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Organisation of the benthic macroinvertebrate assemblage in tropical streams of different orders in North-Eastern Brazil

Jéssica F. Gomes Pio; Tiago da Silva Pereira; Adolfo R. Calor & Carlos E. Copatti✉

Programa de Pós-Graduação em Diversidade Animal, Instituto de Biologia, Universidade Federal da Bahia. Campus de Ondina, Salvador, BA, Brazil.

Abstract. We selected streams located in north-eastern Brazil with the objective of assessing the effect of streams of different orders (1st, 2nd, 3rd) on the distribution and composition of benthic macroinvertebrates in the rainy and dry seasons. A total of 12822 individuals and 62 taxa of benthic macroinvertebrates were obtained, of which Chironomidae was the taxon with the most individuals (60.08% of the total). The benthic macroinvertebrate assemblage ordination demonstrated that streams of the 3rd order had a different assemblage structure compared to streams of the 1st and 2nd order. This distinction may be the result of taxa substitution. The benthic macroinvertebrate assemblage structure was correlated with environmental variables. In conclusion, the variation of the environmental characteristics according to stream order was the most important factor in benthic macroinvertebrate assemblage organisation and replacement of taxa in the streams investigated.

[Keywords: composition, distribution, dry and rainy seasons, Chironomidae, replacement of taxa]

Resumen. Distribución de la comunidad de macroinvertebrados bentónicos en arroyos tropicales de diferentes órdenes en el noreste de Brasil. Se seleccionaron arroyos localizados en el noreste de Brasil con el objetivo de evaluar el efecto de arroyos de diferente orden (1°, 2° y 3r) sobre la composición y la distribución de macroinvertebrados bentónicos durante las estaciones seca y lluviosa. Se obtuvo un total de 12822 individuos y 62 taxones de macroinvertebrados bentónicos, entre los cuales Chironomidae fue el taxón con mayor número de individuos. La distribución de este grupo de macroinvertebrados bentónicos demostró que los arroyos de 3r orden tienen una estructura de comunidad diferente en comparación a los arroyos de 1° y 2° orden. Esta distribución parecería ser el resultado de la sustitución de los taxones. La estructura de la comunidad de macroinvertebrados bentónicos estuvo correlacionada con las variables ambientales. En conclusión, la variación de las características ambientales de acuerdo con el orden del arroyo fue el factor más importante en la organización de la comunidad de macroinvertebrados bentónicos y la sustitución de los taxones en los arroyos investigados.

[Palabras clave: composición, estaciones seca y lluviosa, Chironomidae, sustitución de los taxones]

Introduction

The structure of the benthic macroinvertebrate communities in streams is not defined at random. Streams are heterogeneous environments in relation to their physical characteristics, and this is reflected in the wide variation in the occurrence of organisms (Costa and Melo 2008). Given the ecological importance of streams, it is important to understand the factors that control the distribution and composition of local communities and functional processes (Graça et al. 2015).

In stream ecosystems, classification of the river order has been used to assess changes in the biological community structure (Greathouse and Pringle 2006). The headwater of the river, without influence of tributaries, is considered a stretch of the first order. When two first order streams meet, a second order stream is formed, and so on. Generally, increasing stream order is accompanied by changes in stream width, water flow, depth, temperature, or quantity and type of suspended material transported with the current and its deposition (Greathouse and Pringle 2006; Allan and Castillo 2007), and drained area. These changes can affect the composition and distribution of benthic macroinvertebrates. Thus, river order can be considered a determining factor in benthic macroinvertebrate structure, with markedly different communities between headwater streams (1st and 2nd order) and medium-sized streams (3rd order, for example) (Heino et al. 2005; Bispo and Oliveira 2007).

The medium-sized streams may present several environmental variations, thereby creating ecological niches that can shelter a

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✉ carloseduardocopatti@yahoo.com.br

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greater richness and abundance of benthic macroinvertebrates (Vannote et al. 1980). In addition, medium-sized streams receive tributaries and particulate organic matter due to the unidirectional nature of the water. Therefore, these streams suffer the effect of the accumulation of water, presenting higher water flow than headwaters (Bispo et al. 2006; Tank et al. 2010). Additionally, studies assessing the influence of the river order on benthic macroinvertebrate assemblage organisation in tropical environments, such as streams in Brazil's northeast, are scarce. River basins in north-eastern Brazil have many perennial streams with low water flow and intermittent and irregular or underground water passages (Velloso et al. 2002), possibly as a result of the climate in the region, which is characterised by high temperatures with an alternation of a short rainy season and a prolonged dry season. To date, it is still not known to what extent rainfall fluctuation in this region impacts the structure of the benthic macroinvertebrate assemblage.

The hydrologic regime is a key factor in the distribution and composition of benthic macroinvertebrates (Pereira et al. 2017). In the rainy season, the richness and abundance of benthic macroinvertebrates are expected to decline (Buss et al. 2002) due to substrate disturbance by increased water flow and current velocity, and a consequent drag of organisms (Buss et al. 2004). In the dry season, when water flows are more stable, benthic macroinvertebrates tend to be more abundant (Buss et al. 2002; Nava et al. 2015).

