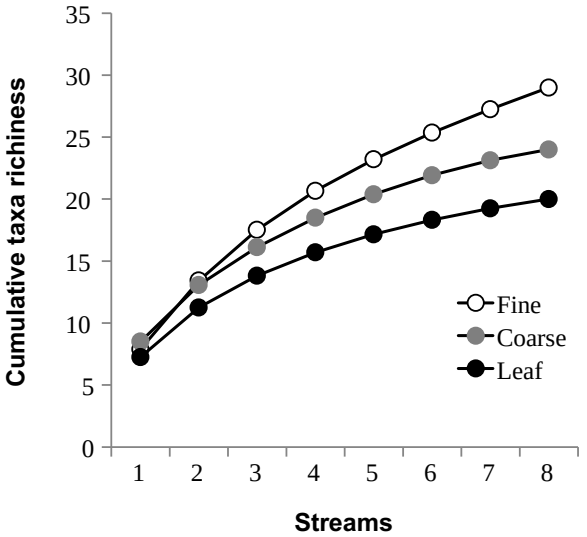

Figure 3. Accumulation curves (Mao Tau) of observed macroinvertebrate richness based on eight streams in São Bartolomeu in three substrate types: fine grain size sediments, coarse grain size sediments and leaf packs. *Curvas de acumulación (Mao Tau) de la riqueza de taxones de macroinvertebrados observada en ocho arroyos en São Bartolomeu en tres tipos de sustrato: sedimentos finos, sedimentos gruesos y paquetes de hojas.*

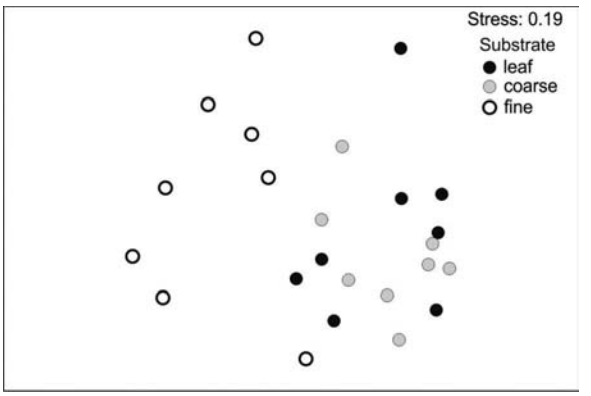

Figure 4. MDS of invertebrate samples taken from leaf packs (L), coarse (C) and fine (F) substrates. *Ordenación MDS de muestras de invertebrados recolectados en depósitos de hojarasca (L), partículas gruesas de sustrato (C) y partículas finas de sustrato (F).*

families was high. Accumulation curves for any of the substrates did not reach asymptote (Fig. 3).

Samples from the sandy substrates differed from the others in terms of taxonomical composition (MDS, ANOSIM; Stress values = 0.19; global R = 0.278; $p < 0.002$; Fig. 4). This was due to the frequency of some taxa in particular substrates; this was the case of Gomphidae, Leptohyphidae and Ceratopogonidae, which were associated with sandy substrates; Leptophlebiidae and Coenagrionidae were associated with cobble substrates, and Perlidae and Hydropsychidae were associated with litter and cobbles when both substrates pooled (Table 2). In terms of functional feeding groups, globally the most abundant were collectors (53%; 10 families), followed by predators (33%; 19 families), shredders (13%; 4 families) and scrapers (1%; 1 family). No estatistics differences were observed across the three substrate types, except for predators, which were proportionally more abundant in leaf packs than in sand ($F = 4.798$; $p = 0.019$).

In experiment 2, we manipulated substrate particle size in patches in a low-order stream. Globally, 55 invertebrate specimens distributed into 14 taxa were sampled after three days, corresponding to 194 individuals/m^2 (vs. 156 individuals/m^2 calculated for the stony substrate in the same river). Hydropsychidae (13%) occurred only in sandy substrates, whereas Helicopsychidae (15%), Leptophlebiidae (15%) and Perlidae (22%) occurred only in stony substrates. Together, these taxa comprised 65% of the sampled invertebrates in the manipulated patches. Sand and cobbles did not differ in terms of individuals/sample (mean = 2.8; t = 0.992; $n = 20$; $p = 0.369$) or taxa/sample (mean = 2.1; t = 0.608, $n = 20$, $p = 0.551$). The mean current velocity ranged from 0.06 to 0.28 m/s, with no difference between substrates (t = 1.047; $n = 20$; $p = 0.309$).

