Responses of aquatic invertebrate assemblages and leaf breakdown to macroconsumer exclusion in Amazonian "terra firme" streams

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With 4 figures and 1 table

Abstract: Many authors have reported a lack of insect shredders in tropical streams and some have suggested that macroconsumers, such as fish and shrimp, are potential substitutes for insect shredders. We experimentally excluded macroconsumers (fish and shrimp) from leaf packs to examine their role in determining the rate of leaf breakdown and their effects on the associated invertebrate community. The experiment was designed in blocks and replicated in two reaches of four streams. Temperature of all stream reaches studied was 24 °C (without variation), and water conductivity was low, varying from 8.8 to 10.8 µs/cm. Fish were never observed near the leaf packs and therefore the effects of the macroconsumer treatment were attributed to shrimps. We found a significant effect on leaf breakdown, with greater leaf breakdown (i.e., less mass remaining after 17 days) in the control (65 % leaf mass remaining) compared to the macroconsumer exclusion (70 % leaf mass remaining). However, the mechanism for this effect was not clear. Considerable variation in leaf decomposition occurred among blocked stream sites, suggesting that some factors differing among these sites, perhaps macroinvertebrate shredder abundance, was contributing to decomposition. Leaves were visually inspected at the conclusion of the experiment and there was no sign of shrimp directly feeding on the leaves. There was no difference in insect shredder abundance between treatments. There was, however, a significantly greater amount of non-mining chironomids in the absence of macroconsumers. This is probably due to the release from predation by shrimp.

Key words: Amazonian streams, shredders, shrimp, fish, electric exclusion.

Introduction

Recent studies have shown the importance of fish, shrimp, and crayfish as keystone species and ecosystem engineers (Jones et al. 1994) in freshwater ecosystems (e.g. Flecker 1996). In streams in Puerto Rico and Ilha Grande (Brazil), shrimp affect community structure of algae and insects (Pringle & Hamazaki 1998, Moulton et al. 2004), the amount and quality of the benthic organic matter (March et al. 2001) and the organic and inorganic sediment cover (Pringle & Blake 1994). Fish, such as *Prochilodus mariae* Eigenmann, have been found to cause similar effects on organic matter and sediment accrual in a Venezuelan stream (Flecker 1996).

Allochthonous organic matter (mainly leaves) has been considered the most important energy source in low-order streams (Cummins et al. 1973). Breakdown

and decomposition of this organic matter involves biotic (microbes and shredders) and abiotic (physical abrasion) factors. In temperate streams, shredders (mostly insects) are responsible for a large amount of leaf breakdown (Graça 2001).

The role of insect shredders in the tropics is controversial. Many authors have reported a paucity of insect shredders in tropical streams (Fittkau 1964, Covich 1988, Benstead 1996, Rosemond et al. 1998, Dobson et al. 2002, Wantzen & Wagner 2006, Rueda-Delgado et al. 2006), although other studies revealed opposite results (e.g. Cheshire et al. 2005). Insect shredding may be less important in tropical systems due to the high concentration of toxic compounds in leaves (Wantzen et al. 2002) and intense microbial activity (Irons et al. 1994). Additionally, it has been suggested that large organisms, such as shrimp and fish (hereafter called macroconsumers), could replace the typical shredders of temperate zones (*Gammarus*, Trichoptera, Plecoptera) in tropical streams (Crowl et al. 2001, Dobson et al. 2002, Wantzen & Wagner 2006).

Previous studies assessing species roles or ecological processes in stream ecosystems have been done using a variety of experimental methods. Experimental design is one of the crucial points in these experiments. Some researchers replicate their studies in a single stream pool or run (Benstead 1996, Moulton et al. 2004, de Souza & Moulton 2005). Others set up their replicates in more than one stream reach (Flecker 1996, Pringle & Hamazaki 1998, Mantel & Dudgeon 2004), or replicates are done in more than one stream (e.g. Ramírez & Pringle 2004). When the experiments are done in more than one stream reach, they rarely were analyzed in blocks (but see Dangles et al. 2001). Variability between sites in the same stream may account for a large part of the total variation (Downes & Hindell 2000) and hide effects under investigation. Additionally, accounting explicitly for variability among stream sites using blocks allows us to evaluate the ecological significance of potential statistical effects (Gotelli & Ellison 2004).

This study was done in the poorly studied Amazonian "terra firme" (not seasonally flooded) blackwater streams (for more details on Amazon river types see Sioli 1984). To our knowledge, this study provides the fist information on leaf breakdown in these streams. We experimentally excluded macroconsumers (fish and shrimp) from leaf packs to examine their role in determining the rate of leaf breakdown and their effects on the associated invertebrate community (other than shrimp). In light of the suggested paucity of insect shredders in tropical streams and the omnivorous behavior of shrimp, we predicted that the exclusion of macroconsumers would result in reduced leaf breakdown rates and increased invertebrate abundance.

