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PRIMARY RESEARCH PAPER

An experimental test of the effects of inorganic sediment addition on benthic macroinvertebrates of a subtropical stream

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Abstract Inorganic sediments of terrestrial origin may impact stream macroinvertebrate communities. Although input of terrestrial sediments to streams may occur naturally, human-induced activities in the catchment amplify this input greatly. We used an in-stream experiment to investigate whether short-term additions of terrestrial sediments of two size classes affected stream macroinvertebrates. The experiment was designed in blocks to minimize the influence of flow velocity and other environmental variables. Four treatments were employed: (i) addition of fine sand (0–0.24 mm), (ii) coarse sand (0.25–0.8 mm), (iii) fine+coarse sand, and (iv) control (water only). Macroinvertebrates were sampled immediately after the addition of sediments (or water). The experiment consisted of 20 blocks. We analyzed the response of the macroinvertebrate fauna in terms of abundance and species richness. Since species richness is strongly dependent on number of individuals sampled, we also analyzed rarefied species richness. Community structure was evaluated using a distance-based Manova on presence/absence and abundance data. The addition of coarse and fine+coarse sand reduced the abundance and species richness of macroinvertebrates in relation to the control. The response in terms of rarefied species richness in the treatments did not differ from the control, indicating that reduction in species richness was a sampling artifact resulting from decreased sample abundance. The Manova analyses indicated that coarse-sand addition caused changes in both species composition and community structure. Addition of fine and fine+coarse sand affected only slightly species composition and community structure. We concluded that even short-term input of terrestrial sediments causes impacts on benthic macroinvertebrates, and recommend that land-use management of tropical catchments should employ practices that reduce input of terrestrial sediments to streams.

Keywords Substrate · Disturbance · Erosion · Aquatic insects

Introduction

Inorganic sediments of terrestrial origin may impact aquatic ecosystems in a number of ways. The origin of this sediment can be natural or a result of human activities (Waters, 1995; Wood & Armitage, 1997). Most of the natural sources of sediments are the result of intense rain events in the drainage basin and consequent surface transport into streams. This is particularly intense in deforested landscapes, like

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those created after forest fires (Beaty, 1994). In addition, the floods resulting from these intense rain events may cause erosion of the stream bank and channel (Lane & Sheridan, 2002; Silva et al., 2003). Input of sediment by human activities results mainly from agriculture and urban land use (Buss et al., 2002), deforestation (Wantzen, 1998, 2006), logging activities (Fransen et al., 2001), mining (Quinn et al., 1992), and road construction and usage (Cline et al., 1982; Waters, 1995; Fossati et al., 2001). High sediment loads make water treatment more costly, modify the morphology and depth of the channel (Wantzen, 2006), and can damage the turbines of hydroelectric power stations (Carvalho, 1994).

For aquatic communities, sediment smaller than 2 mm is most harmful (Waters, 1995). High concentrations of fine sediment increase the water turbidity, decreasing light penetration, and consequently decreasing the productivity of these ecosystems (Davies-Colley et al., 1992). Also, the deposited sediment reduces epiphytic algae biomass (Francoeur & Biggs, 2006), diversity, and productivity, indirectly affecting fish (Cordone & Kelley, 1961; Shaw & Richardson, 2001) and grazing invertebrates (Cline et al., 1982; Rier & King, 1996) that feed on these algae.

Previous studies examining the effects of terrestrial sediment on stream macroinvertebrates reported reductions in abundance, richness, and diversity (Marchant, 1989; Angradi, 1999; Shaw & Richardson, 2001; Wantzen, 2006; Connolly & Pearson, 2007). Suspended and deposited sediment modify the habitat structure of the streambed. For instance, large quantities of deposited sediment limit the interstitial space for some aquatic organisms (Angermeier et al., 2004), decreasing their abundances (Gayraud & Philippe, 2001). Also, large quantities of sediment reduce interstitial velocity (Angradi, 1999). On the other hand, burrowing organisms, such as some Chironomidae and Coleoptera larvae as *Oulimnius* sp. and *Stenelmis* sp. may benefit (Gayraud & Philippe, 2001). Suspended sediment adheres to the filter structure of organisms, such as Simuliidae (Diptera) and Hydropsychidae (Trichoptera), causing reduction in quantity and quality of food captured (Wood & Armitage, 1997; Fossati et al., 2001). For instance, Strand & Merritt (1997) added sediments to microcosms and found that survivorship of the Trichoptera *Hydropsyche betteni* Ross declined from 90% in controls to 60% in sedimentation treatments. In the same experiment, the survivorship of the Trichoptera *Ceratopsyche sparna* Ross in controls was 40% and declined to 32% in treatment with sediments. Suspended particles might also reduce the capture success of visually oriented fish, such as trout and cause reduced fitness and growth rates (Shaw & Richardson, 2001).