Experiment 3: invertebrates in sandy and stony substrates in a larger river

In experiment 3, we sampled fine and coarse substrates in a larger river where sandy substrates cover a large area. A total of 192 macroinvertebrates was collected in the ten Surber samples. The mean densities of invertebrates in stony and sandy substrates were 18 and 21 individuals per Surber sampler respectively, with no significant differences between substrates (t = 0.402, $n = 10$, $p = 0.799$), whereas the number of taxa was 6 in both substrates (t = 0.122, $n = 10$, $p = 0.906$).

However, assemblages colonizing both substrates were significantly different (MDS, ANOSIM, Stress = 0.05; R global = 0.322; $p = 0.008$). Chironomidae (~ 11%) and Gomphidae (7%) occurred exclusively in the sandy substrate, whereas Simuliidae (9%) occurred only in the stony substrate. Baetidae (31%) and Leptophlebiidae (15%) occurred in both substrates. These taxa comprised 73% of the sampled invertebrates; the remaining invertebrates were distributed among 23 taxa, with < 5% of the sampled invertebrates in each taxa.

In terms of functional feeding groups, the most abundant groups were collectors (65%; 6 families), followed by predators (28%; 12 families) and shredders (7%; 4 families), but no differences in functional feeding groups occurred between the two substrates ($0.469 > p > 0.056$).

Aquatic hyphomycetes survey

The densities of spores in the water column ranged from zero (streams 2 and 4) to ~ 1350/l (stream 5; Fig. 5). The sporulation rates in leaf substrates incubated in the field station for 2 days ranged from 100 to 7500 spores cm^{-2} of leaf material day^{-1}. Almost all spores belonged to a single species cf. *Lunulospora curvura*. Occasionally, other tetra radiate spores occurred as well as a species similar to *Hesliscus submersus*, an unidentified sigmoid and cf. *Tripospermum*. There was no relation between spore counts

Table 2. Indicator taxa according to the IndVal method (Dufrêne & Legendre, 1997). *Taxones indicadores de acuerdo con el método de IndVal (Dufrêne & Legendre, 1997)*

Three substrates in 8 streams			
Taxa	Substrate	*p*	IndVal
Gomphidae	Sand	0.004	0.625
Leptophlebiidae	Cobbles	0.009	0.639
Coenagrionidae	Cobbles	0.012	0.568
Leptohyphidae	Sand	0.021	0.500
Ceratopogonidae	Sand	0.021	0.500
Tipulidae	Litter	0.062	0.534
Cobbles + Litter treated as a single substrate in 8 streams			
Taxa	Substrate	*p*	IndVal
Perlidae	C+L	0.001	0.795
Gomphidae	Sand	0.002	0.644
Hydropsychidae	C+L	0.005	0.688
Leptohyphidae	C+L	0.006	0.500
Ceratopogonidae	Sand	0.007	0.500
Leptophlebiidae	C+L	0.027	0.608
Elmidae	C+L	0.036	0.644
Corydalidae	C+L	0.053	0.438
Sediment manipulation in Mata Pau stream			
Taxa	Substrate	*p*	IndVal
Hydropschidade	Sand	0.010	0.600
Helicopschidae	Cobbles	0.034	0.500
Perlidae	Cobbles	0.034	0.583
Velhas River			
Taxa	Substrate	*p*	IndVal
Chironomidae	Sand	0.016	0.952

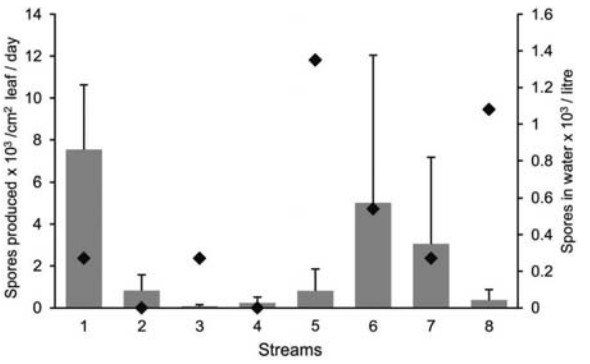

Figure 5. Spore production (mean and SD) of natural leaf substrates sampled from eight streams in the São Bartolomeu forests (bars, left) and spore density in the water (dots, right). *Producción de conidios (media y DS) en substratos naturales de hojas, muestreadas en ocho arroyos en el bosque de São Bartolomeu (barras, izquierda) y densidad de esporas en el agua (puntos, derecha).*

in sporulation tests and in the stream waters ($r = 0.0859$, $p = 0.794$).