Study area

The study area was the "Km 41" Reserve, an experimental area managed by the Biological Dynamics of Forest Fragments Project (BDFFP). The area is located 100 km north of Manaus, Amazonas, Brazil (02° 25′ S, 59° 43′ W) and is covered by rainforest known locally as "terra firme" forest. The annual average precipitation in the study area varies from 1900 to 2500 mm and is concentrated in the period from December to May (Gascon & Bierregaard 2001).

Blackwater streams drain the area. They have acidic water (pH 3.8–4.4) due the presence of humic and fulvic acids and are generally nutrient-poor (Sioli 1984, Junk & Furch 1985). Streams in undisturbed areas are covered by tall forests with dense canopies and receive about 10 % of the incident sunlight (Sioli 1975). As a consequence of low light incidence and scarce nutrients, primary productivity is low and food webs depend heavily on allochthonous materials from the surrounding forest (Henderson & Walker 1986). Our experiment was done in four blackwater streams (three 1st-order and one 2nd-order) in the Urubu River drainage basin. The streams were 1–2.5 m wide and had streambeds composed of sand and submerged leaves. Mean water temperature was 24 °C (annual range 23–26 °C) and water conductivity ranged from 8.8 to 10.8 µS/cm.

Streams in Central Amazonia harbor a diversified fish fauna composed largely of small Characiforms (tetras) and Siluriforms (catfishes) (Sabino & Zuanon 1998, Lowe-McConnell 1999, Mendonça et al. 2005). Common species in leaf litter at the study sites include *Helogenes marmoratus* Günther, *Ituglanis amazonicus* Steindachner and *Apistograma* aff. *steidachneri* Regan.

Shrimp assemblages at the studied sites include three species: *Macrobrachium inpa* Kensley and Walker, *Macrobrachium nattereri* Heller and *Pseudopalaemon amazonensis* Porto-Ramos. These species are omnivorous and feed mostly on aquatic and terrestrial insects (notably chironomid larvae, Walker & Ferreira 1985), microcrustaceans and small fish (Henderson & Walker 1986).

Material and methods

Experimental design

The experiment was run from 8 to 24 May 2005 using replicate controls and exclusion treatments. In order to reduce environmental variability, the experiment was designed in blocks (Box et al. 1978). Within blocks, treatments were allocated to similar places in terms of water velocity, depth and stream-bottom type. Electric fences were used to exclude access to treatment sites by fish and shrimp (or other large organisms) as first described in Pringle and Blake (1994). Recently, water conductivity has been shown to have no effect on the efficiency of electric fence devices (Ramírez & Pringle 2004).

Fences were electrified by an electric charger (JFL – Equipamentos Eletrônicos Ind. Com. Ltda, Santa Rita do Sapucaí, MG, Brazil – model Shock 8 Express), which was powered by a 12-volt car battery. When used in the air, the charger emits one

Fig. 1. Illustration of the electric fence device. Each fence was constructed with PVC pipes (20-mm diameter), forming a square with 40-cm sides. The electric field of exclusion was created inside the fence by the stripped wires. The grounding wire acted as a positive electrode in the exclusion device, thereby forming an electric field inside the fence. Four wooden stakes were attached to each fence corner to fix the fence to the stream bottom.
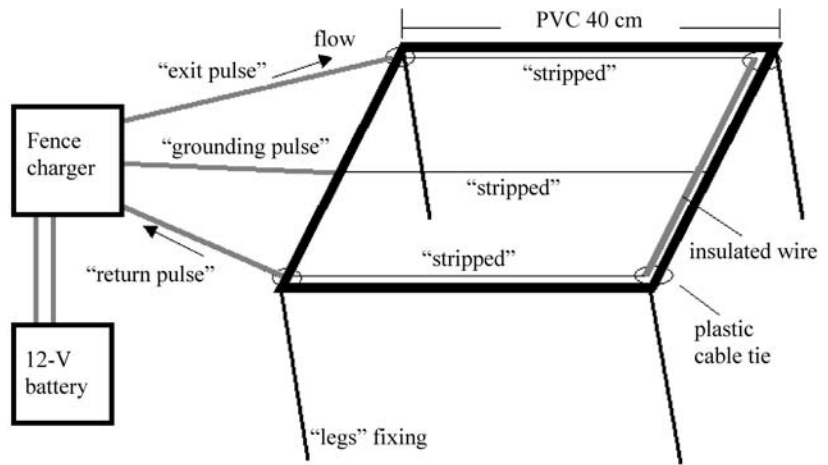
8000-volt electric pulse per second with a duration of 110 μs, exit-pulse energy of 1.2 joules and maximum current of 1.4 amperes; these values, however, are unknown under the water and could be variable. Electric fields for exclusion in aquatic systems depend on the intensity of the field (e.g. Brown et al. 2000, de Souza & Moulton 2005). The chargers we used do not emit high-intensity pulses, so the fence was able to exclude only larger organisms (> 1 cm) such as fish and shrimp (although shrimp and fish smaller than 1 cm are not excluded). As observed in pilot experiments, electric pulses were enough to exclude shrimp and fish, but not insects. A stronger charger would be necessary to exclude insects and small fishes and shrimps.