In this study, streams located in the North-East of Brazil were selected, with the aim of assessing the effect of streams of different orders (1st, 2nd, 3rd) on the distribution and composition of benthic macroinvertebrates during the rainy and dry seasons. It was hypothesised that: a) streams of the 3rd order differ in terms of distribution of organisms, taxa, and functional feeding groups (FFG) in relation to streams of the 1st and 2nd order, and b) given the greater stability of the hydrologic regime, richness of organisms, taxa and FFG are higher during the dry season compared with the rainy season.

Materials and Methods

Study sites

The study was conducted in streams of the Santo Antônio Sub-Basin, which belongs to the upper Paraguaçu Basin. All streams are located within the Chapada Diamantina National Park (152400 ha), Lençóis, BA, North-Eastern Brazil, an Integral Protection Conservation Unit. Vegetation of this region is a mosaic of different phytogeographic formations known as the "Chapada Diamantina Complex", including typical features of the following ecosystems: Caatinga, Cerrado, Atlantic Forest and, Campo Rupestre.

The climate in the region is classified as Aw in the Köppen system, tropical, Alternating prolonged dry seasons (winter) with short and irregular rainy seasons (summer). The region has a mean annual rainfall of 1600 mm and an average annual air temperature of 22.9 °C, with mean monthly temperatures between 15 and 26 °C (Alvares et al. 2013).

Nine sampling sites were selected in streams of 1st, 2nd, 3rd orders (three at each stream order) (Table 1) based on the presence of stone substrates and riparian vegetation preserved, pristine conditions and a depth of 20-40 cm.

Table 1. Characteristics of the sites sampled in Chapada Diamantina National Park, Brazil. D: dry season and R: rainy season.

Tabla 1. Características de los sitios muestreados en el Parque Nacional da Chapada Diamantina, Brasil. D: estación seca y R: estación lluviosa.

Order	Stream	Geographic coordinates	Height (m)	Width (m)	Date of sampling D	S
1st	Lapão	12°32'30.1" S; 41°22'56.1" W	377	1.5	19/08/2014	21/01/2015
1st	Lapão	12°32'33.6" S; 41°22'52.8" W	353	1.5	19/08/2014	21/01/2015
1st	Ribeirãozinho	12°29'37.0" S; 41°19'44.0" W	295	0.75	20/08/2014	22/01/2015
2nd	Lençóis	12°00'06.4" S; 41°30'24.3" W	397	2	18/08/2015	20/01/2015
2nd	Lençóis	12°33'40.5" S; 41°23'35.6" W	397	2	18/08/2015	20/01/2015
2nd	São José	12°32'33.4" S; 41°22'51.1" W	335	3	19/08/2014	21/01/2015
3rd	Utinga	12°29'36.9" S; 41°19'43.9" W	356	13.5	19/08/2014	21/01/2015
3rd	Ribeirão	12°35'11.1" S; 41°22'59.9" W	334	11	20/08/2014	22/01/2015
3rd	Ribeirão	12°29'36.9" S; 41°19'43.9" W	340	9	20/08/2014	22/01/2015

All streams are perennial throughout the year, since many streams in the region do not follow this pattern and flow underground during the dry season.

Sample design and identification

Sampling was carried out in the dry season (August 2014) and the rainy season (January 2015). Mean monthly precipitation in the dry and rainy season was 20.5±3.19 and 103.7±50.10 mm, respectively, while air temperature was 22.23±0.60 and 24.26±0.39 °C, respectively (INMET 2014, 2015). Benthic macroinvertebrates were collected using a Surber sampler (mesh size=0.25 mm; area=0.1 m²), which is the easiest method of obtaining purely quantitative samples, according to Copatti et al. (2013) and Pereira et al. (2017). The Surber sampler was deployed at random locations in the stream (centre and margins). At each site, a distance of ~50 m was traversed and 10 sub-replicates (sub-samples), for a total of 1 m^2, were sampled where each sub-replicate was at least 1 m away from any other sub-replicate. We added up the total number of individuals in each sub-sample (individuals/m^2). The samples were placed in plastic containers and preserved in a 10% formaldehyde solution at -4 °C. Screening was performed in the Laboratório de Estudo e Fisiologia de Fauna Aquática da Universidade Federal da Bahia (LEFFA-UFBA), Brazil. The identified benthic macroinvertebrates were preserved in 80% ethanol. The specimens and their FFG were identified to the genus level for Ephemeroptera, Plecoptera and Trichoptera, to the subclass level for Annelida, and to the family level for other groups, using the taxonomic keys of Cummins et al. (2005) and Domínguez and Fernández (2009).

Physical and chemical variables

The following physical and chemical water properties were analysed: dissolved oxygen (mg/L O_2) and water temperature (°C) were determined with a digital oxygen meter, pH was measured with a pH meter, and total ammonia (mg/L N-NH_3), nitrite (mg/L N-NO_2), alkalinity (mg/L $CaCO_3$) and hardness (mg/L $CaCO_3$) were determined using a kit (Alfatecnoquímica, Florianópolis, SC, Brazil). Current velocity (m/s) was calculated as the time taken for a floating object to travel 3 m. To calculate water flow (m^3/s), the speed of flow was multiplied by the area of the stream section (Table 2). Dissolved oxygen, water temperature, pH, current velocity, and water flow were determined in situ, and the other

Table 2. Physical and chemical water variables of the streams of different orders in the Chapada Diamantina National Park, Brazil. A and B: Lapão. C: Ribeirãozinho. D and E: Lençóis. F: São José. G: Utinga; H and I: Ribeirão.

