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Research Paper

Experimental Assessment of the Effects of Environmental Factors and Longitudinal Position on Alpha and Beta Diversities of Aquatic Insects in a Neotropical Stream

key words: substrate heterogeneity, partial redundancy analysis, Ephemeroptera, Plecoptera, Trichoptera

Abstract

We evaluated the effect of environmental factors on the abundance and genera richness of aquatic insects colonizing artificial substrates. We also assessed the relative effects of environmental factors on assemblage variation (beta diversity), and contrasted its magnitude with the variation associated with the spatial position of reaches in a Neotropical stream. Homogeneous and heterogeneous bricks were installed in seven stream reaches and removed after 60d. Substrate type and organic matter showed significant effects on abundance. For observed richness, substrate type was the only important variable. For rarefied richness, no explanatory variable was important. A NMDS ordination of bricks revealed the effect of spatial position and substrate type. A partial Redundancy Analysis (pRDA) indicated that the environmental matrix explained 12.44% of the total variation, while reach position explained 7.41%. We conclude that at local scale (reaches 430–920 m apart), environmental factors, especially substrate heterogeneity, were important in determining the alpha and beta diversities of the insect assemblage in the stream.

1. Introduction

The total diversity in a region, or gamma diversity, can be partitioned into two components: (i) the local component, termed alpha diversity, and (ii) the changes in species composition (turnover) among local assemblages, termed beta diversity (Whittaker, 1960; Loreau, 2000). The alpha component can be estimated as the local average richness or

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values from a diversity index (Magurran, 2004). The beta diversity component represents the turnover in species composition among sites (Whittaker, 1960), and can be estimated by dissimilarity indexes (Koleff *et al.*, 2003) or simply as the difference between regional and local diversities (Crist *et al.*, 2003). Recently, Anderson *et al.* (2011) suggested researchers should recognize two main types of beta diversity, directional turnover along a gradient (spatial and/or environmental) and variation among communities. In the first case (turnover), the idea is to measure how community changes from one sample unit to another in a gradient in terms of loosing and gaining species, while in the second case (variation) the idea is to assess overall variation among sampling units in a given spatial extent (Anderson *et al.*, 2011).

In addition to the estimation of beta diversity, ecologists usually are interested in the relationship of beta diversity and environmental or spatial predictor variables. Legendre *et al.* (2005) pointed out that variation in community composition do not necessarily translate in variation in dissimilarities and suggested that constrained ordination methods should be used to evaluate relationships between variation in community composition (or abundance) and predictor variables. Tuomisto and Ruokolainen (2006) recognized that the term ‘beta diversity’ is used to describe variation in community composition and variation in dissimilarities. Accordingly, they distinguished two main classes of methods to analyze or explain beta diversity, each one used to assess distinct hypotheses. Methods such as the Mantel or distance-based regressions aim to model variation in biological dissimilarities in relation to environmental or spatial dissimilarities/distances. On the other hand, methods of constrained ordination (Canonical Correspondence Analysis, Redundancy Analysis) explain variation in community composition or abundance.

According to Clarke *et al.* (2008), beta diversity is a fundamental aspect for understanding diversity patterns and is mostly determined by i) environmental dissimilarities, and ii) geographical distance among places. At biogeographical scales, evolutionary processes such as speciation are the key factors in the differentiation of biota. Recently, emphasis has been given to (i) the quantification of changes in diversity among assemblages (Koleff *et al.*, 2003; Magurran, 2004), (ii) how the total diversity in a region is partitioned among lower spatial scales and habitats (Crist *et al.*, 2003; Ligeiro *et al.*, 2010), and (iii) the determination of factors that explain variation in beta diversity (Legendre *et al.*, 2005; Soininen *et al.*, 2007; Costa and Melo, 2008).