The fence design used in our study (Fig. 1) was similar to that used by Moulton et al. (2004). Two pieces of insulated copper wire (6 and 3 m long) were attached to the charger. The longer wire was connected to the charger on the "exit high-voltage" connector. The current passed through three sides inside the fence before returning to the charger through the "return high-voltage" connector. The wires were stripped in the sections that covered two of the three sides of the fence (each side was 40 cm long), located on opposing sides of the plot. The shorter wire was connected to the grounding connector, after which its final 40 cm was stripped and fixed to the center of the fence parallel to the stripped parts of the longer wire. The shorter wire acted as the positive electrode, while the stripped parts of the longer wire acted as negative electrodes, thereby forming an electric field inside the fence.

Each fence was constructed with PVC pipes (20 mm diameter), forming a square with 40-cm sides. To prevent dislodgement, PVC pipes were attached to four wooden stakes (0.3 cm diameter, 30 cm long) and fixed in the stream bottom. Electrified and non-electrified (control) fences were installed in two reaches, at least 100 m apart, in each of four streams, totaling eight replicated blocks. Within each block, treatments were placed 30 cm apart, one upstream of the other, in pool areas (varying in depth from 8 to 30 cm and in width from 1 to 2.5 m). Control and electrified fences were constructed in the same way and the flip of a coin was used to define which quadrat would receive electricity.

Leaf-packs

In order to evaluate macroconsumer effects on leaf breakdown, eight leaf packs were placed inside each experimental unit. Leaf packs were made with green leaves of *Mabea speciosa* J.

Müller Argoviensis (Euphorbiacea) collected from a single tree before leaf abscission on 2 May 2005. This procedure assured high similarity among the leaves used in each experimental unit and prevented potential effects of variation in chemical composition of leaves from different trees. Leaves of *Mabea speciosa* were used because the species was commonly found along the streams, was common in submerged litter and, in a preliminary experiment, showed rapid breakdown.

In the laboratory, leaves were washed under running water to remove dust and other material from the leaf surface, placed on a table to drain excess water, and then assembled in packs of 7.021 ± 0.010 g (wet weight), usually consisting of 6–8 leaves. Leaf packs were assembled by binding petioles using a plastic cable tie and attaching a plastic label. In a preliminary experiment, we observed that *M. speciosa* leaves did not lose fragments during the experiment (Fig. 2), and thus we had no problem in using leaf packs rather than leaf bags. Randomly selected leaf packs were attached to experimental units using monofilament-fishing line.

The initial dry weight of each leaf pack was estimated using a correction factor obtained from 16 leaf packs weighed after drying at 55 °C for 48 hours. The correction factor was calculated using the formula $CF = 1 / (mean (WW_i/DW_i))$, where CF is the correction factor, WW_i is the wet weight and DW_i is the dry weight of leaf pack i. Mean dry weight of the 16 leaf packs was 3.325 ± 0.111 g and the correction factor calculated was 0.461.

During the experiment, nocturnal and diurnal visits were carried out to observe the presence of macroconsumers in the controls, verify the efficiency of the exclusion device, measure the drawdown of the batteries and replace them when necessary. During nocturnal visits, stream sites were approached carefully and shrimp inside the control plots were rapidly counted using a flashlight. We do not use a flashlight covered with red filters as they hamper shrimp counts and because shrimps also appear to be sensitive to red light, mainly when using low quantities of red filters.

In order to decide when to stop the experiment (i.e. after a considerable amount of leaf breakdown but before breakdown was complete), 24 extra leaf packs were placed at four sites (6 packs per site) in one stream. These leaf packs were collected after 1, 2, 4, 7, 10 and 13 days. These extra packs were not included in the analysis, and were only used to visually observe the physical state of the leaves (i.e. presence of shredding activities). The experiment was terminated after 17 days,
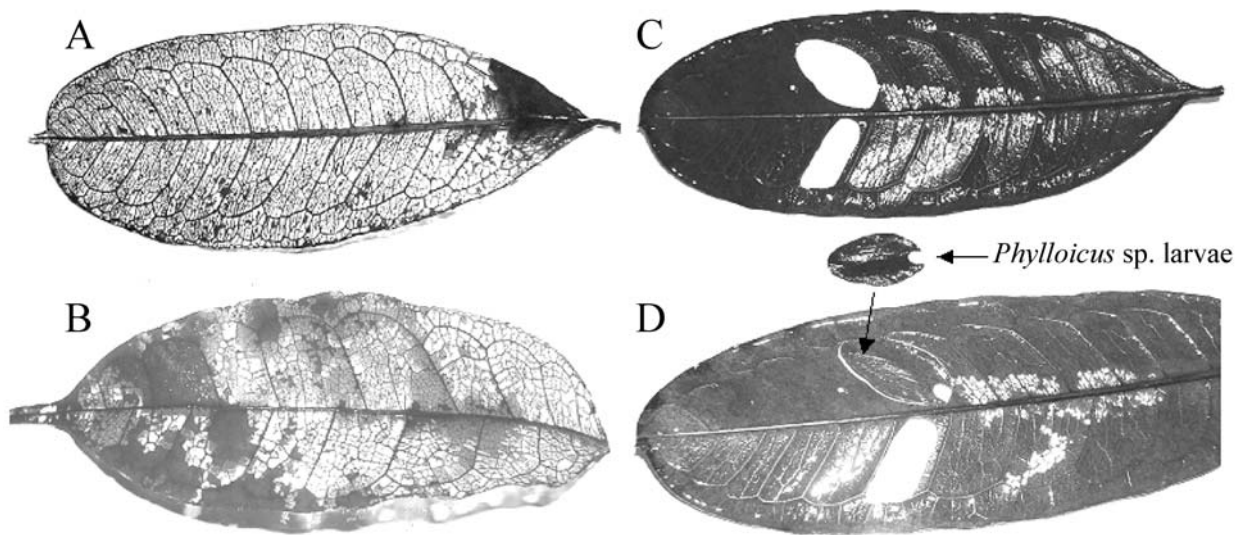
Fig. 2. Examples of leaf condition at the end of the experiment. Notice that leaves did not lose large fragments (A–D). *Phylloicus* spp. and *Triplectides* spp. created the feeding pattern observed in **A** and **B** (the same pattern on leaves was observed in laboratory feeding trials). Leaf discs used by *Phylloicus* spp. in its case building (**C** and **D**).