Tabla 2. Variables físicas y químicas del agua de los arroyos de diferentes órdenes en el Parque Nacional da Chapada Diamantina, Brasil. A y B: Lapão. C: Ribeirãozinho. D y E: Lençóis. F: São José. G: Utinga; H e I: Ribeirão.

Variables	1st order			2nd order			3rd order		
Dry season	A	B	C	D	E	F	G	H	I
Current velocity	0.1	0.14	0.05	0.12	0.05	0.19	0.37	0.5	0.15
Water flow	0.02	0.01	0.03	0.06	0.06	0.02	1.22	0.9	0.75
Temperature	22.0	22.0	21.0	22.0	22.0	21.5	22.5	21.0	22.0
Dissolved oxygen	8.50	9.00	8.00	7.50	8.00	9.00	9.00	9.00	9.00
pH	5.00	4.50	8.00	4.50	4.80	5.30	8.00	5.30	5.00
Nitrite	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.02	0.00
Total ammonia	0.20	0.10	0.20	0.15	0.15	0.15	0.20	0.15	0.15
Hardness	10.0	10.0	10.0	20.0	20.0	10.0	50.0	10.0	10.0
Alkalinity	5.00	5.00	10.0	5.00	5.00	10.0	100.0	5.00	5.00
Rainy season									
Current velocity	0.44	0.21	0.05	0.34	0.06	0.317	0.33	0.30	0.13
Water flow	0.10	0.01	0.01	0.17	0.02	0.11	1.27	0.40	0.79
Temperature	29.0	28.5	23.5	30.0	28.0	30.0	30.5	26.5	27.5
Dissolved oxygen	7.50	9.00	9.00	7.50	9.00	9.00	9.00	7.50	9.00
pH	5.50	4.80	6.00	5.00	5.00	5.80	7.50	7.50	5.00
Nitrite	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.01	0.02
Total ammonia	0.25	0.20	0.15	0.10	0.15	0.15	0.10	0.15	0.15
Hardness	10.0	10.0	10.0	15.0	20.0	20.0	70.0	10.0	10.0
Alkalinity	10.0	10.0	10.0	5.00	5.00	10.0	90.0	5.00	5.00

Values are means. Current velocity is expressed as m/s. Water flow is expressed as m^3/s. Temperature is expressed as °C. Dissolved oxygen is expressed as mg/L O_2. Nitrite is expressed as mg/L N-NO_2. Total ammonia is expressed as mg/L N-NH_3. Water hardness and alkalinity are expressed as mg $CaCO_3$/L.

Los valores son promedios. La velocidad de la corriente se expresa en m/s. La temperatura se expresa en °C. El oxígeno disuelto se expresa en mg/L O_2. Los nitritos se expresan en mg/L N-NO_2. El amoníaco total se expresa en mg/L N-NH_3. La dureza y la alcalinidad del agua se expresan en mg $CaCO_3$/L.

parameters were measured at LEFFA-UFBA. In each stream, water was collected with litter bottles on the same day as the sampling of benthic macroinvertebrates. The litter bottles were stored in icebox for further analysis of the parameters.

Data analysis

Principal components analysis (PCA) was performed on the four environmental water variables (stream width, current velocity, water flow, and temperature), obtaining several synthetic axes that capture the majority of the structural variation observed from the original data (McCune and Grace 2002). Only non-redundant environmental variables that significantly characterised the order of streams were used. Prior to analysis, data were square root transformed. To evaluate whether differences were significant, ANOSIM was performed.

Total richness, rarefied richness, and abundance of benthic macroinvertebrates were estimated considering a taxonomic uniformity at the family level. Additionally, abundance and richness of the FFG of benthic macroinvertebrates were measured. Prior to analysis, abundance values were logarithmically transformed [y=log(x+1)]. The Levene test showed the homoscedasticity of the variances. To test the hypothesis that streams of the 3rd order have a more distinct benthic macroinvertebrate assemblage than streams of the 1st and 2nd order, these data were analysed using a two-way analysis of variance bidirectional (ANOVA) (order vs. season), followed by a post hoc Tukey test. The significance level was set at 95% (*P*<0.05).

To evaluate the best dimension for representing most of the original information about the variation in benthic macroinvertebrates composition and distribution obtained from the abundance data of each stream site (n=3, dry and rainy seasons), non-metric multidimensional scaling (NMDS) was used. As a metric of similarity among replicates, the Bray-Curtis distance was used as it incorporates abundance data and gives less weight to outliers. To improve the convergence of ordering and reduce variation among taxa, abundance data were transformed into a weighted average. Initially, an automatic procedure to evaluate the best solution was performed. Subsequently, two dimensions were selected, which were indicated as the most appropriate, and a Monte-Carlo test was used to check if the axis extracted from NMDS to represent the original matrix was stronger than expected by chance. The significance level was set at 95% (*P*<0.05). The analyses were performed with PC-ORD software v. 4.25 (McCune and Grace 2002).

