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ORIGINAL ARTICLE

Influence of bryophyte biomass and organic matter quantity on the abundance and richness of oligochaetes in forest streams with different phytophysiognomies in southeastern Brazil

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ABSTRACT

Aquatic oligochaetes can be found associated with different types of substrates; including bryophytes (mosses and liverworts) adhered to stones in lotic ecosystems. However, little information is available on the association between oligochaetes and bryophytes in tropical region. Therefore, the aims of this study were: to investigate whether the assemblages of oligochaetes respond to variation in the biomass of bryophytes and quantity of particulate organic matter (POM) present on these plants; verify differences in the composition of oligochaetes in streams flowing through areas with two phytophysiognomies (rocky field and seasonal semideciduous forest). The samples were collected from five first-order streams in the southeast of the state of Minas Gerais. Six samples of bryophytes adhered to stones were obtained from each stream. A total of 1586 oligochaetes were collected and 11 taxa were identified, belonging to the families Naididae and Enchytraeidae. The most abundant taxa were Bothrioneurum (37.95%) and Enchytraeidae (33.01%). A positive relation was observed between oligochaete abundance and POM in two streams (Ibitipoca I and Ibitipoca III) and a positive relation was found between oligochaete abundance and bryophyte biomass in one stream (Ibitipoca I). The composition of the fauna varied within and between the phytophysiognomies studied. The results show that the oligochaetes find favorable conditions to establish themselves in bryophytes, evidencing the ecological importance of these plants as habitat for invertebrates.

Key words: Atlantic forest; Enchytraeidae; liverwort; lotic systems; mosses; Naididae.

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INTRODUCTION

The bryophytes associated with stones in low-order lotic environments accumulate particulate organic matter (Habdija *et al*., 2004) and are used as substrate for colonization of periphyton (Stream Bryophyte Group, 1999), providing food and shelter against the water current (Rosa *et al*., 2011, 2013; Rodrigues *et al.*, 2013) and refuge from predators (Glime and Clemons, 1972; Parker *et al*., 2007; Alvarez and Peckarsky, 2013) for various species of aquatic invertebrates. Several studies (Percival and Whitehead, 1929; Suren, 1993; Linhart *et al*., 2002; Tokeshi and Arakaki, 2012) have shown that these plants contribute to the dimensional complexity of the habitat within the stream flow, because they increase the surface area that can be colonized, resulting in a positive variation in the density and diversity of invertebrates.

The Oligochaeta are among the most diverse invertebrates that can be found in bryophytes (Gorni and Alves, 2007; Rodrigues *et al*., 2013b). Their presence is positively related to the biomass of these plants and the quantity of particulate organic matter retained among the phyllids and caulids (Suren, 1991; Suren and Winterboun, 1992; Linhart *et al*., 2002). The Oligochaeta species that colonize bryophytes are for the most part those present in the sediment of streams. In different phytophysiognomies, for example, streams can differ greatly regarding the morphology, sediment granulometry and type of plant matter that enters the beds. Therefore, the fauna present is a reflection of the local environmental conditions. In Brazil, specific studies of the oligochaete fauna associated with bryophytes are still rare, with the exceptions being the works of Gorni and Alves (2007) and Rodrigues *et al*. (2013b). Also, Rosa *et al*. (2011) investigated macroinvertebrates in bryophytes and also reported the presence of oligochaetes, with the most abundant family being Naididae.

The aim of this study was to learn the composition and structure of the assemblages of aquatic oligochaetes associated with bryophytes in first-order streams, to find out whether these invertebrates respond to the variation in the quantity of particulate organic matter retained in these plants and the different phytophysiognomies through which the streams flow. For this purpose, we tested the following hypotheses: i) habitats with larger bryophyte biomass contain a greater quantity of particulate organic matter, and consequently, greater abundance and richness of oligochaetes; and ii) the composition of the oligochaete fauna associated with bryophytes is distinct between streams located in different phytophysiognomies (seasonal semideciduous forest and rocky field).