when the leaf breakdown pattern was similar to that shown in Fig. 2 (approximately 35 % of the leaves were broken down and decomposed). As the conductive tissues were not involved in the initial breakdown process, this means that most of the mesophyll tissues were removed, and only conductive tissues remained.

At the end of the experiment all leaf packs (eight) inside each fence were collected. Each leaf pack was carefully collected by hand and placed individually in a sealable plastic bag. The material was stored in an icebox until processing (approximately 12 hours later). In the laboratory, leaves were washed on a 6 μm-mesh sieve to separate insects from leaves. Insects were stored in 80 % ethanol for later identification and counting. Given the poor taxonomic knowledge of aquatic invertebrates in Amazonia, individuals were sorted into families except for Trichoptera, for which the availability of a key made determination to genus possible (Pes et al. 2005).

Before drying and weighing, leaves were examined under a dissecting microscope (40×, transmitted light) to count the number of leaf-mining chironomids. Leaves in each pack were then dried at 55 °C for 48 hours. Breakdown was estimated from the mean dry mass remaining in the eight leaf packs set out per treatment.

Statistical analysis

Differences in leaf breakdown, insect shredder and chironomid (Chironomidae) abundance (separated as miners and non-miners) between the two treatments were tested using a randomized block Analysis of Variance (a paired *t* test). Pearson correlation was used to determine if the abundances of these taxa were related to the dry mass remaining. Data were log-transformed when necessary to improve homogeneity of variance and normality of residuals.

Non-metric multidimensional scaling (NMDS) was used to compare species composition between treatments. The raw data initially were standardized by dividing each observation by the *block* total abundance (e.g. Control 1 + Exclusion 1). This standardization removed the variability between blocks, facilitating visualization of potential differences between treatments. NMDS also was carried out after down-weighting common species (division by the maximum abundance of each species). The Bray-Curtis similarity index was used to measure the resemblance between treatments. How much the result of an NMDS analysis can be adjusted to the original similarity matrix (goodness of fit) among samples is frequently measured by the STRESS statistic (S); values lower than 0.2 are considered acceptable (Clarke 1993).

A Multivariate Analysis of Variance (Manova) was used to test the significance of differences on the invertebrate fauna between treatments and to verify the relative importance of treatments and blocks in explaining the total variance. We used a distance-based Manova with Euclidian distance and log-transformed data. The statistic used was the Sum of Squares Between groups (SSB) (Pillar & Orlóci 1996) and its significance was evaluated using 9999 randomizations of groups.

The distance-based Manova was carried out in Multiv software (Pillar 2006). All remaining analysis were done using the R statistical software (R Development Core Team 2007). The NMDS analysis was carried out using function *metaMDS* in the *vegan* package (Oksanen et al. 2007).