Transport of sediment is dependent upon hydraulic parameters, such as flow, streambed slope, and the local bed composition (Wood & Armitage 1997; Amos et al., 2004). These parameters can modify the effect of sediment on aquatic ecosystems (Culp et al., 1986; Carvalho, 1994; Francoeur & Biggs, 2006). High sediment loads are associated with high discharge events, and some studies found a linear relationship between flow and suspended sediment concentration (Amos et al., 2004). High flows during spates, and the associated increase in sediment transport, usually reduce density of benthic organisms (Melo et al., 2003a; Bond & Downes, 2003). This effect can be amplified in impacted sites, where abundant terrestrial sediments are available (Wantzen, 1998, 2006).

Negative effects of sediments may be dependent on particle size, although only a few studies have treated this aspect. For instance, Runde & Hellenthal (2000) added particles of four size classes in the range 0.126–2.000 mm, and observed that drift behavior of the Trichoptera *Hydropsyche sparna* Ross was highest for the smallest particles and that no drift occurred in treatments with addition of particles of the largest size class.

Most of the previous studies of sedimentation in streams investigated the source of sediments in the landscapes, particularly those resulting from human impacts (e.g., Mol & Ouboter, 2004). Additionally, most of the studies that investigated the impact of terrestrial sediments on the stream biota were carried out in the laboratory (microcosms) (e.g., McClelland & Brusven, 1980; Strand & Merritt, 1997; Runde & Hellenthal, 2000; Wood et al., 2001) or in temperate streams (e.g., Angradi, 1999; Kreutzweiser et al., 2005; but see Wantzen, 1998, 2006; Connolly & Pearson, 2007). As species adapted to different environmental conditions may respond differently to human or natural disturbance, results obtained in temperate regions may not be valid for tropical regions. In this study we evaluated experimentally in situ the effect of the addition of terrestrial sediment of two size classes on benthic macroinvertebrates inhabiting a subtropical stream.

Materials and methods

Study area

The experiment was conducted in the Forqueta River, a fourth order tributary of the Maquiné River, located in Maquiné, Rio Grande do Sul, Brazil (29°31′57″S and 50°14′55″W). The Forqueta River has a catchment area of 116.1 km^2 and baseflow discharge of 1.42 m^3/s. We studied one stream reach 1.5 km long, with a streambed composed of stones, pebbles, cobbles, wood debris, and leaf litter. Most of the original riparian vegetation is still present, although impacted by human activities, particularly small-scale farming and ranching (Becker et al., 2004). The land-use in the area is not enough to cause great input of terrestrial sediment in the river. The experiment was conducted in riffles and in this microhabitat stones were not covered by sediments. Information on fish and other large-bodied animals occurring in a first-order stream of the same catchment can be found in Vilella et al. (2004). The area receives 1.400 mm of rainfall scattered along the year. The experiment was always done during baseflow and at least three days after a rain event. Physical and chemical data was colleted during the experiment in the studied reach and are presented in Table 1.

Methods

We evaluated the effect of inorganic sediment on benthic macroinvertebrates using a experiment designed in blocks and replicated 20 times. The factors were the addition or not of two fraction of sediment: fine sand (0–0.24 mm) and coarse sand (0.25–0.8 mm). The resulting four treatments consisted of no sediment (control); with both fine and coarse sand; with fine sand only; or with coarse sand only. The experiment consisted thus of 80 experimental units (4 treatments * 20 blocks). The treatments were randomized within each block and aligned to stream flow distant at least 1 m of each other (Fig. 1). The stream sections chosen to place each block were riffles at least 5 m long and with homogeneous substrate composition (cobbles and stones). The study was conducted in several days in the period March–June 2006.