METHODS

Study area

The study was conducted in two remnant Atlantic Forest areas in the state of Minas Gerais (southeastern Brazil) with different phytophysiognomies: Particular area-Fazenda Floresta (21°43' to 21°44'S and 43°16' to 43°17'W - Juiz de Fora), where we selected two streams in an area of seasonal semideciduous forest (Floresta I and II), located at altitude between 800 and 890 m; and Ibitipoca State Park (21°40' to 21°43' S and 43°52' to 43°54' W- Lima Duarte), with altitude from 1300 to 1400 m, where we selected two streams flowing through rocky fields (Ibitipoca I and III) and one stream in a seasonal semideciduous forest fragment (Ibitipoca II).

Rocky field areas are characterized for having sandy soils (spodosols), with predominance of grasses, bushes and trees. The vegetation is typically xeromorphic due to the oligotrophy and acidity of the soil and water restrictions (Rapini *et al.,* 2008). In Ibitipoca State Park, this phytophysiognomy includes gentle slopes with sandy/stony soil, rocky outcrops with steep slopes, mountain tops and swampy lowlands. In wetter areas near watercourses, it is common to find rocks covered by lichens and mosses. On the other hand, semideciduous seasonal forest areas are characterized by vegetation with an arboreal, arbustive and herbaceous strata, where the trees and bushes shed part of their leaves during the dry season, thus acting to regulate the water balance in the two climate seasons (dry and rainy). Argissols and latossols are the main soil types related to this type of forest (Dias *et al.*, 2002).

The five streams sampled (Tab. 1) have well preserved riparian vegetation, without influence of human activities. The sediment in the streams located in the seasonal semideciduous forest areas consisted by coarse (58%), medium (34%) and fine (6%) sand. The sediment in streams located in the rocky field areas were composed mainly by coarse (83%) sand, follow by medium (15%) and fine (5%) sand. All streams had well oxygenated water with low electrical conductivity, characteristic of preserved streams. The streams at higher altitudes had cooler water.

Sampling

In each stream, along a stretch of about 300 m, we sampled six stones with approximate diameter of 20 cm that were fixed in the bed and totally or partially covered by bryophytes. The approximate distance between the stones was 40 m and all were collected in areas of riffles. A metal structure measuring 10x10 cm was placed over each stone, about 1 to 2 cm above the water surface, and the bryophytes were scraped with a spatula, placed individually in plastic jars and fixed in a 4% formaldehyde solution. The samples were gathered in May-July 2010.

In the laboratory, the bryophytes were carefully washed in running tap water over a sieve with mesh of 53 µm to remove the fauna and retained particulate matter. This material was sorted under a stereoscopic microscope and the oligochaetes were preserved in 70% alcohol. The bryophytes were dried in an oven at 60°C for 48 h and weighed on a precision balance (0.1 mg) to calculate the biomass (g). The same procedure was applied to the particulate matter, which was then incinerated in a muffle furnace at 550°C for 4 h to obtain the quantity of organic matter (g), calculated by the difference between the initial weight (before burning) and the final weight (after burning). For identification of the oligochaete species, slides were prepared of the samples stained with lactophenol, as described by Brinkhurst (1971). The slides were examined under a light microscope to identify the oligochaetes to the lowest taxonomic level possible, according to Righi (1984) and Brinkhurst and Marchese (1989), using the updated nomenclature proposed by Reynolds and Wetzel (2014). The bryophytes were identified by specialists using the works of Frahm (1991), Sharp *et al*. (1994), Buck (1998), Gradstein and Costa (2003) and Pursell (2007) as references.

Tab. 1. Geographic localization, mean and standard deviation of limnological variables of the streams in Fazenda Floresta and Ibitipoca State Park, Minas Gerais, Brasil.