To evaluate whether there was an association between environmental variables (PCA synthetic axes) and benthic macroinvertebrate assemblage composition (NMDS ordination axis), a multiple regression analysis was performed, with a significance level of 95% (*P*<0.05). In cases of multiple comparisons with the same data set, the critical value of *P* was corrected through Bonferroni's method, in which the significance level is divided by the number of tests performed (adapted from Xavier et al. 2015).

Results

In general, the physical and chemical water variables (e.g., dissolved oxygen and acidity) were similar for the different orders of streams and seasons investigated. However, water flow was higher in streams of the 3rd order and water temperature was higher in the rainy season.

The ordination of environmental variables resulted in two axes with eigenvalues above 1.0 (Table 3). Axes 1 (60.29%) and 2 (25.87%) of the PCA explained 86.16% of the total variance. Axis 1 was correlated with current velocity, water flow and stream width. Axis 2 was correlated with water temperature

Table 3. Summary of the results of principal components analysis (PCA) of the environmental water variables of the streams of different orders in the Chapada Diamantina National Park, Brazil. Variables with a correlation coefficient above 0.7 (R>0.7) relative to the first and second axis of ordination are marked with an asterisk.

Tabla 3. Resumen de los resultados del análisis de componentes principales (PCA) entre las variables ambientales del agua de arroyos de diferentes órdenes en el Parque Nacional da Chapada Diamantina, Brasil. Las variables con coeficiente de correlación superiores a 0.7 (R>0.7) en relación con el primer y segundo ejes del ordenamiento están marcados con un asterisco.

	Axis 1	Axis 2
Eigenvalues	2.41	1.03
Variation proportion (%)	60.29	25.87
Variables	Correlation coefficient	
Temperature	0.31	0.90*
Stream width	0.90*	-0.32
Current velocity	0.76*	0.27
Water flow	0.96*	-0.33

(Figure 1). The streams of the 3rd order were ordered along the first axis in both periods and differed from the streams of the 1st and 2nd order (ANOSIM, R=0.72, P=0.0001 and R=0.57, P=0.001, respectively).

In total, 12822 benthic macroinvertebrate individuals were recorded (4101 and 8721 in the dry and rainy season, respectively) distributed among 62 taxa (48 and 46 taxa in the dry and rainy season, respectively). Chironomidae was the taxon with the most individuals (7704, or 60.08% of the total) (see online supplementary material). There was no difference in richness ($F_{2,12}$=0.209; P=0.814) and abundance ($F_{2,12}$=2.702; P=0.107) of taxa. There was no difference in the rarefied richness estimated among streams of different orders ($F_{2,12}$=1.133; P=0.354): 10.81±0.7 and 12.86±0.6 for 1st order streams, 10.82±0.3 and 9.29±1.8 for 2nd order streams, and 15.72±1.9 and 14.36±1.5 for 3rd order streams in the dry and rainy seasons, respectively. Some groups were recorded exclusively for 1st order (Gyrinidae and Halipidae), 2nd order (Dryopidae), or 3rd order (Hydrobiidae, Planorbidae, Physidae, Thiaridae, *Brachycercus*, *Hexagenia*, *Traverhyphes*, *Askola*, and *Hagenulopsis*) streams. The functional feeding group "predators" had the greatest richness (20). The number of taxa was similar for collector-gatherers (8), scrapers (7), filter-collectors (4), and shredders (4). There was no difference in richness or abundance among streams of different orders, or between the dry and rainy season for the same FFG ($F_{2,12}$<2.71 and P>0.10 and $F_{2,12}$<1.94 and P>0.18, respectively).

Grouping of the streams in streams of 1st, 2nd, and 3rd order, based on the composition and distribution of benthic macroinvertebrates from two-dimensional NMDS analysis, resulted in a minimal stress of 9.35, showing high quality of the ordination due to low distortion of the original data when reduced to a single axis. According to the Monte Carlo test, the axis extracted from NMDS was stronger than expected by chance (P=0.005). The order generated by NMDS showed greater proximity among the streams of the 3rd order (points g, h and i) in relation to streams of the 1st and 2nd order. The sites of streams of the 3rd order tended to have greater scores in axis 2 (Figure 2).

Multiple regression analysis among synthetic axes generated by the PCA, and of the second axis of the NMDS, indicated an association between the benthic macroinvertebrate assemblage structure and environmental variables that characterise different stream orders ($F_{2,15}$=12.84; R^2=0.63; P=0.0005). However,