The sediment used in the experiment was obtained from a dirt road parallel to the stream and sieved through 0.25 and 0.8 mm mesh. In order to add the sediment to each treatment, we used a bucket with a connected hose (12 mm inner diameter) in its side (Fig. 2). After placing the sediment in the bucket, we continuously added water while stirring the deposited sediment to completely suspend it in the water. The suspended sediment was then drained through the hose into the experimental area. The volume of sediment added in each experimental unit was 3100 cm^3. In the

Table 1 Mean, minimum, maximum, and standard deviation of physical and chemical variables in the studied reach of the Forqueta River obtained for each of the 80 experimental units in several days during the period of study (March–June 2006)

	Mean	Min	Max	SD
Oxygen (mg/l)	6.7	5.6	8.1	0.5
Oxygen (%)	71.7	60.4	97.2	8.8
pH	6.9	6.4	7.2	0.2
Conductivity (μS/cm)	37.2	31.7	45.0	2.2
Temperature (°C)	17.4	13.0	24.0	4.0
Flow (cm/s)	23.4	9.4	45.6	7.9
Depth (cm)	23.6	12.0	45.0	7.9

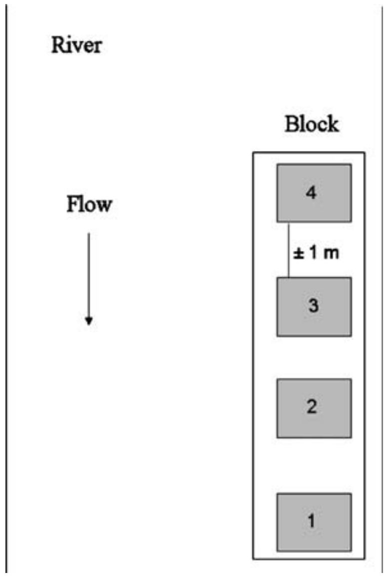

Fig. 1 Blocks used in the experimental addition of sediment. The boxes represent the samples, and the numbers inside the boxes represent the sample sequence

Fig. 2 Addition of fine sediment to the stream. (**A**) addition of sediment to the bucket. (**B**) addition of water to the bucket with sediment. (**C**) slurry fed into the stream through a hose. (**D**) dispersal of the sediment in the stream water

control treatment, we added only water. The addition of sediments (or water in the control) to each experimental unit lasted for 15 min. Around 180 l of water were added to the control treatment and 70–90 for the treatments containing inorganic sediment. Water was obtained from the same stream site. Treatments within each block were always applied downstream to upstream, taking care to not disturb the upstream block area during sample collection.

Immediately after adding the sediments, we collected one benthic sample using a Surber net (25 cm × 25 cm; mesh size 0.5 mm). The sample was obtained about 30–40 cm downstream from the sediment source. This distance was sufficient for the suspended sediment to disperse over the area where the Surber sample was later collected. The collected macroinvertebrates were stored in plastic containers with 80% ethanol. At the laboratory, the macroinvertebrates were sorted, counted and identified to the lowest taxonomic level possible, usually genus or morphospecies, following published taxonomic keys (Pitoni et al., 1976; Merritt & Cummins, 1996; Domíngues et al., 1992; Angrisano, 1995; Olifiers et al., 2004; Pes et al., 2005; Dias et al., 2006). Diptera and non-insects were usually sorted to family.

Data analysis

We assessed the effect of sediment addition on the abundance and species richness of macroinvertebrates. Species richness is strongly affected by the number of individuals in a sample (Gotelli & Colwell, 2001). Accordingly, patterns obtained using raw species richness may reflect high density in the patch and thus a sampling artifact (McCabe & Gotelli, 2000; Melo et al., 2003a). In order to remove the effect of sample abundance on species richness, we also carried out the analysis using rarefied species richness as the response. Rarefied species richness was obtained for samples of three treatments within each block, standardized to the number of individuals in the smallest sample (the fourth treatment).