	Coordinates	Water temperature (°C)	Oxygen (mgL^{-1})	pH	Conductivity (µScm^{-1})
Floresta I	21°44'7"S 43°18'2"W	18.5±0.10^a	10.8±0.90^a	8.23±0.57^a	21.16±7.59^a
Floresta II	21°44'59"S 43°17'28"W	18.3±0.26^a	11.07±0.66^a	8.12±0.52^a	16.60±1.60^a
Ibitipoca I	21°42'11"S 3°53'34"W	14.4±0.17^b	10.83±0.05^a	7.60±0.26^a	11.10±0.96^a
Ibitipoca II	21°42'25"S 43°53'9"W	15.2±1.00^b	11.06±0.90^a	7.66±0.30^a	16.63±5.60^a
Ibitipoca III	21°42'16"S 43°53'13"W	14.08±0.80^b	11.0±0.60^a	7.60±0.20^a	13.90±4.70^a

Different letters in the first column means that the values are significantly different (P<0.05).

Data analysis

The assemblages of oligochaetes were analyzed to determine the total abundance and taxonomic richness. Each bryophyte was considered a sample unit and each stream was considered a replicate. For the statistical analyses, the data on fauna were tested for normality (Shapiro-Wilk test) and homogeneity of the variance (Levene test) (α=0.05).

To check for differences in bryophyte biomass, quantity of particulate organic matter (POM), total abundance and richness of oligochaetes between streams, we used analysis of variance- one way ANOVA (or the Kruskal-Wallis test for data without normal distribution). The values of abundance and richness were transformed in log (x+1). We used simple linear regression to test the relations: bryophyte biomass and POM; abundance and POM; abundance and bryophyte biomass; richness and POM; and richness and bryophyte biomass. We also used simple linear regression to check the relation between the abundance of oligochaetes and richness of bryophyte families and richness of oligochaetes and richness of bryophytes families. These tests were performed in Statistica program (Statsoft, 2004).

To test our second hypothesis, we applied the nonparametric Multi-Response Permutation Procedure (MRPP) to verify differences in the composition of taxa between the streams in the two phytophysiognomies studied, employing the PC-ORD program (McCune and Mefford, 2006).

RESULTS

We identified 1586 oligochaetes, belonging to the families Naididae and Enchytraeidae (Tab. 2). The most abundant taxa were *Bothrioneurum* (37.95%) and Enchytraeidae (33.1%). The Naididae specimens belonged to the subfamilies Naidinae (1.88%), Pristininae (41.38%) and Rhyacodrilinae (56.74%), with Pristininae being the most diverse, containing eight species

We also identified 17 families of bryophytes, of which the most representative were Pilotrichaceae Kindb. (moss), Aneuraceae H. Klinggr. (liverwort) and Plagiochilaceae Müll. Frib. & Herzog (liverwort). We recorded 30 taxa (genera or species) of bryophytes, and in general each sample contained more than one species. In the Floresta I stream, only one species was found, while Ibitipoca I stream had the greatest species richness (S=12), of which eight were only found in this stream. There was no relation between the abundance of oligochaetes and richness of bryophyte families (F=0.534; P=0.517; r^2=0.151) or between the richness of oligochaetes and richness of bryophyte families (F=0.417; P=0.564; r^2=0.122). The oligochaete abundance was highest in Ibitipoca I and III streams (df=4; H=15.130; P=0.004) while the taxonomic richness was greatest in Ibitipoca II and III streams (df=4; F=4.091; P=0.018) (Fig. 1 A,B). With respect to bryophyte biomass, Floresta I stream had the lowest values (df=4; F=5.857; P=0.001) (Fig. 1 C). The total quantity of POM in the bryophyte samples differed only in relation to Floresta I stream (df=4; F=3.011; P=0.037) (Fig. 1 D).

The linear regression results showed there was no significant relationship in the majority of the streams between the oligochaete abundance and POM quantity as well as between the oligochaete abundance and bryophyte biomass. The same applies to the oligochaete richness. The relationships were only significant for Ibitipoca I stream (except for richness x bryophyte biomass). Besides this, for Floresta I stream there was a significant relationship between POM quantity and bryophyte biomass, and for Ibitipoca III stream between oligochaete abundance and POM (Tab. 3). According to the MRPP results, the composition of oligochaetes varied within and between

Tab. 2. Numerical abundance of the taxa collected in the streams in Fazenda Floresta and Ibitipoca State Park, Minas Gerais, Brazil.