Streams are heterogeneous in terms of physical characteristics, and this is reflected in the high variability of assemblages only a few meters apart from each other (Heino *et al.*, 2004; Costa and Melo, 2008). Most of the physical variations within streams are caused by slope, which in turn determines current velocity and, together with the geological constitution of the area, substrate type (Heino *et al.*, 2004). Substrate type and heterogeneity are key factors in the determination of the stream biota, and have been studied extensively (Hynes *et al.*, 1976; Hart and Finelli, 1999; Graça *et al.*, 2004; Jiang *et al.*, 2010). For instance, substrate heterogeneity allows the coexistence of an increased number of species by providing distinct microhabitats or refuges (Johnson *et al.*, 2003; Mykrä *et al.*, 2007; Schneck *et al.*, 2011) and may cause high persistence of macroinvertebrate at small stream patches (Brown, 2007). Habitat heterogeneity is also the basis of many current models of community structure in streams (Winemiller *et al.*, 2010).

In addition to differences in physical characteristics, variability of the stream insect fauna can be caused by restrictions on dispersal that slow the colonization of disturbed areas (Death, 1996; Watanabe *et al.*, 2008), or those in which only a few or even no females were able to successfully deposit eggs (Bunn and Hughes, 1997). Despite the recognition of potential patchiness in stream fauna caused by dispersal restrictions, even among sites less than 30 km apart (Watanabe *et al.*, 2008), few experimental studies have evaluated geographical distance as an explanatory variable and its importance in relation to environmental factors (Soininen, 2004; Munn *et al.*, 2009; Landeiro *et al.*, 2011).

The concept of beta diversity has been used extensively in the study of spatial gradients (HARRISON *et al.*, 1992; MAGURRAN, 2004), although turnover in gradients is the result of environmental dissimilarities and geographical distances. The intrinsic correlation between environmental and spatial components represents a problem for the determination of the exclusive contribution of each component (DINIZ-FILHO *et al.*, 2003; GENNER *et al.*, 2004; LEGENDRE *et al.*, 2005). Studies that evaluate both environmental and spatial factors, usually partition total variability in species composition into four fractions: [a] variability explained exclusively by the environment, [b] variability explained by the environment and space (shared fraction), [c] explained exclusively by space, and [d] unexplained (residual) variance.

We studied the diversity of insects of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) colonizing clay bricks in several reaches of a single stream. First, we investigated the effect of brick heterogeneity, water flow, and trapped coarse detritus on genera richness (alpha diversity). Previous studies reported higher richness in heterogeneous substrates containing large amounts of coarse detritus (JOHNSON *et al.*, 2003; HEINO *et al.* 2004; GRAÇA *et al.*, 2004; GONZÁLEZ and GRAÇA, 2005). However, substrate heterogeneity and organic matter cause an increase in the density of organisms, and thus it is not clear whether high genera richness in heterogeneous substrates associated with coarse detritus is an indirect effect of high abundance. We therefore evaluated the effect of these three factors, taking into account abundance using rarefaction. Second, we investigated the effects of local factors (brick heterogeneity, current velocity, and amount of coarse detritus) on assemblage variation (beta diversity), and contrasted its magnitude with the variation associated with the spatial position of reaches in the stream.

2. Methods

2.1. Study Area

Our experiment was set up in the Forqueta stream, a tributary of the Maquiné River located in Maquiné county, state of Rio Grande do Sul, Brazil (29°31′57″ S, 50°14′55″ W) (Fig. 1). The region is inserted in the extreme southern portion of the Atlantic Rain Forest. The Forqueta stream has a catchment area of 116.1 km^2 mostly covered by old-growth and secondary forests, base flow discharge of 1.42 $m^3 s^{-1}$, and annual rainfall of 1400 mm. The water is well oxygenated (dissolved oxygen > 7 mg L^{-1}), and has lightly acid pH (6.5), and low electrical conductivity (< 50 µS cm^{-1}). The streambed is mostly rocky and more than 70% of the area is covered with particles in the range 10–40 cm (maximum diameter).