The analysis of the data for each response was done initially using two-way blocked Analysis of Variance. The two predictor variables were fine and coarse sand, and were entered into the model as categorical variables with two levels each (presence or absence of sediment). We were most interested to assess the potential interaction of sediment types. None of the three analyses (abundance, species richness, and rarefied richness) revealed a significant interaction between sand types. For simplicity, we thus opted to use a one-way blocked Analysis of Variance, where the explanatory variable contained four treatment levels (i) fine sand, (ii) coarse sand, (iii) fine and coarse sand, and (iv) control (water only; see above). Abundance data were log-transformed before the Anova analysis. In case of differences among treatments, we employed a Dunnett test to compare the control to each of the three sediment-addition treatments.

We also evaluated the effect of sediment addition on relative abundance and species composition of macroinvertebrates using a distance-based MANOVA. A triangular Euclidian distance matrix was obtained from $\log(x + 1)$ transformed abundance data. We then obtained the sum of squares of the distances among sampling units in different treatments. In case of differences among treatments, this statistic should be high (Pillar & Orlóci, 1996). The significance of the statistic was assessed using a permutation test. Since our experiment was designed in blocks, permutations were restricted to those experimental units belonging to the same block. A total of 10,000 permutations were carried out to compute the *P*-value. For the species composition data, we obtained a dissimilarity matrix using the Jaccard index. The same statistical and permutation procedure used for the abundance data was employed for the presence/absence data. All multivariate analyses were done using the Multiv program (Pillar, 2006).

Results

Composition of community

One of the blocks contained a few individuals (3–17) for a reliable analysis, and we opted to exclude it from further analysis. The remaining 19 blocks (76 Surber samples) contained 6,128 individuals distributed in 79 taxa. Ephemeroptera with 2,084 individuals, Coleoptera with 1,597 individuals, and Trichoptera with 998 individuals were the most abundant groups. They represented 76.34% of all individuals collected.

Effect of terrestrial sediment on benthic macroinvertebrate

Macroinvertebrate abundance was significantly affected by addition of sediments ($F_{3,54} = 4.99$, $P = 0.004$) (Fig. 3a). The Dunnett test indicated a reduction in abundance with addition of coarse sand ($P = 0.006$) and coarse+fine sand ($P = 0.003$) in relation to the control. Fine sand affected abundance only slightly ($P = 0.089$). Response of species richness to sediment addition was similar to that of

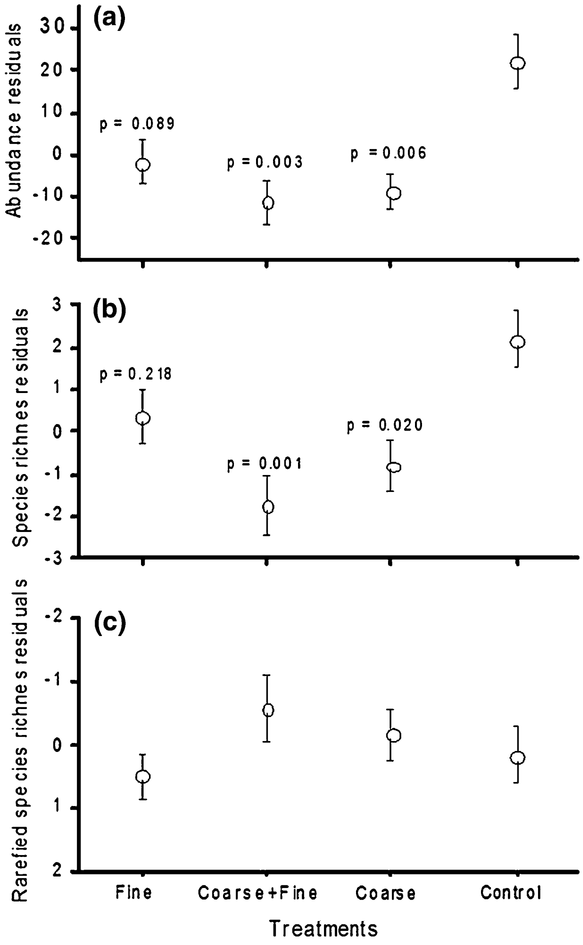

Fig. 3 Mean and standard error of residuals from block for the effect of sediment addition treatments on abundance (**a**), species richness (**b**), and rarefied species richness (**c**). Abundance residuals differed among treatments ($F_{3,54} = 4.99$, $P = 0.004$). Species richness residuals differed among treatments. There was no difference among mean values of rarefied species richness residuals ($F_{3,54} = 0.84$, $P = 0.48$). The *P*-values refer to the comparison of the treatment to the control using the Dunnett test