	Floresta I	Floresta II	Ibitipoca I	Ibitipoca II	Ibitipoca III
NAIDIDAE					
Naidinae					
Chaetogasther diastrophus (Gruithuisen, 1828)	-	-	-	20	-
Pristininae					
Pristina aequiseta Bourne, 1891	-	-	-	-	13
Pristina jenkinae (Stephenson, 1931)	8	2	-	1	-
Pristina leidyi Smith, 1896	-	-	-	-	4
Pristina osborni (Walton, 1906)	-	2	110	8	27
Pristina proboscidea Beddard, 1896	-	-	-	-	5
Pristina menoni (Aiyer, 1929)	-	-	13	-	-
Pristina sp. 1	1	5	-	18	-
Pristina sp. 2	-	-	78	-	144
Rhyacodrilinae					
Bothrioneurum	-	-	-	-	602
ENCHYTRAEIDAE	15	12	209	57	232
Total abundance	24	21	410	104	1027

the phytophysiognomies studied. As shown in Tab. 4, there was a difference between Floresta I and Ibitipoca III streams (different phytophysiognomies), but not between Floresta I and Ibitipoca I and Ibitipoca I and Ibitipoca II, which also have different phytophysiognomies. Besides this, the analysis showed a difference in the composition between two streams with the same phytophysiognomy (Ibitipoca I and Ibitipoca III).

DISCUSSION

The predominant species recorded in this study were from the Naididae family. Previous studies of the macrofauna associated with bryophytes have also found this family to be the main component of the oligochaete assemblage in this habitat (Percival and Whitehead, 1929; Habdija *et al*., 2004; Gorni and Alves, 2007; Rosa *et al*., 2011). The Naididae are able to swim freely (Martin *et al*., 2008), favoring the exploitation of different habitats, such as bryophytes, where they find an abundant and steady source of food (Suren, 1992a).

Enchytraeidae is a cosmopolitan family (Martin *et al*., 2008), and in this study it presented the highest abundance in four of the five streams investigated. These oligochaetes are commonly found in bryophytes (Suren, 1993; Rosa *et al*., 2011; Glime, 2012) and are abundant in streams with well oxygenated water (Johnson and Ladle, 1989). The streams studied all fit this description, so this may have contributed to the presence of Enchytraeidae in the bryophytes analyzed. The species of oligochaetes collected in this study are also associated with other microhabitat types, such as fine sediments, sand and stones, in both riffles and pools (Schenková *et al*., 2001; Alves *et al*., 2008; Gorni and Alves, 2008; Behrend *et al*., 2009; Baturina,