2.2. Field Experiment

To evaluate substrate heterogeneity on EPT, we constructed substrates using hollow clay bricks, 25.5 × 12 × 10.5 cm. The bricks were filled with bags of sand to assure substrate stability in the streambed, and were closed by attaching two unglazed clay tiles with silicone glue. Two substrate types, differing in heterogeneity, were constructed (Fig. 2). The heterogeneous substrates were confectioned by carving lines ca. 3 mm deep in the lateral surfaces using an electric concrete saw and by attaching tiles with their grooved sides facing out. In addition, we attached two pieces of tiles (ca. 5 × 5 cm) on each side of the bricks and two plastic strips (ca. 5 × 2 cm) on the top and bottom surfaces (grooved tile surfaces, see Fig. 2). To avoid losing bricks downstream, we attached them to nylon lines anchored to iron bars buried ca. 40 cm deep and located 1 m upstream from the brick. The major axis of the brick was installed parallel to the stream flow and with the tile sides facing up and down.

One set of six bricks (three heterogeneous and three homogeneous bricks) was installed in each of seven consecutive reaches (430–920 m apart). Within reaches, bricks were installed in similar riffles. The bricks were installed in the stream in July 2007 and removed after 60 d. We used a U-net (mesh size 250 µm) to retain invertebrates during brick removal. The bricks were washed and carefully examined for attached insects. The organisms were fixed with 70% ethanol. In the laboratory, insects of the orders

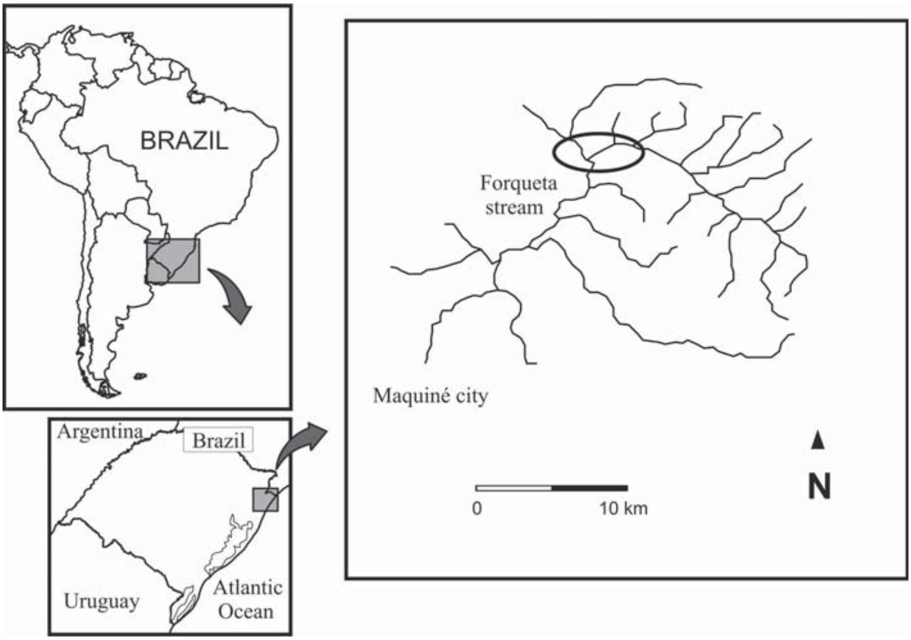

Figure 1. Map of the studied region indicating the studies sites in the Forqueta stream.

Ephemeroptera, Plecoptera, and Trichoptera (EPT) were separated from the detritus and identified to genus according to MERRITT and CUMMINS (1996), FERNÁNDEZ and DOMÍNGUEZ (2001), SALLES *et al.* (2004), and COSTA *et al.* (2006). Previous studies have showed the effectiveness of using EPT groups and quantitative data to uncover even subtle patterns in stream ecology studies (MELO, 2005).