abundance. Addition of sediment reduced species richness in relation to the control ($F_{3,54} = 5.00$, $P = 0.004$) (Fig. 3b). There were significant differences between coarse sand (Dunnett test, $P = 0.020$) and fine+coarse sand in relation to the control ($P = 0.001$). Fine sand did not affect species richness ($P = 0.218$). However, the response of stream macroinvertebrates in terms of rarefied species richness was not affected by addition of sediment ($F_{3,54} = 0.84$, $P = 0.48$) (Fig. 3c), indicating that differences in observed species richness were due mostly due to differences in abundance among treatments.

The distance-based Manova using abundance data indicated that treatments differed (SSQ = 9.2, $P = 0.003$). This difference was most evident for the comparison control and coarse sand (SSQ = 5.41, $P < 0.001$). The addition of fine sand (SSQ = 3.1, $P = 0.070$) or fine+coarse sand (SSQ = 3.22, $P = 0.046$) caused moderate change in community structure. In terms of species composition only, the Manova using Jaccard distance indicated differences among treatments (SSQ = 1.95, $P = 0.010$), although of lower magnitude than that found for abundance data. Similarly to that found for abundance data, the most distinct effect was caused by addition of coarse sand (SSQ = 0.75, $P = 0.012$). Addition of fine+coarse sand resulted in moderate change of species composition (SSQ = 0.68, $P = 0.043$), whereas addition of fine sediment did not result in changes (SSQ = 0.55, $P = 0.450$).

Discussion

Experimental studies are important to exclude competing factors affecting the response of interest (Bond & Downes, 2003; Matthaei et al., 2006). They are particularly useful in assessing the effect of terrestrial sediment in streams, because their input usually occurs associated with other potential disturbance factors resulting from general land use (Buss et al., 2002). We showed that the experimental addition of sediment decreased the abundance and the richness of benthic macroinvertebrates in relation to the control. However, rarefied richness did not show differences between the locations with or without sediment addition. This latter result indicates that sediment addition mostly affects abundance, and that the reduction in observed species richness is likely a sampling artifact. Sediments also affected the community structure of the stream in terms of relative abundance and species composition.

Most of the previous studies have reported negative effects on benthic fauna (Angradi, 1999; Shaw & Richardson, 2001; Suren & Jowett, 2001; Wantzen, 2006; Connolly & Pearson, 2007). For instance, Shaw & Richardson (2001) showed a reduction in the abundance of macroinvertebrates with a sedimentation pulse, attributing this reduction to the increase in drift caused by physiological stress. Similarly, Suren & Jowett (2001) found a reduction in benthic abundance and a concomitant increase in drift of *Paracalliope fluviatilis* Thomson, *Oxyethira albiceps* McLachlan, *Hydrobiosis* sp. and larvae of Chironomidae, resulting from sedimentation. Rosenberg & Wiens (1978) observed that the addition of sediment to in-stream channels increased drift of Oligochaeta, Simuliidae, Plecoptera, Ephemeroptera, Hydracarina, and Chironomidae.

The negative impact of inorganic sediments of terrestrial origin on density of benthic macroinvertebrates seems to be independent of their origin. In our experiment, sediments were collected from a nearby dirt road. Cline et al. (1982) studied the effect of sediments resulting from road construction, and found a decrease in density of stream macroinvertebrates. In New Zealand, Quinn et al. (1992) observed that the input of sediment from gold mining was responsible for reduction of the density of invertebrates in six streams. Similarly to abundance, previous studies have found a reduction in the taxonomic richness of invertebrates in locations with high sediment input (Marchant, 1989; Quinn et al., 1992; Matthaei et al., 2006; Wantzen, 2006; Connolly & Pearson, 2007). Shaw & Richardson (2001) observed a reduction in the richness of invertebrate families with the increase of the sedimentation pulse. Fernández-Aláez et al. (2002) also found a decrease in the taxonomic richness of Trichoptera with the release of sediments from a coal mine.