DISCUSSION

Invertebrate assemblages and substrates

Our main finding was that, unlike our initial hypothesis, fine substrates were not poorer in number of invertebrates and taxa richness than coarse substrates at the patch scale. However, differences were observed in the invertebrates inhabiting different substrate types. This observation was consistent across our three experiments. Our second finding was that aquatic hyphomycetes were generally present in submerged leaf substrates. We will first analyze the macroinvertebrate assemblages and then address the aquatic hyphomycetes and the potential relationship between both.

The first finding was unexpected and apparently in opposition to the literature because cobble substrates are structurally more complex than sand (Barnes *et al*., 2013) and therefore capable of housing a large number and variety of individuals when compared with sandy substrates, as confirmed by other studies (Graça *et al*., 2004; Vasconcelos & Melo, 2008).

The lack of differences may be related to the natural low densities of macroinvertebrates. The density of invertebrates in our eight low-order streams ranged from 150 to 300 individuals/m^2. These values were very low when compared with the literature: 500-3700 in alpine streams (Robinson & Doering, 2012), ~ 10000 (median of 177 samples) across Portugal (Graça *et al*., 2004), 1500-11 000 in Alaska (Wipfli *et al*., 1999) and up to 15 000 in Spain (Ortiz & Puig, 2007). Low numbers may indicate that local energetic resources are scarce or that environmental conditions are adverse and unpredictable. Hence, substrate heterogeneity will not be a limiting factor. Further studies should address the observed low densities and the resource levels (quantification of fine and coarse particulate organic matter and benthic algae).

Regarding the identity of invertebrates, caddisfly and mayfly assemblages colonized preferentially coarser, well-sorted substrates, whereas invertebrates living within substrate particles, such as many chironomids and oligochaetes, were more abundant in fine substrates. This pattern was consistent across the three experiments. This segregation of taxa across substrates was also reported in the literature. For example, Vasconcelos & Melo (2008) found that in sandy substrates, the number of invertebrates was lower than that of the control and that the community structure differed. Additionally, Larsen *et al*. (2009) and Buendia *et al*. (2011) reported that patches with fine and coarse sediments differed in invertebrate composition, with a lower EPT (Ephemeroptera, Plecoptera, Trichoptera) richness in fine sediments. In a similar experiment, Larsen *et al*. (2011) experimentally added fine sediments to trays for three weeks and observed a consequent reduction of some taxa such as mayflies and stoneflies, as well as a reduction in diversity.

Shredders and aquatic hyphomycetes

Regarding functional feeding groups, our results showed a low abundance of shredders as reported in other studies in the tropics (Wantzen & Wagner, 2006; Gonçalves *et al.*, 2007; Boyero *et al.*, 2011), with a predominance of collector-gatherer organisms in the low-order streams and the

Velhas River (higher order). This low abundance of shredders and distribution groups has been related to the nutritional quality of leaves (Tomanova *et al*., 2006; Ferreira *et al*., 2014). Regarding aquatic hyphomycetes, sporulation rates ranged from 5-10 conidia cm^{-2} day^{-1}, which is below the 150-500 reported by Artigas *et al*. (2008). Further comparisons are difficult because the results are normally expressed in terms of leaf dry mass (unavailable in our case because of field station restrictions) and because we used natural substrates; in the literature, measurements are generally performed in leaves incubated in rivers. Spore densities in the water ranged from 0 to 1-400 conidia/l, which is in the lower range of the reported from temperate streams: ~200-3500 in North America (Suberkropp & Wallace, 1992), 600-8500 in Australia (Kenneth, 1993), 1000-8000 in the UK (Iqbal & Webster 1973), and ~10-10000 in France (Laitung *et al*., 2002).