Results

No macroconsumers were observed inside the electric fences during diurnal (n = 17) and nocturnal (n = 11) observations. Nocturnal observations on 11 nights indicated a mean of 2.1 (range 0–5) shrimp in control plots. Fish were observed swimming in the middle
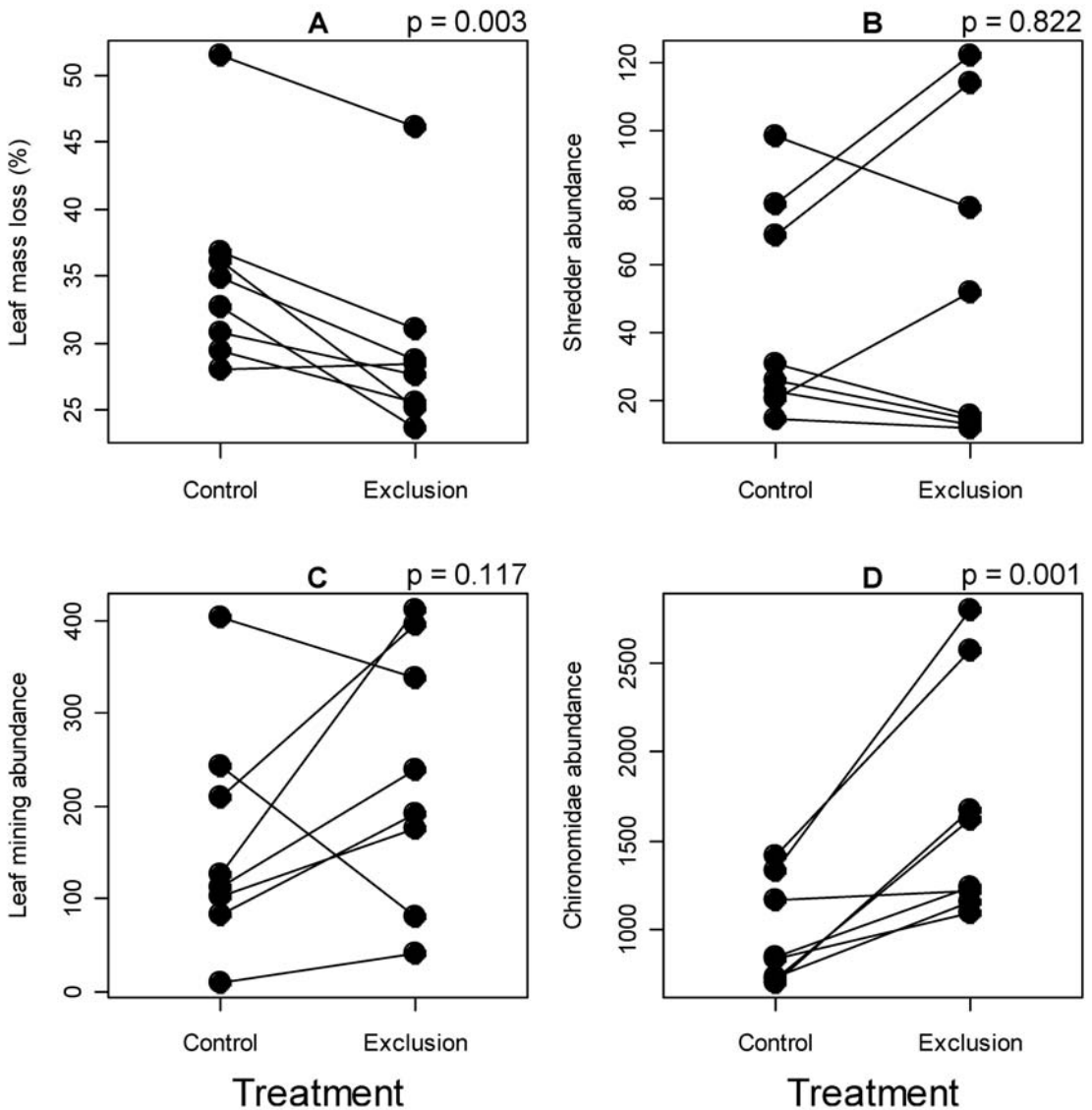
Fig. 3. Effects of macroconsumer exclusion on leaf mass loss (**A**), abundance of shredders (*Triplectides* spp. and *Phylloicus* spp.) (**B**), mining chironomids (**C**) and non-mining chironomids (**D**). The *p*-values indicate probability of the null hypothesis of no difference between exclusion and control treatments. Lines indicate blocks.

and upper portions of the water column in the electric fence, but never at the stream bottom or next to the leaf packs.

Macroconsumer effects on leaf breakdown

No leaf pack was lost during the experiment and all of the leaves set out at the beginning of the experiment were recovered. Leaves from both treatments showed the same fragmentation pattern (Fig. 2A, B). Only non-conductive tissues were consumed. Leaves usually had small holes (~3 mm) and holes left by the

caddisfly *Phylloicus* spp. (Fig. 2C, D) after cutting leaf discs used in their case construction. Marks of shredding activities were observed after one day of immersion in water in the extra leaf packs used to monitor degradation over time.