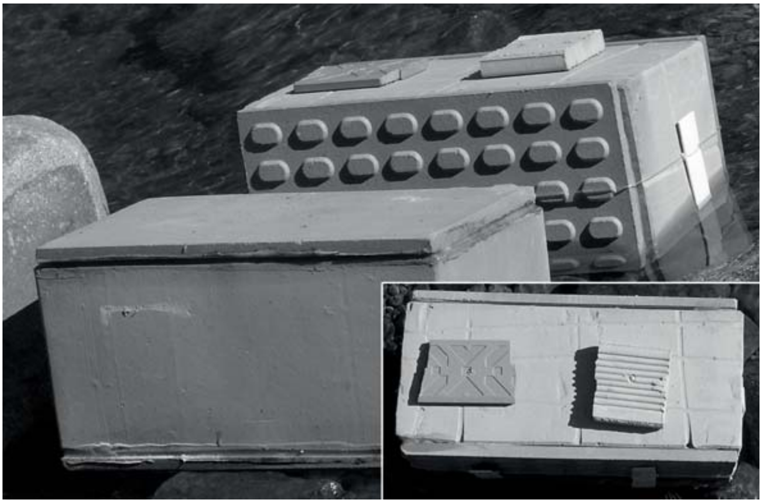

Figure 2. Bricks used as substrate in the experiment. In detail, complementary plates glued to the brick surface to increase heterogeneity.

The current velocity and organic matter associated with each brick were quantified during its removal. The current velocity was measured before removal of the brick positioning a mechanical flowmeter (General Oceanics, USA) on top of brick. The coarse particulate organic matter adhered to the bricks was removed and incinerated in a muffle oven (550 °C for 5 h). The organic matter (ash-free dry mass) was obtained by the difference of the total mass and the remaining mass.

2.3. Data Analysis

2.3.1. Environmental Effects on Abundance, Richness and Rarefied Richness

We employed a Generalized Linear Model (GLM) to evaluate the effect of flow velocity, organic matter, and brick heterogeneity on abundance and on the number of genera at each brick. The model included reaches as blocks. We removed non-significant variables ($P > 0.05$ for deviance difference using a Chi-square distribution) and refit the model until the partial effects of explanatory variables were important. The models were initially fit using the Poisson distribution, appropriate for count data (O'HARA and KOTZE, 2010). However, the residual variance of these models were much larger than the residual degrees of freedom, indicating overdispersion (CRAWLEY, 2005, p. 230). Accordingly, we employed a quasipoisson distribution in the GLM. We also analyzed rarefied genera richness, in order to test whether the effect of the three variables on richness was an indirect effect of abundance (MCCABE and GOTELLI, 2000; GOTELLI and COLWELL, 2001). Because the comparisons were done within blocks (reaches), we applied rarefaction to each of the five bricks in a reach to obtain a richness estimate for the subsample size (number of individuals) similar to that observed in the brick harboring the lowest abundance. Given that rarefied richness is non-integers, we employed the Gaussian distribution in the analysis. We removed non-significant variables ($P > 0.05$ for residual difference using a F-test) and refit the model until the partial effects of explanatory variables were important.

2.3.2. Importance of Environmental and Distance Factors on Assemblages

We initially explored the relative importance of environmental conditions and space on the resemblance among the faunas colonizing individual bricks using Non-Metric Multidimensional Scaling (NMDS) ordination on two axes (results not shown). A single brick harbors a limited number of individuals and genera, and thus may not represent very well the potential fauna available. We therefore opted to run a second NMDS ordination using pooled samples including the three bricks of the same heterogeneity type and reach. This analysis included 14 pooled samples (7 reaches × 2 brick types). The resemblances among objects were obtained using the Bray-Curtis dissimilarity index on log-transformed (log[abundance + 1]) data.

The relative importance of the environmental variables (at brick scale) and geographical distance (at reach scale) on variation in community structure was analyzed using a partial Redundancy Analysis (pRDA). The analysis is aimed at the second level of abstraction of TUOMISTO and RUOKOLAINEN (2006) and assesses beta diversity as the variation in the raw-data table. The environmental data matrix used in pRDA included three variables: (i) flow velocity at bricks (continuous), (ii) organic matter (continuous), and (iii) brick type (categorical, two levels). We employed a single geographical variable, distance from the most upstream reach through the stream corridor, to model the effect of space on assemblage variability. The relative importance of the environmental and spatial matrices was contrasted using adjusted R^2 (PERES-NETO *et al.*, 2006). This approach was used because the environmental matrix contained three variables versus only one variable (distance from the first upstream reach) in the spatial matrix. Analyses were done in the *R* environment (R DEVELOPMENT CORE TEAM, 2011) using the "vegan" package (OKSANEN *et al.*, 2009) for the rarefaction and multivariate analyses.