The main finding of this survey is that aquatic hyphomycetes were present in the streams where they are expected to decompose leaves, increasing their quality for consumers. However, we found few leaves in the advanced decomposition stage, which could be easily consumed by shredders. In nearby neotropical savanna streams, Gonçalves *et al*. (2006, 2007) also found a low biomass of aquatic hyphomycetes in submerged leaves. Further, other authors have reported low fungal biomass, sporulation and species diversity in other South American streams (Ferreira *et al*., 2012; Jabiol *et al*., 2013). The low abundance of aquatic hyphomycetes and invertebrates could be attributed to the low quality of leaves in the tropics in terms of chemical defenses (Wantzen *et al*., 2002) and leaf toughness (Graça & Cressa, 2010).

The low microbial colonization may result in low substrate quality for invertebrate consumption, limiting the resources available for invertebrates. Finally, given the high level of shade in this habitat, it is plausible that primary production in these streams is also low, but this hypothesis needs to be tested in future studies.

In summary, the sampled streams had a low density but high diversity of macroinvertebrates. Aquatic hyphomycetes were present in the submerged leaf litter, but shredders were not common. If these conclusions can be generalized to other systems in the region, two further questions need to be addressed: (1) why the invertebrate densities are low and (2) why shredders are uncommon.

ACKNOWLEDGEMENTS

The authors acknowledge CAPES (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*), CNPq (*Conselho Nacional de Desenvolvimento Científico e Tecnológico*), FAPEMIG (*Fundação de Amparo a Pesquisa do estado de Minas Gerais*) for financial support. MG was awarded an international visiting researcher grant by the CNPq (CNPq No. 400029/2013-8). MC was awarded a research productivity grant by the CNPq (CNPq No. 302960/2011-2) and a Minas Gerais researcher grant by FAPEMIG-Fundação do Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG PPM-00077/13). We thank the research license by Instituto Estadual de Florestas (IEF No. UC 053/13) for the acess to the Floresta Estadual do Uaimii, and to Silvia Magalhães and Clecio Magalhães for kindly receiving us at São Bartolomeu. We also thank Janaina Uchôa's field support.

REFERENCES

ABELHO, M. 2001. From litterfall to breakdown in streams: a review. *ScientificWorld Journal*, 1: 656–80.

ALLAN, D. & M. CASTILLO. 2007. *Stream Ecology-Structure and Function of Running Waters*. Springer, Dordrecht.

APHA. 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st ed. American Public Health Association, Washington, DC.

ARTIGAS, J., A. M. ROMANÍ & S. SABATER. 2008. Effect of nutrients on the sporulation and diversity of aquatic hyphomycetes on submerged substrata in a Mediterranean stream. *Aquatic Botany*, 88: 32–38.

BÄRLOCHER, F. 1992. *The Ecology of Aquatic Hyphomycetes*. Springer-Verlag, Berlin.

BARNES, J. R., I. P. VAUGHAN & S. J. ORMEROD. 2013. Reappraising the effect of habitat structure on river macroinvertebrates. *Freshwater Biology*, 58: 2154–2167.

BENFIELD, E. F. & J. R. WEBSTER. 1985. Shredder abundance and leaf breakdown in an Appalachian Mountain stream. *Freshwater Biology*, 15: 113–120.

BOYERO, L., R. G. PEARSON, M. O. GESSNER, L. A. BARMUTA, V. FERREIRA, M. A. S. GRAÇA, D. DUDGEON, A. J. BOULTON, M. CALLISTO, E. CHAUVET, J. E. HELSON, A. BRUDER, R. J. ALBARIÑO, C. M. YULE, M. ARUNACHALAM, J. N. DAVIES, R. FIGUEROA, A. S. FLECKER, A. RARNIREZ, R. G. DEATH, T. IWATA, J. M. MATHOOKO, C. MATHURIAU, J. F. GONÇALVES, M. S. MORETTI, T. JINGGUT, S. LAMOTHE, C. M'ERIMBA, L. RATNARAJAH, M. H. SCHINDLER, J. CASTELA, L. M. BURIA, A. CORNEJO, V. D. VILLANUEVA & D. C. WEST. 2011. A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. *Ecology Letters,* 14: 289–294.

BUENDIA, C., C. N. GIBBINS, D. VERICAT, J. A. LOPEZ-TARAZON & R. J. BATALLA. 2011. Influence of Naturally High Fine Sediment Loads on Aquatic Insect Larvae in a Montane River. *Scottish Geographical Journal*, 127: 315–334.