The mean percentage of dry mass remaining in the controls was 65 % (SD 7.35), while in the electrified treatments it was 70.5 % (SD 7.10). Leaf breakdown was significantly faster in controls than in the exclusion treatments (arcsine of the square-root of the proportion of remaining dry mass, $F_{1,7} = 19.51$, $p = 0.003$; Fig. 3A). There was higher variation in the

Table 1. Partitioning of the Total Sum of Squares. Breakdown = arcsine of square root of leaf weight loss; shredders, leaf-miners and chironomids abundance were log(x + 1) transformed. Percentage of variation explained was calculated by dividing the sum of squares within treatments or blocks by the total sum of squares.

	breakdown	shredders	leaf-miners	chironomids
Treatment SSQ	0.014 **	0.001	0.187	0.204 **
% explained	15 %	0.0006 %	7.2 %	42 %
Block SSQ	0.074 ***	1.669 **	1.977 *	0.229 *
% explained	79.5 %	88.6 %	76.7 %	47.1 %
Residual SSQ	0.005	0.213	0.411	0.052
% explained	5.4 %	11.4 %	15.9 %	10.8 %
Total SSQ	1.594	1.884	2.576	0.486

Significance codes: *** 0.001; ** 0.01; * 0.05

dry mass remaining among blocks than between treatments (range 48.5–71.9 % in controls; 53.8–76.3 % in electrified treatments) (Fig. 3A). In fact, partitioning of the total variance (Table 1) indicated that treatments accounted for only 15 % of the variance (coefficient of determination, 1 df) while blocks accounted for 79.5 % (7 df).

Macroconsumer effects on insect shredders and chironomids

A total of 29,514 invertebrates (aquatic insects, copepods, cladocerans and ostracods) were collected, 11,794 of which were in the control quadrats and 17,720 in the electrified quadrats. The most common taxon was Chironomidae (21,135 non-miners and 3161 miners), and the most common insect shredder was the caddisfly *Triplectides* spp. (560 individuals).

Individuals of *Triplectides* spp. (2 species) and *Phylloicus* spp. (3 species) accounted for 15 % of the total invertebrate abundance (excluding chironomids). We grouped and analyzed them as shredders. Chironomids were sub-divided into leaf miners and non-miners (leaf miners were considered shredders, but were analyzed separately). Abundances of insect shredders and leaf-mining chironomids (log[x+1]) did not differ between treatments (respectively, $F_{1,7} = 0.04$, $p = 0.822$ and $F_{1,7} = 3.18$, $p = 0.117$) (Fig. 3B, C). Exclusion of macroconsumers caused a mean increase of 703 individuals of non-mining chironomids as compared to the controls ($F_{1,7} = 27.2$, $p = 0.001$) (Fig. 3D). Treatment effects were more intense on chironomids than on leaf breakdown. While treatments accounted for only 11.1 % of the variation in leaf breakdown (and 84.7 % for blocks), treatments accounted for approximately 42 % (coefficient of determination, 1 df) of the variation in chironomid abundance (and 47 % in blocks, 7 df, Table 1).

Relationship of insect abundance to dry mass remaining

Abundances of insect shredders and leaf-mining chironomids at the end of the experiment were related to the percentage of dry mass remaining. Insect shredder abundance was high in quadrats with low dry mass remaining (raw data, pooled control and exclusion treatments, $n = 16$, $r = –0.58$, $p = 0.019$), indicating that they are important agents in leaf breakdown. On the other hand, leaf-mining chironomids were positively related to remaining dry mass (raw data, $n = 16$, $r = 0.66$, $p = 0.006$). Non-mining chironomids were not related to remaining dry mass (raw data, $n = 16$, $r = 0.36$, $p = 0.172$), indicating that their low abundance in control treatments (Fig. 3D) was not due to the low amount of dry mass remaining at the end of the experiment.