3. Results

Samples from the seven reaches contained 16,299 individuals and 34 genera of EPT (Table 1). Ephemeroptera was the most abundant order, and included 14,153 individuals (86.8%) and 11 genera. Trichoptera included 1931 individuals (11.8%) and 18 genera, and Plecoptera 215 individuals (1.4%) and six genera. *Baetodes* (Ephemeroptera, Baetidae), *Farrodes* (Ephemeroptera, Leptophlebiidae), and *Smicridea* (Trichoptera, Hydropsychidae) were the most abundant genera.

Table 1. Average (± standard deviation) of total individuals, observed and rarefied richness, current velocity, and organic matter in homogeneous and heterogeneous substrates in Forqueta stream, Maquiné, Brazil.

Variables	Substrate type	
	Homogeneous	Heterogeneous
Total organisms	4665	11,634
Mean of organisms per brick	222.1 (± 195.3)	554 (± 735.2)
Observed richness	13.1 (± 2.5)	16.8 (± 3.8)
Rarefied richness	9.9 (± 2.9)	9 (± 3)
Current velocity (cm s^{-1})	23.6 (± 20.8)	20.8 (± 8.8)
Organic matter (g)	0.94 (± 1.11)	1.69 (± 1.8)

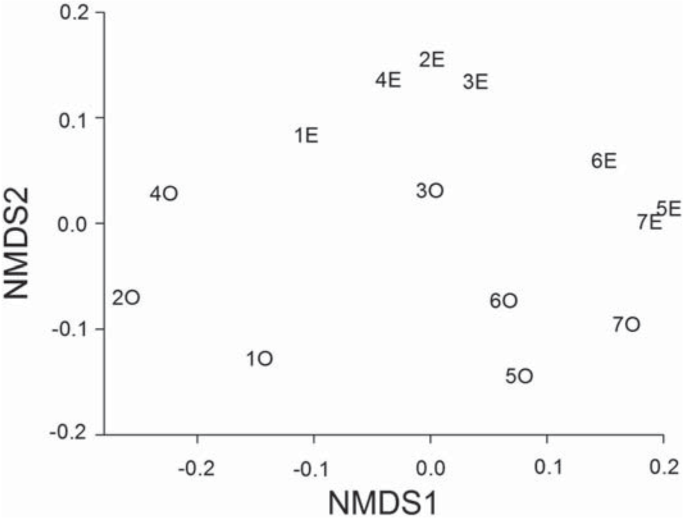

Figure 3. Non-Metric Multidimensional Scaling ordination of 14 pooled samples (stress = 12.7%) in seven reaches of the Forqueta stream. Codes 1 to 7 indicate the stream reach. The reaches are numbered consecutively from downstream to upstream. O = homogeneous substrate. E = heterogeneous substrate.

The reduced GLM model for abundance data retained organic matter and substrate type. Abundances observed on heterogeneous bricks were higher than on homogeneous bricks (difference in deviance = 2452.7, *df* = 1, $P < 0.001$) and on those harboring large quantities of organic matter (difference in deviance = 640.2, 1 *df* = 1, $P = 0.024$). For the observed genera richness, substrate type was the only important variable, and, similarly to abundance, more genera were found on the heterogeneous bricks (difference in deviance = 6.5, *df* = 1, $P = 0.003$). No explanatory variable was important to explain the variation in rarefied genera richness.