CLARKE, K. R. & R. M. WARWICK. 2001. *Change in marine communities: as apporach to statistical analysis and interpretation*. 2nd. Edition. PRIMER-E., Plymouth.

COLWELL, R. K. 2006. *StimateS: Biodiversity Estimation Software*. Version 8.2.

DOWNING, J. A., J. J. COLE, C. M. DUARTE, J. J. MIDDELBURG, J. M. MELACK, Y. T. PRAIRIE, P. KORTELAINEN, R. G. STRIEGL, W. H. MCDOWELL & L. J. TRANVIK. 2012. Global abundance and size distribution of streams and rivers. *Inland Waters*, 2: 229–236.

DUFRENE, M. & P. LEGENDRE. 1997. Species assemblages and indicator species: the need for flexible asymmetrical approach. *Ecological Monographs.,* 67: 345–366.

FERREIRA, V., A. C. ENCALADA & M. A. S. GRAÇA. 2012. Effects of litter diversity on decomposition and biological colonization of submerged litter in temperate and tropical streams. *Freshwater Science*, 31: 945–962.

FERREIRA, W. R., R. LIGEIRO; D. R. MACEDO, R. M. HUGHES, P. R. KAUFMANN, L. G. OLIVEIRA & M. CALLISTO. 2014. Importance of environmental factors for the richness and distribution of benthic macroinvertebrates in tropical headwater streams. *Freshwater Science*, 33: 860–871.

FLORES, L., J. R. DÍEZ, A. LARRAÑAGA, C. PASCOAL & A. ELOSEGI. 2013. Effects of retention site on breakdown of organic matter in a mountain stream. *Freshwater Biology*, 58: 1267–1278.

GESSNER, M. O. & E. CHAUVET. 1994. Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology*, 75: 1807–1817.

GONÇALVES, J. F., M. A. S. GRAÇA & M. CALLISTO. 2006. Leaf-litter breakdown in 3 streams in temperate, Mediterranean, and tropical Cerrado climates. *Journal of the North American Benthological Society,* 25: 344–355.

GONÇALVES, J. F., M. A. S. GRAÇA & M. CALLISTO. 2007. Litter decomposition in a Cerrado savannah stream is retarded by leaf toughness, low dissolved nutrients and a low density of shredders. *Freshwater Biology*, 52: 1440–1451.

GRAÇA, M. A. S. & C. CRESSA. 2010. Leaf quality of some tropical and temperate tree species as food resource for stream shredders. *International Review of Hydrobiology*, 95: 97–41.

GRAÇA, M. A. S., R. C. F. FERREIRA & C. N. COIMBRA. 2001. Litter processing along a stream gradient: the role of invertebrates and decomposers. *Journal of the North American Benthological*, 20: 408–420.

GRAÇA, M. A. S., P. PINTO, R. CORTES, N. COIMBRA, S. OLIVEIRA, M. MORAIS, M. J. CARVALHO & J. MALO. 2004. Factors affecting macroinvertebrate richness and diversity in Portuguese streams: a two-scale analysis. *International Review of Hydrobiology*, 89: 151–164.

HOFER, N. & J.S. RICHARDSON. 2007. Comparisons of the Colonisation by Invertebrates of Three Species of Wood, Alder Leaves, and Plastic "Leaves" in a Temperate Stream. *International Review of Hydrobiology*, 92: 647–655.

IQBAL, S. H. & J. WEBSTER. 1973. Aquatic hyphomycete spora of the River Exe and its tributarias. *Transactions of the British Mycological Society*, 61: 331–346.

JABIOL, J., A. BRUDER, M. O. GESSNER, M. MAKKONEN, B. G. MCKIE, E. T. H. M. PEETERS, V. C. A. VOS & E. CHAUVET. 2013. Diversity patterns of leaf-associated aquatic hy-

phomycetes along a broad latitudinal gradient. *Fungal Ecology*, 6: 439–448.

JONES, J. I., J. F. MURPHY, A. L. COLLINS, D. A. SEAR, P. S. NADEN & P. D. ARMITAGE. 2012. The Impact of Fine Sediment on Macro-Invertebrates. *River Research and Applications*, 28: 1055–1071.