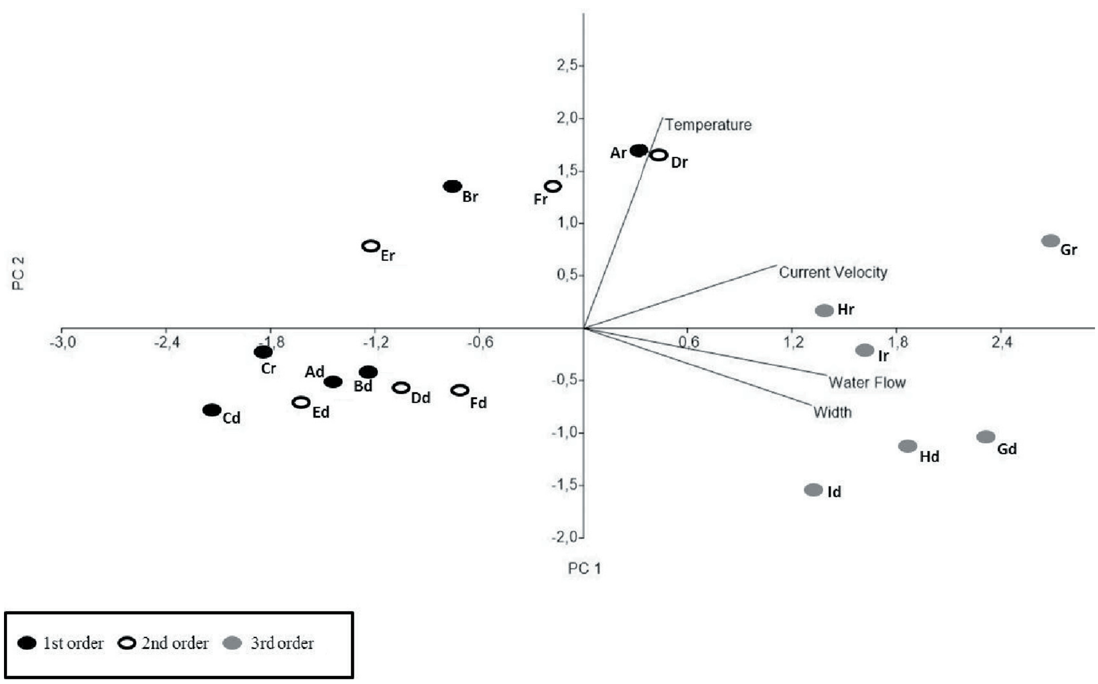

Figure 1. Principal components analysis (PCA) between seasons and environmental variables of streams of different orders in the Chapada Diamantina National Park, Brazil. 1: 1st; 2: 2nd; 3: 3rd. d: dry season; r: rainy season.

Figura 1. Análisis de componentes principales (PCA) entre las estaciones del año y las variables ambientales de arroyos de diferentes órdenes en el Parque Nacional da Chapada Diamantina, Brasil. d: estación seca; r: estación lluviosa.

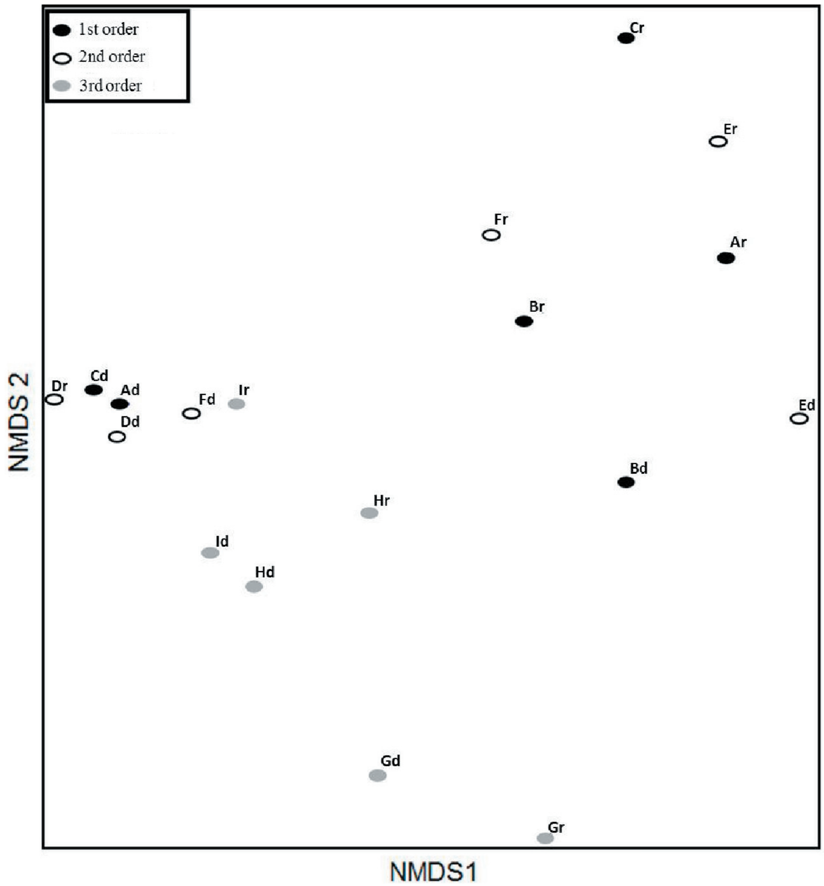

Figure 2. NMDS analysis between seasons and composition of the streams of different orders in the Chapada Diamantina National Park, Brazil. d: dry season; r: rainy season.

Figura 2. Análisis NMDS entre las estaciones del año y la composición de arroyos de diferentes órdenes en el Parque Nacional da Chapada Diamantina, Brasil. d: estación seca; r: estación lluviosa.