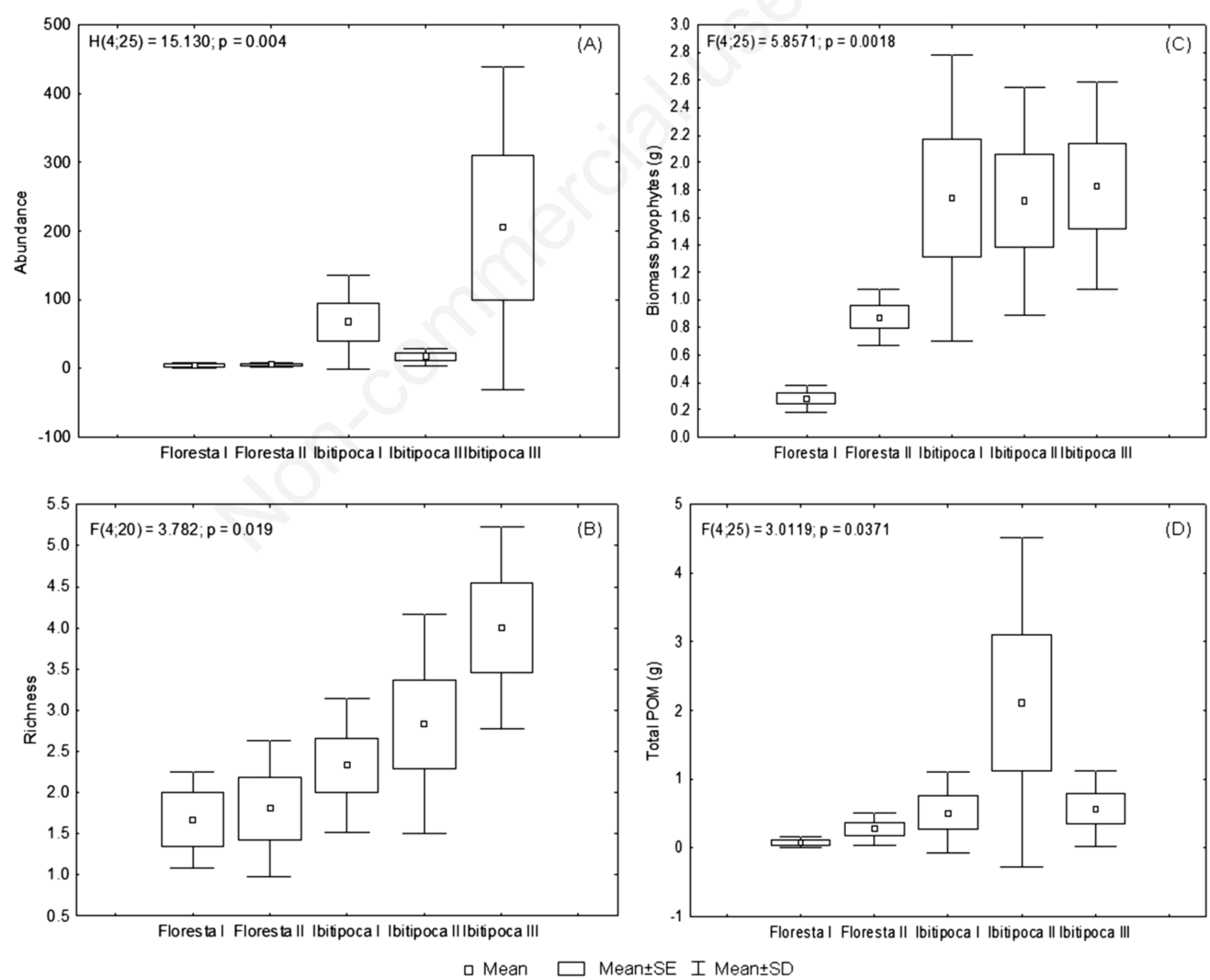

Fig. 1. Box plot for the values of abundance, richness, bryophyte biomass and particulate organic matter in the streams sampled in Fazenda Floresta and Ibitipoca State Park, Minas Gerais, Brazil.

2012; Gorni and Alves, 2012; Rodrigues *et al*., 2013a). Hence, there are no indications that a particular species is restricted to inhabiting bryophytes.

Some studies have shown that the numerical abundance of invertebrates is associated with the availability of food resources. Suren and Winterbourn (1992) reported that the abundance of the majority of taxa identified in bryophytes was related to the quantity of periphyton or detrital biomass, while Habdija *et al*. (2004) found the density of invertebrates to be positively associated with the quantity of particulate organic matter retained in these plants. Linhart (2002) and Vlcková *et al*. (2002) also found

Tab. 3. Simple linear regression analysis of the relations between abundance, richness, total quantity of particulate organic matter and bryophyte biomass of streams in Fazenda Floresta and Ibitipoca State Park, Minas Gerais, Brazil. Values underlined are significantly different.