The NMDS ordination of pooled samples (sum of three bricks) reflected substrate type and spatial position (Fig. 3). The first axis separated samples according to spatial position, and scored downstream reaches 1–4 in the left-hand portion of the ordination diagram. The second axis separated samples according to substrate type, with heterogeneous bricks scored in the upper part of the diagram (Fig. 3).

The pRDA indicated that the environmental matrix explained 12.44% of the total faunal variation (beta diversity) on bricks (adjusted R^2) ($P < 0.001$; Fig. 4), while space, measured as distance from the first upstream reach, explained 7.41% ($P < 0.001$). The shared explained variation by the two groups of variables was near zero (negative adjusted R^2), indicating that the environmental and spatial effects were independent from each other. Most of the variation in species composition and relative abundances remained unexplained (80.8%). The ordination of sites after taking into account the effect of space showed that the three

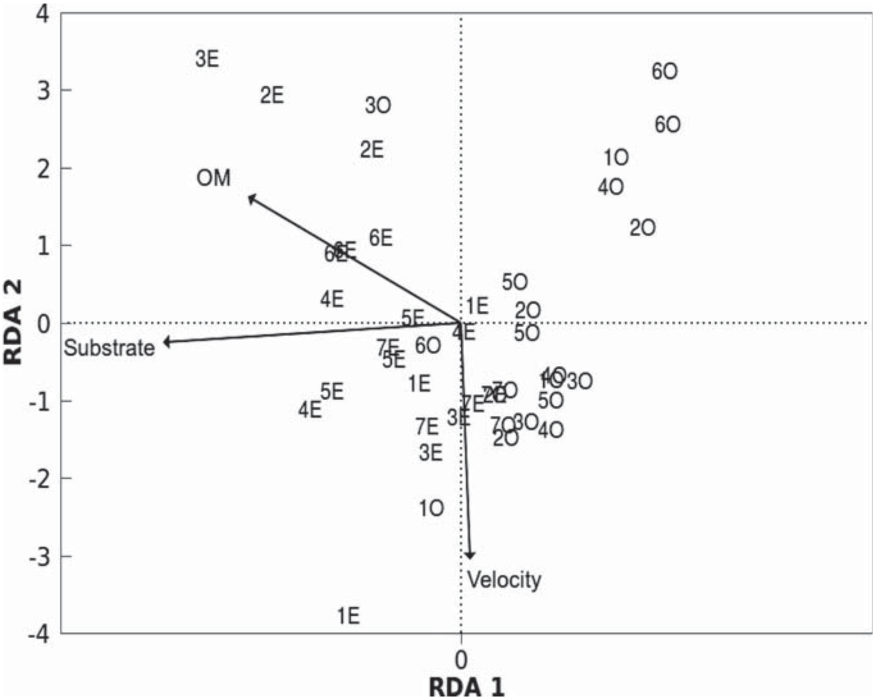

Figure 4. Partial Redundancy Analysis (pRDA) of Ephemeroptera, Plecoptera and Trichoptera (EPT) associated to 42 artificial bricks in the Forqueta stream. The analysis was done taking into account the effect of space. Codes 1 to 7 indicate the stream reach. The reaches are numbered consecutively from downstream to upstream. O = homogeneous substrate. E = heterogeneous substrate. OM = Organic matter trapped in bricks.

environmental variables presented similar correlation with the first two axes of the ordination (Fig. 4). Substrate type was correlated to axis 1 and provided a good distinction of homogeneous and heterogeneous bricks. Axis 2 was mostly correlated to flow velocity.