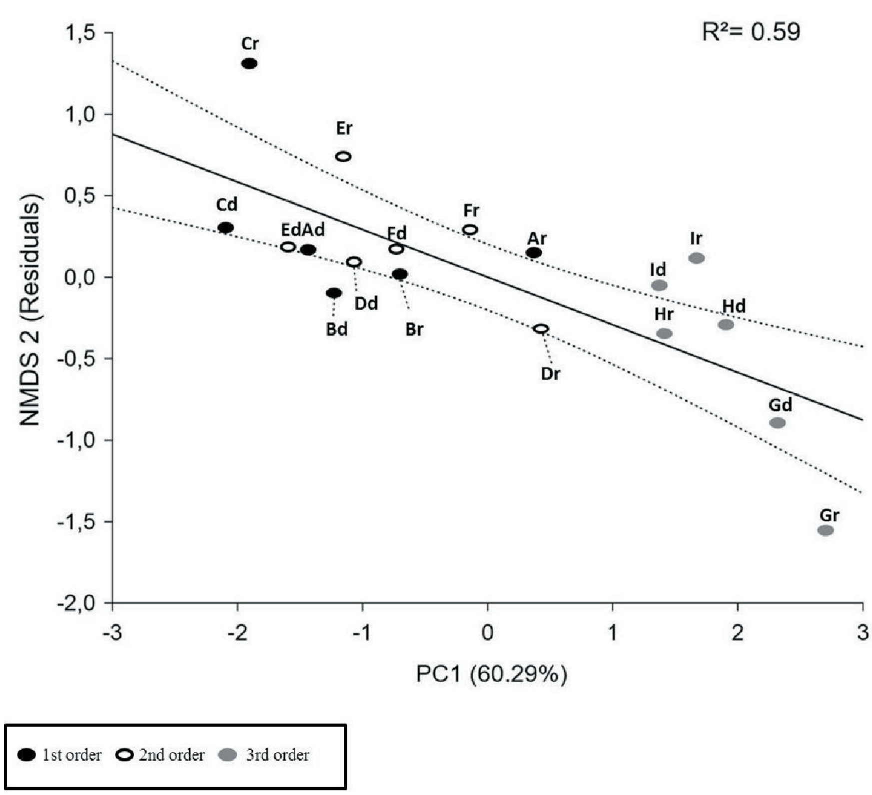

Figure 3. Partial regression of the PCA1 (environmental variables) in the NMDS 2 axis (benthic macroinvertebrate assemblage ordination) of streams of different orders in the Chapada Diamantina National Park, Brazil. d: dry season; r: rainy season.

Figura 3. Regresión parcial del PCA1 (variables ambientales) en el eje NMDS 2 (ordenamiento de la comunidad de macroinvertebrados) de los arroyos de diferentes órdenes Parque Nacional da Chapada Diamantina, Brasil. d: estación seca; r: estación lluviosa.

when the effect of each independent factor (PCA's) on the other factor was isolated from the analysis of the possible partial regressions, only PCA 1 vs. NMDS 2 was significant at a level of $P<0.025$ after Bonferroni correction ($F_{2,15}=12.84$; $R^2=0.59$; $P<0.0004$). The benthic macroinvertebrate assemblage structure was correlated with environmental variables that contribute to PCA 1 (current velocity, water flow and stream width) (Figure 3). Clearly, the distinction recorded in benthic macroinvertebrate assemblage structure among streams of different orders occurred between streams of the 3rd order and streams of headwater (1st and 2nd order).

Discussion

The composition and distribution of benthic macroinvertebrates in streams of the Chapada Diamantina National Park was not influenced by season. The similarity in assemblage structure between the dry and rainy season was supported by the results of the NMDS analysis. Even though season is a determining factor for benthic macroinvertebrate communities, it appears that season is less important in determining the distribution and composition of benthic macroinvertebrates in tropical streams (Pereira et al. 2017), even under varying hydrologic regime amounts among seasons. According to Melo and Froehlich (2001), this lack of season influence suggests a temporal stability in assemblage composition as well as resistance and resilience capacity of benthic macroinvertebrates in response to increased precipitation during the rainy season (Robinson 2012; Rocha et al. 2012).

The analyses presented here allowed the verification of the hypothesis that the benthic macroinvertebrate assemblage structure is related to the order of the stream and habitat physical characteristics, where streams of the 1st and 2nd order have greater similarity in their assemblage structure than streams of the 3rd order (points g, h, and i of the NMDS). The streams of 1st and 2nd order had more areas of headwater which tend to accumulate sediment. In contrast, streams of the 3rd order receive tributaries and are subjected to water accumulation; Therefore, they are characterised by higher current velocity and water flow (Bispo et al. 2006). According to Vannote et al. (1980), medium-sized streams are highly variable, providing the creation of new niches and more food resources. This is consistent with the data since assemblage structures were different in 3rd order streams.

The high abundance of the family Chironomidae (60.08%) may influence the abundance of the predators and collector-gatherers in this study, possibly because these FFGs include few species of small sizes (Zilli et al. 2008). The diet of the predators is composed of other benthic macroinvertebrates, such as collector-gatherers (Copatti et al. 2013), which were abundant in this study. In this case, the availability of prey is an attraction for predators, explaining their abundance (Ligeiro et al. 2010).