JUN, Y. C., N. Y. KIM, S. J. KWON, S. C. HAN, I. C. HWANG, J. H. PARK, D. H. WON, M. S. BYUN, H. Y. KONG, J. E. LEE & S. J. HWANG. 2011. Effects of land use on benthic macroinvertebrate communities: Comparison of two mountain streams in Korea. *International Journal of Limnology*, 47: S35–S49.

KENNETH, T. 1993. *The ecology of aquatic hyphomycetes in an Australian upland stream*. Australian National University.

LAITUNG, B., J. L. PRETTY, E. CHAUVET & M. DOBSON. 2002. Response of aquatic hyphomycete communities to enhanced stream retention in areas impacted by commercial forestry. *Freshwater Biology*, 47: 313–323.

LARRAÑAGA, S., J. DÍEZ, A. ELOSEGI & J. POZO. 2003. Leaf retention in streams of the Agüera basin (northern Spain). *Aquatic Sciences*, 65: 158–166.

LARSEN, S., G. PACE & S. J. ORMEROD. 2011. Experimental effects of sediment deposition on the structure and function of macroinvertebrate assemblages in temperate streams. *River Research and Applications*, 27: 257–267.

LARSEN, S., I. P. VAUGHAN & S. J. ORMEROD. 2009. Scale-dependent effects of fine sediments on temperate headwater invertebrates. *Freshwater Biology*, 54: 203–219.

MATHOOKO, J. 1995. Colonization of artificial substrates by benthos in a second-order high altitude river in Kenya. *Hydrobiologia*, 308: 229–234.

MIYAKE, Y., T. HIURA, N. KUHARA & S. NAKANO. 2003. Succession in a stream invertebrate community: A transition in species dominance through colonization. *Ecological Research*, 18: 493–501.

OLOMUKORO, J. O. & U. OKOLOGUME. 2008. Macroinvertebrate colonisation of artificial substrates in a Nigerian river I: gravel and leaf litter. *African Journal of Aquatic Science*, 33: 143–148.

ORTIZ, J. D. & M. A. PUIG. 2007. Point source effects on density, biomass and diversity of benthic macroinvertebrates in a Mediterranean stream. *River Research and Applications*, 23: 155–170.

PÉREZ, J., M. MENÉNDEZ, S. LARRAÑAGA & J. POZO. 2011. Inter- and intra-regional variability of lLeaf litter breakdown in reference headwater streams of Northern Spain: Atlantic versus Mediterranean streams Streams. *International Review of Hydrobiology*, 96: 105–117.

ROBERTS, D. W. 2013. *Labdsv: Ordination and multivariate analysis for Ecology*. R package version 1.6-1.

ROBINSON, C. T. & M. DOERING. 2012. Spatial patterns in macroinvertebrate assemblages in surface-flowing waters of a glacially-influenced floodplain. *Aquatic Sciences*, 75: 373–384.

SARR, A. B., C. J. BENETTI, M. FERNÁNDEZ-DÍAZ & J. GARRIDO. 2013. The microhabitat preferences of water beetles in four rivers in Ourense province, Northwest Spain. *Limnetica*, 32: 1–10.

SUBERKROPP, K. & J. B. WALLACE. 1992. Aquatic hyphomycetes in insecticide-treated and untreated streams. *Journal of the North American Benthological*, 11: 165–171.

TOMANOVA, S., E. GOITIA & J. HELEŠIC. 2006. Trophic levels and functional feeding groups of macroinvertebrates in neotropical streams. *Hydrobiologia*, 556: 251–264.

VASCONCELOS, M. C. & A. MELO. 2008. An experimental test of the effects of inorganic sediment addition on benthic macroinvertebrates of a subtropical stream. *Hydrobiologia*, 610: 321–329.

VON BERTRAB, M. G., A. KREIN, S. STENDERA, F. THIELEN & D. HERING. 2013. Is fine sediment deposition a main driver for the composition of benthic macroinvertebrate assemblages? *Ecological Indicators*, 24: 589–598.

WANTZEN, K. M. & R. WAGNER. 2006. Detritus processing by invertebrate shredders: a neotropical-temperate comparison. *Journal of the North American Benthological*, 25: 216–232.

WANTZEN, K. M., R. WAGNER & W. J. JUNK. 2002. How do plant-herbivore interactions of trees influence coarse detritus processing by shredders in aquatic ecosystems of different latitudes? *Verhandlungen des Internationalen Verein Limnologie*, 28: 815–821.