4. Discussion

4.1. Environmental Effects on Abundance, Richness and Rarefied Richness

We found that substrate heterogeneity increased the observed abundance of individuals and alpha diversity, the later measured as observed genera richness. Also, high abundances were found in bricks with larger amounts of organic matter. These findings corroborates previous studies of other stream types and regions evaluating substrate heterogeneity and availability of coarse organic matter on abundance (Johnson *et al.*, 2003; Heino *et al.*, 2004; Graça *et al.*, 2004; González and Graça, 2005). Substrates with high heterogeneity contribute to organic-matter retention, reduce negative interactions (predation and competition), create flow refuges (Munn *et al.*, 2009), and protect from disturbance events (Brown, 2007). We observed a positive effect of brick heterogeneity on alpha diversity measured as observed richness; however, this effect was not observed for rarefied richness (*i.e.*, removing the effect of abundance). Previous studies evaluating the effect of substrate heterogeneity in streams have reported a positive relationship between richness (alpha diversity) and heterogeneity (Beisel *et al.*, 2000; Boyero, 2003). However, these studies did not remove the effect of abundance on richness, and therefore the effect they observed may in fact be caused by an indirect effect of larger sample size (*i.e.*, higher abundance) (Graça *et al.*, 2004; González and Graça, 2005). McCabe and Gotelli (2000) studied the effect of small-scale disturbance in streams, and found that its effects on observed richness were actually an indirect effect of abundance. They showed that the use of rarefied richness resulted not only in different conclusions, but also in a straightforward interpretation of results. An unimportant effect of high heterogeneity on rarefied richness indicates that each taxon is affected similarly by the increase in abundance. However, it would be plausible to expect that high heterogeneity allows not only an abundance increase, but also colonization by species that are not able to persist in homogeneous substrates. Accordingly, the excess of taxa on heterogeneous substrates would be composed by two sets of species. The first set would be composed by those rare species that have a higher probability of being sampled because of the increased sample effort (number of individuals). The second set would be comprised by taxa that are not commonly found on a homogeneous substrate (*e.g.*, Schneck *et al.*, 2011). Although we did not observe such a true taxa increase at the scale of the bricks, it would be plausible to expect such a possibility at higher spatial scales.

4.2. Importance of Environmental and Spatial Factors on Assemblage Variation

The effect of substrate heterogeneity on abundance and genera richness observed in the GLM models was corroborated by the NMDS ordination. Variation or beta diversity among pooled samples in the NMDS ordination best reflected the effect of spatial position (dimension 1) and brick heterogeneity (dimension 2) on the assemblage. Some studies report that the substrate and the accumulated organic matter are key factors in the distribution of organisms at small spatial scales (González and Graça, 2005; Feld and Hering, 2007; Zilli *et al.*, 2008; Jiang *et al.*, 2010). Therefore, the coarse organic matter accumulated in the substrate increases the substrate heterogeneity and results in the increase of abundance and richness of organisms.

The pRDA analysis showed that both environmental variables and reach position had effects on variation of assemblage structure (beta diversity). Environmental variables explained 12.4% of the total variation. In Central European streams, FELD and HERING (2007) observed that environmental variables explained 22% of the variance of benthic assemblage structure at the reach scale. At micro-scale (25 × 25 cm), the variance explained by environmental variables decreased to 15.8%, a percentage of explanation similar to that found in our study. The importance of environmental factors should likely increase as different habitats are included. For example, COSTA and MELO (2008) showed that environmental factors, expressed as categories of habitats, was the main driver of beta diversity and explained 42% of the total variation in the structure of macroinvertebrate assemblages present in riffles, pools, moss attached to rocks, and submerged roots of terrestrial plants, while space (stream sites in the same catchment, also coded as a categorical variable) explained 22% of the total variation. The high percentage of unexplained variation is common in ecological studies and is due, at least in part, to important variables that are missing in the analysis (GENNER *et al.*, 2004).

In conclusion, the abundance of organisms is higher in heterogeneous substrates, but the alpha diversity (richness of taxa) appeared not to differ with substrate heterogeneity. The observed alpha diversity was related to substrate heterogeneity, but this relationship was spurious, as observed in the analysis of rarefied richness. The heterogeneity of substrate and the amount of organic matter had a direct positive effect on insect abundances, causing an increase in the observed EPT genera richness. The beta diversity among assemblages was partially explained by environmental variables at the brick scale, and by the spatial position of reaches (scale of km). Our study included a single and relatively fine spatial scale. Future studies should partition variation at multiple scales to identify on which ones environmental or spatial factors are most important.

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