In general, FFGs should primarily follow variation in the amount or quality of suitable food or factors determining the distribution of food resources (Wright and Li 2002). Although autochthonous food sources may be a more important part of the diets of benthic macroinvertebrates in forested streams (Torres-Ruiz et al. 2007), the abundance of collector-gatherers in this study may be due to the high contribution of allochthonous material, which can be more important in terms of energy than the production of aquatic algae and macrophytes in the streams investigated. In addition, the shading of riparian vegetation can influence a lower abundance of algae. At the study sites, the riparian vegetation was preserved and well-developed, with a constant supply of energy and abundant leaf litter decaying at the bottom.

Allochthonous material is also important for shredders, mainly in headwater streams (Gopal and Chauhan 2013), and the abundance of scrapers increases where primary productivity is favoured by light and nutrients (Uieda and Motta 2007). In addition to the reduced availability of algae, the low abundance of shredders and scrapers could have been influenced by the low nutritional quality of leaves and biomass hyphomycetes (Ferreira et al. 2014; Graça et al. 2015). However, despite the high litter deposition in the study sites, the nutritional quality of the leaves was not assessed in this study.

It was also observed that the distinction between the streams of the 3rd order and the headwater streams in this study is probably a result of the substitution of taxa of the benthic macroinvertebrates (Baptista et al. 1998) and does not represent changes in richness and/or abundance. There was no significant addition of taxa in streams from 1st to 3rd order, but the replacement of some taxa among streams of different orders was recorded, which may have influenced the differences found in the structure of the benthic macroinvertebrate assemblages between the 3rd order streams and the headwater streams. This is very relevant information as, in a scenario of climate change, the richness of species tends to remain the same, but beta diversity is changing rapidly. This was also observed in previous studies in streams in the Neotropical region (Baptista et al. 2001; Bispo and Oliveira 2007).

The benthic macroinvertebrate assemblage was correlated with environmental variables (current velocity, water flow, and stream width) that influence their selection of a suitable habitat with appropriate conditions for benthic macroinvertebrates (Ferreira et al. 2014). With increasing stream order, hydrodynamic changes (e.g., stream width, water flow, and current velocity) are expected (Allan and Castilo 2007). Taxa of the benthic macroinvertebrate quickly respond to these changes, reflecting the different assemblage structures in streams of different orders (Heino et al. 2005).

In terms of spatial scale, in this study, the benthic macroinvertebrate assemblage responded to an environmental gradient between headwater streams (1st and 2nd order) and medium-sized streams (3rd order). Similarly, Bispo and Oliveira (2007) verified a gradient in the EPT fauna structure between headwater streams and medium-sized streams in the Central West region of Brazil, where the environmental variables current velocity and water flow were decisive for this separation. Melo and Froehlich (2001) verified the separation of Neotropical streams in groups along a size gradient due to distribution of benthic macroinvertebrates related to the physical structure of the channel, which may also have contributed to the separation observed in this study.

Differences in the resident fauna among streams of different orders confirm the importance of environmental variation for different taxa of benthic macroinvertebrates (Ligeiro et al. 2013). Differences found in benthic macroinvertebrate assemblages among streams of different orders can be related to niches and food availability. The environmental gradient observed among streams of different orders in Chapada Diamantina National Park is the result of the interaction of physical variables of the stream, and naturally impacts benthic macroinvertebrate organisation in accordance with the stream order. Thus, it is suggested that the environmental gradient formed by the variables correlated with stream order is crucial for the benthic macroinvertebrate assemblage structure and contributes to the understanding of the dynamics of these streams.

Our findings suggest that the variation of the biological and environmental characteristics according to stream order was an important factor for assemblage structure and replacement of taxa in streams of the Chapada Diamantina National Park, distinguishing streams of the 3rd order with respect to streams of the 1st and 2nd order. However, future studies that encompass a larger the spatial dimension (such as the number of streams) should be developed to show spatial generality of the results.

We could also show that, despite the variation of precipitation between dry and rainy seasons, there was no difference in the richness and abundance of benthic macroinvertebrates among streams of different orders and/or between seasons. Therefore, an obvious implication of our study is that future biomonitoring programs should focus more on assemblage variability according to stream order. Consequently, this will allow a more accurate detection of anthropogenically caused changes in stream ecosystems.

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Online Supplementary Material 1

Benthic macroinvertebrates of streams of different orders in the Chapada Diamantina National Park, Brazil. D = Dry season; R = Rainy season.

Macroinvertebrados bentônicos de riachos de diferentes ordens no Parque Nacional da Chapada Diamantina, Brasil. D = período seco; R = período chuvoso.