	F	P	R^2
Floresta I			
POM x bryophyte biomass	12.361	0.025	0.694
Abundance x POM	0.072	0.794	-0.227
Abundance x bryophyte biomass	1.915	0.238	0.154
Richness x POM	0	0.979	-0.249
Richness x bryophyte biomass	1.333	0.313	0.062
Floresta II			
POM x bryophyte biomass	2.686	0.175	0.252
Abundance x POM	1.101	0.373	0.002
Abundance x bryophyte biomass	0.013	0.911	-0.246
Richness x POM	2.055	0.224	0.174
Richness x bryophyte biomass	0.872	0.594	-0.026
Ibitipoca I			
POM x bryophyte biomass	25.480	0.008	0.830
Abundance x POM	11.960	0.026	0.686
Abundance x bryophyte biomass	7.527	0.050	0.566
Richness x POM	12.545	0.024	0.697
Richness x bryophyte biomass	6.923	0.051	0.542
Ibitipoca II			
POM x bryophyte biomass	0.133	0.730	-0.209
Abundance x POM	0	0.977	-0.249
Abundance x bryophyte biomass	0.186	0.687	-0.194
Richness x POM	1.518	0.285	0.094
Richness x bryophyte biomass	0.118	0.743	-0.214
Ibitipoca III			
POM x bryophyte biomass	0.083	0.780	-0.224
Abundance x POM	34.652	0.005	0.870
Abundance x bryophyte biomass	0.020	0.886	-0.243
Richness x POM	0.051	0.824	-0.234
Richness x bryophyte biomass	1.243	0.328	0.046

POM, particulate organic matter.

Tab. 4. Result of the Multi-Response Permutations Procedures (MRPP) between streams in Fazenda Floresta and Ibitipoca State Park. Values underlined are significantly different.

			T	A	P
Floresta I	*vs*	Floresta II	1.294	-0.082	0.927
Floresta I	*vs*	Ibitipoca I	-1.868	0.083	0.051
Floresta I	*vs*	Ibitipoca II	-0.535	0.023	0.262
Floresta I	*vs*	Ibitipoca III	-2.661	0.143	0.016
Floresta II	*vs*	Ibitipoca I	-3.003	0.105	0.014
Floresta II	*vs*	Ibitipoca II	-0.799	0.029	0.199
Floresta II	*vs*	Ibitipoca III	-4.229	0.186	0.003
Ibitipoca I	*vs*	Ibitipoca II	-1.180	0.036	0.121
Ibitipoca I	*vs*	Ibitipoca III	-3.596	0.114	0.004
Ibitipoca II	*vs*	Ibitipoca III	-4.472	0.137	0.001

a significant correlation between oligochaete abundance and amount of particulate organic matter. Although we found higher values for organic matter and numerical abundance in bryophytes in the streams in Ibitipoca State Park, the linear regression analysis showed that the oligochaete abundance was only related to the quantity of organic matter in Ibitipoca I and III streams. For the other streams, this relation was not significant, indicating that other factors, such as current speed and quantity of periphyton (not investigated in this study), might be related to the presence of oligochaetes in these plants (Habdija *et al*., 2004). The retention of detritus by bryophytes is related to their morphology and the entrance of allochthonous material in the aquatic system (Suren, 1992b). Percival and Whitehead (1929) observed higher oligochaete abundance levels where the concentration of mosses was higher. Suren (1993) reported significant relations between bryophyte biomass and density of invertebrates. The morphological and structural complexity provided by the diversity of aquatic plant species (Tokeshi and Arakaki, 2012), such as bryophytes, that occupy spaces on rocks that are near to each other is an important factor for maintenance of the species diversity of oligochaetes and other invertebrates. In the streams in Ibitipoca State Park, the presence of stones covered with a thicker carpet of bryophytes, thus having greater biomass, also contributed to the higher oligochaete richness values.

The species of oligochaetes identified in this study presented wide distribution and high adaptive capability to different environmental conditions. Although rocky field and seasonal semideciduous forest areas have different phytophysiognomies regarding the type of vegetation and geomorphological characteristics (*e.g*., soil and rocks), those differences were not sufficient or exclusive to the point of causing changes in the composition of oligochaetes, since we observed variation both within and between the two phytophysiognomies.

CONCLUSIONS

The results of this study show that despite the absence of significant pairwise relations between the bryophyte biomass, POM, abundance and richness of oligochaetes in all the streams, these organisms find favorable conditions to establish themselves among bryophytes, since these plants shelter an abundant and diverse fauna. Therefore, this work provides important information on the distribution of oligochaetes in preserved streams, besides demonstrating the ecological importance of aquatic bryophytes as habitat for invertebrates.

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