Species richness observed in a sample is highly dependent on the number of individuals present. This relationship is widely recognized in the ecological literature (Gotelli & Colwell, 2001). The most straightforward way to deal with this dependency is to remove the effect of abundance by standardizing samples to the size of the smallest sample using rarefaction (an alternative is extrapolation; see Melo et al., 2003b). Despite the wide recognition and methods to control for this dependency, most of the previous studies assessing the effects of sediments on benthic assemblages rely on observed species richness only. McCabe & Gotelli (2000), in a study evaluating the frequency and intensity of disturbance events on number of species, found that different conclusions are reached using observed or rarefied species richness, and that the most realistic was that using rarefied richness. In our study, we did not detect a reduction in rarefied species richness following addition of sediments. Accordingly, the impact of sediments acts on individuals rather than on the species. If there is a reduction in raw species richness, this is due to a sampling artifact, resulting from a reduced number of individuals. The alternate mechanism, not observed in our study, would be the effect of sediments on species. The decrease in abundance would be most intense for some species, which in fact has been observed before (McClelland & Brusven, 1980). In this case, not only abundance but also rarefied richness would be reduced in treatments.

Cline et al. (1982) studied the effect of fine sediments derived from road construction on stream macroinvertebrates, and found reduced density downstream from the input source. However, the pattern was not as clear for diversity, measured using the Shannon index. Interestingly, one of the best characteristics of diversity indexes, in relation to observed species richness, is its low dependence on sampling effort (Magurran, 2004, p. 134). In other words, the index values tend to stabilize in increased sample sizes. Our results are thus in agreement with those of Cline et al. (1982).

In relation to the control treatment, coarse sand (0.25–0.8 mm) caused a stronger negative effect on total abundance than did fine sand (0–0.24 mm). This effect was evident both in the treatment with coarse sand only or in the combination fine+coarse sand. Similarly, the effects of coarse or fine+coarse sand were evident in both Manova analyses using abundance or presence/absence data. In these two multivariate analyses, addition of fine sand did not affect community composition and structure in relation to the controls. On the other hand, Wood et al. (2001) noted that the cased Trichoptera *Melampophylax mucoreus* (Hagen) was most affected by the finest particle size class in the study (0.125–0.249 mm). However, the quantity of sediments added in the study of Wood et al. (2001) was enough to completely cover the substrate (5 or 10 mm deep) and was added instantly, burying the Trichoptera larvae. In our study, the sediment remaining on the surface of the substrate was not enough to cover invertebrates, particularly in treatments with fine sand, due to water transport. The quantity of fine sand used in our study and that deposited in the streambed perhaps did not attain the threshold level suggested by Kaller & Hartman (2004) for the expression of negative effects. Similar to the results of Wood et al. (2001) and opposite to ours, Runde & Hellenthal (2000) found that drift of *Hydropsyche sparna* increased more with small particles (0.126–0.250 mm) than with medium particles (0.251–1.000 mm), and there was no drift with large particles (1–2 mm). At least two mechanisms may be involved in the negative effect of terrestrial sediment in streams: attrition and covering of substrate (Waters, 1995; Strand & Merritt, 1997; Fossati et al. 2001). Our experiment consisted of a very short input of sediments, insufficient to cover the substrate. Accordingly, the likely mechanism involved in the loss of individuals from our experimental areas was abrasion. This is consistent with our finding that coarse sand caused grater loss of individuals than fine sand.

The experimental input of inorganic sediment caused a negative effect on the abundance and raw richness, but not on the rarefied species richness of benthic macroinvertebrates. The negative impacts on abundance and changes in community structure were stronger for coarse sand (0.25–0.80 mm) than for fine sand (0–0.24 mm) and likely reflect the high attrition of the large particle on invertebrates. Our results indicate that even short-term input of terrestrial sediments is sufficient to reduce abundance and change community structure. Accordingly, management of tropical catchments should adopt practices that reduce the input of terrestrial sediments to streams.

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