TAXA	1st Order		2nd Order		3rd Order		Total
	D	**R**	**D**	**R**	**D**	**R**	**D+R**
INSECTA							
Coleoptera							
Dryopidae	0	0	0	1	0	0	1
Dytiscidae	1	2	5	4	6	13	31
Elmidae	14	20	70	87	93	20	304
Gyrinidae	0	1	0	0	0	0	1
Haliplidae	4	0	0	0	0	0	4
Hydrophilidae	1	1	5	1	18	6	32
Psephenidae	0	0	1	7	3	5	16
Staphylinidae	0	0	1	10	8	25	44
Diptera							
Ceratopogonidae	64	13	58	34	42	34	245
Chironomidae (Tanypodinae)	213	48	152	441	120	63	1037

Chironomidae (Others)	789	340	1304	3600	233	401	6667
Empididae	4	14	34	134	1	4	191
Simuliidae	39	461	58	1589	2	40	2189
Tipulidae	10	15	34	54	9	5	127
<u>Ephemeroptera</u>							
Baetidae							
Camelobaetidius	0	1	2	2	0	16	21
Not identified	11	25	6	42	14	109	207
Caenidae							
Brachycercus	0	0	0	0	1	0	1
Caenis	2	1	0	0	5	0	8
Ephemeridae							
Hexagenia	0	0	0	0	1	0	1
Leptohyphidae							
Traverhyphes	0	0	0	0	4	1	5
Tricorythodes	0	0	0	1	3	0	4
Not identified	0	0	1	0	22	7	30
Leptophlebiidae							
Askola	0	0	0	0	3	0	3

Farrodes	0	0	0	1	7	5	13
Hagenulopsis	0	0	0	0	0	3	3
Microphlebia	3	2	0	3	0	0	8
Miroculis	0	1	0	0	0	2	3
Not identified	0	1	0	4	14	7	26
Hemiptera							
Gerridae	1	3	0	0	0	2	6
Mesoveliidae	0	5	2	2	1	3	13
Naucoridae	9	17	45	74	18	7	170
Notonectidae	0	0	1	0	1	0	2
Veliidae	8	0	3	6	2	5	24
Lepidoptera							
Crambidae	5	1	5	6	5	4	26
Megaloptera							
Corydalidae	2	20	6	9	2	1	40
Odonata							
Anisoptera							
Calopterygidae	1	4	0	1	0	3	9
Coenagrionidae	12	12	6	21	6	8	65
Zygoptera							

Gomphidae	0	3	5	5	2	6	21
Libellulidae	5	4	0	7	10	0	26
<u>Plecoptera</u>							
Perlidae							
Anacroneuria	2	2	9	1	6	1	21
Not identified	1	0	2	1	1	0	5
<u>Trichoptera</u>							
Helicopsychidae							
Helicopsyche	0	3	0	2	1	0	6
Hydrobiosidae							
Atopsyche	0	0	3	1	0	1	5
Hydropsychidae							
Leptonema	0	3	0	1	0	0	4
Smicridea	123	135	49	80	19	56	462
Not identified	4	11	3	0	0	7	25
Hydroptilidae							
Oxyethira	13	4	0	0	1	0	18
Not identified	2	0	0	0	0	0	2
Odontoceridae							
Marilia	3	5	3	8	0	0	19

Not identified	0	0	0	0	5	0	5
Philopotamidae							
Chimarra	91	188	28	140	0	4	451
Not identified	4	0	4	2	0	0	10
Polycentropodidae							
Cyrnellus	0	0	2	0	0	2	4
Polycentropus	2	0	11	0	0	14	27
Polyplectropus	1	6	1	3	0	1	12
Not identified	0	0	1	0	0	0	1
ANNELIDA							
Oligochaeta	9	15	3	9	2	4	42
Hirudinea	3	3	0	0	3	0	9
MOLLUSCA							
Hydrobiidae	0	0	0	0	8	36	44
Planorbidae	0	0	0	0	1	0	1
Physidae	0	0	0	0	3	0	3
Thiaridae	0	0	0	0	16	6	22
Total species	34	36	35	38	42	39	62
Total individuals	1,456	1,390	1,923	6,394	722	937	12,822

Online Supplementary Material 2

ANOVA and Tukey´s test for richness and abundance of the functional feeding groups of benthic macroinvertebrates of streams of different orders in the Chapada Diamantina National Park, Brazil. D = Dry season; R = Rainy season.

Anova e teste de Tukey para riqueza e abundância do grupo functional alimentar de macroinvertebrados bentônicos de riachos de diferentes ordens no Parque Nacional da Chapada Diamantina, Brasil. D = período seco; R = período chuvoso.

Analysis	**1st Order**		**2nd Order**		**3rd Order**	
	D	**R**	**D**	**R**	**D**	**R**
			Richness			
ANOVA	$F_{4.10} = 15.58$	$F_{4.10} = 16.92$	$F_{4.10} = 8.04$	$F_{4.10} = 59.04$	$F_{4.10} = 12.90$	$F_{4.10} = 20.67$
	SS = 1.60	SS = 2.00	SS = 4.93	SS = 0.93	SS = 3.07	SS = 2.13
Tukey test	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
			Abundance			
ANOVA	$F_{4.10} = 8.43$	$F_{4.10} = 10.42$	$F_{4.10} = 9.43$	$F_{4.10} = 10.90$	$F_{4.10} = 8.59$	$F_{4.10} = 10.76$
	SS = 0.14	SS = 0.16	SS = 2.27	SS = 0.27	SS = 0.15	SS = 0.13
Tukey test	$p < 0.003$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.002$	$p < 0.001$