Macroconsumer effects on invertebrate assemblages

Using the full dataset standardized by total abundance within blocks, the NMDS ordination separated control samples from those in the electrified quadrats (Fig. 4A). However, the effect of treatments disappeared when non-mining chironomids were removed (Fig. 4B). A subsequent standardization to remove the influence of dominant species (i.e. division by the total for each species) resulted in the partial separation of blocks but did not distinguish between the treatments (Fig. 4C). The lack of treatment effects was observed both using the full datasets and the datasets without non-mining chironomids (Fig. 4C,D). Additionally, blocks within the same stream or in an adjacent one were not grouped together, suggesting that the variation among blocks was enough to interpret them as independent of each other (i.e. not spatially autocorrelated; Fig. 4C,D).
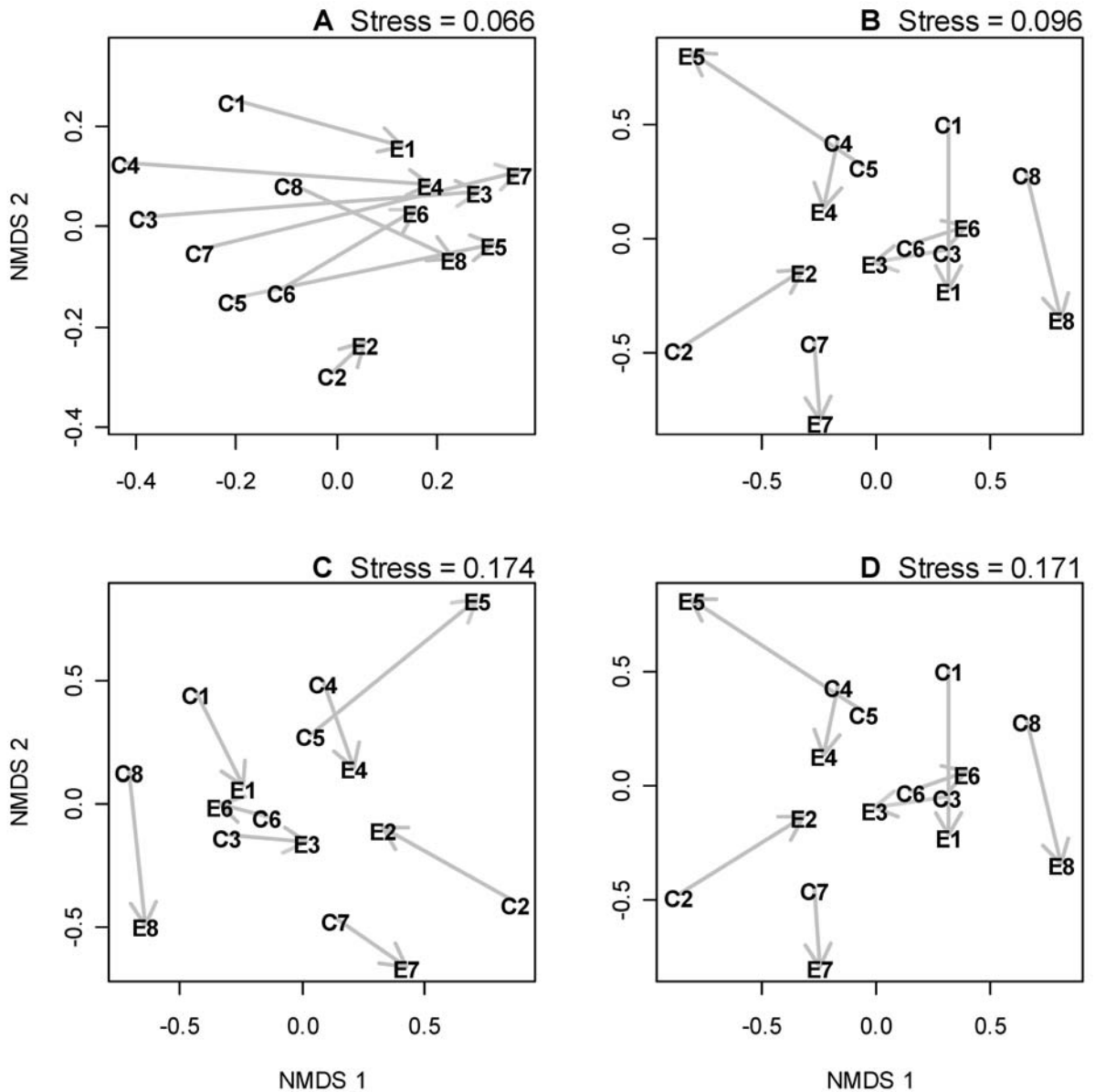
Fig. 4. Non-Metric Multidimensional Scaling ordination (NMDS) of invertebrate community structure in control and exclusion treatments. Each point on the NMDS plot represents an experimental unit of control (C) and electrified (E) treatments. Numbers indicate blocks of the experiment; 1-2, 3-4, 5-6, 7-8 are the blocks in the same stream. The lines joining the treatments of the same block are draw for illustrative purposes only. Control and exclusion treatments formed two distinct groups when using the full data set **(A)**. However, the distinction between treatments disappears after exclusion of non-miner chironomids **(B)**. After a 2nd standardization to down-weight common species, blocks (and not treatments) are usually evident either in datasets with **(C)** or without **(D)** non-mining chironomids.

Despite no clear evidence of a treatment effect in the NMDS ordination, the null hypothesis of no difference in community structure was rejected by the MANOVA test using the dataset without non-mining chironomids (SSB statistic = 2.98, $p = 0.038$). However, the variance explained by treatments (6.7 %, 1 df, partition of sums of squares) was much lower than that accounted for by blocks (65.3 %, 7 df).

Discussion

Our study demonstrated that macroconsumers increased leaf breakdown and reduced non-mining chironomid densities. However, mining-chironomids and insect shredders (*Triplectides* spp. and *Phylloicus* spp.) were unaffected. Invertebrate community structure was affected, although the magnitude of the effect

was small when compared to natural variation among stream reaches (evaluated as variation accounted for by blocks). Despite the statistically significant effect of macroconsumers on leaf breakdown, its ecological relevance was low when compared to natural variation among stream reaches (blocks).

Shredding by shrimp is a key factor affecting leaf breakdown in some tropical streams (Crowl et al. 2001, March et al. 2001, Wright & Covich 2005), but not in others (Rosemond et al. 1998, Mantel & Dudgeon 2004). These studies showed that effects depend on the specific macro-fauna found in streams or stream reaches (March et al. 2001). For example, studies concerning the shrimp *Xiphocaris elongata* showed strong effects on leaf breakdown (Pringle et al. 1993, Crowl et al. 2001, 2006, March et al. 2001). However, the usually abundant tropical shrimp *Macrobrachium* spp. has only a slight effect on leaf breakdown (March et al. 2001, Mantel & Dudgeon 2004). In our study, *Macrobrachium* spp. were the most abundant shrimp species, and, as shown by previous studies in Amazonian streams, these shrimps and most fishes are mainly predaceous, notably on chironomid larvae (Kensley & Walker 1982, Walker & Ferreira 1985, Henderson & Walker 1986).

Accordingly, the low ecological significance of macroconsumers on leaf breakdown in our study may reflect the feeding preferences of the dominant *Macrobrachium* spp. shrimps. Considering the gut contents of Amazonian shrimps and fishes (Kensley & Walker 1982, Walker & Ferreira 1985), we speculate that the effects we found on leaf weight loss caused by macroconsumers were mostly due to the feeding and foraging habits of shrimps and fishes (bioturbation) than due to the direct consumption of leaves.

Many authors have noted a pronounced lack of insect shredders in tropical streams (Fittkau 1964, Covich 1988, Benstead 1996, Rosemond et al. 1998, Dobson et al. 2002, Wantzen & Wagner 2006, Rueda-Delgado et al. 2006, Gonçalves et al. 2006). Accordingly, Wantzen & Wagner (2006) pointed out that most studies from neotropical lotic systems have concluded that shredding (at least by aquatic insects) is of minor importance. Irons et al. (1994) found temperature to be an important factor controlling the distribution of insect shredders, suggesting that shredder richness decreases with decreasing latitude and that microbial activity increases with increasing temperature. However, no study has evaluated microbial activity in Amazonian streams, and only speculation about the importance of fungi is available (Walker 1986). On the other hand, tropical streams harbor a great diversity of generalist and omnivorous insect species (Covich 1988, Pringle & Hamazaki 1998, Wantzen & Wagner 2006, Wantzen et al. 2006), and more refined studies have been able to identify many tropical insect shredder species, including both generalists and specialists (Cheshire et al. 2005). Despite the low diversity of insect shredders found in our study, they were abundant and accounted for 15 % of the total invertebrates (excluding chironomids). Additionally, the insect shredders found (*Triplectides* spp. and *Phylloicus* spp.) are among the largest invertebrates found in our study. Although our experiment was not designed to evaluate the relative importance of insect shredders, we speculate that they should account for a large part in the leaf breakdown process. Future studies in tropical streams should evaluate this speculation and the relative importance of microbes.

As observed in other studies (Flecker 1996, Pringle & Hamazaki 1997, March et al. 2002, Creed & Reed 2004), macroconsumer assemblages significantly reduced non-mining chironomid abundance. At least three mechanisms might be involved. Shrimp usually disturb the substrate and thus could dislodge non-mining chironomids from leaf packs (March et al. 2002). Reduced densities of non-mining chironomids could also be due to the low amount of leaves that remained in the control quadrats (i.e. an indirect cascade effect). However, we did not observe a significant relationship between non-mining chironomid abundance and remaining dry mass. March et al. (2002) suggested that the sessile behavior of chironomids makes them easily affected by predation. Indeed, Henderson & Walker (1986) analyzed gut-content of Amazonian fishes and shrimps and observed that chironomids were a common item.

In contrast to non-mining chironomids, macroconsumers did not affect mining chironomids and insect shredders (the caddisflies *Phylloicus* spp. and *Triplectides* spp.). Lack of effect on the abundance of these insects may be due to their protective cases, which impeded predation. Mining-chironomids live in leaf mesophyll while the caddisflies build portable cases made of hollow twigs (*Triplectides* spp.) and leaf discs (*Phylloicus* spp). Previous studies have shown that, at least for caddisflies, cases usually confer protection against predation (Johansson 1991, Nislow & Molles 1993, Wissinger et al. 2006).

Community structure was only slightly affected by macroconsumers. Despite the significant p-value (i.e. $p < 0.05$) obtained in the MANOVA analysis, effects were not clearly evident in the NMDS ordination diagram, which usually grouped blocks but not

treatments. Indeed, partitioning the sum of squares in the MANOVA analysis indicated that natural variance among stream reaches (evaluated by our blocks) was much higher than that accounted for by the exclusion treatment.

Macroconsumers had high impacts on non-mining chironomids, decreasing their abundance by an average of 25 %. Our experimental design has the advantage of accommodating environmental heterogeneity, i.e. the natural variance among sites. In this case, the environmental noise (variability among sites) was corrected by paired comparisons for testing macroconsumer exclusion, giving our treatments greater effectiveness and increasing the robustness of our test of macroconsumer effects. In addition, we were able to calculate the natural variability among stream reaches. We found that the natural variability accounted for much more of the leaf breakdown and invertebrate community structure variation than did macroconsumer effects. The low macroconsumer effects on leaf breakdown may reflect the feeding preferences of predatory fishes and *Macrobrachium* spp. shrimps. The rapid leaf breakdown observed might be due to the abundant insect shredders. This is partially supported by shredding marks commonly observed on leaves.

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