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Influence of Habitat and Land Use on the Assemblages of Ephemeroptera, Plecoptera, and Trichoptera in Neotropical Streams

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ABSTRACT. Insects of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) are often used to assess the conditions of aquatic environments, but few studies have examined the differences in these communities between riffles and pools. Our objective was to test whether riffles shelter greater richness and abundance of EPT, as well as to assess the sensitivity of these insects for detecting impacts from different land uses in streams in southeastern Brazil. Samples were collected in the dry season of 2012 with a Surber sampler in riffles and pools of nine streams (forest, pasture, and urban areas). Principal component analysis distinguished the streams according to different land uses as a function of percentage of plant cover and water oxygenation level and showed partial distinction between riffles and pools as a function of current speed and percentage of ultrafine sand. Detrended correspondence analysis indicated the distinction in EPT composition between riffles and pools, except in urban streams. The results of this study confirm the expected differences in the EPT fauna structure between riffles and pools, especially in forest and pasture environments. The individual metrics of riffle and pool assemblages showed significantly different responses to land use. Therefore, we suggest individual sampling of riffles and pools, since the metrics of these assemblages' insects can differ between these habitats and influence the results of assessments in low-order streams.

Key Words: aquatic insect, conservation, lotic ecosystem, mesohabitat

The ecological integrity of aquatic ecosystems is threatened by anthropic factors associated with different types of land use (Goulart and Callisto 2003, Allan 2004). Changes such as removal of streamside plant cover, sedimentation, loss of woody detritus, hydrological alterations, entry of pollutants, and enrichment by nutrients can compromise the health of aquatic ecosystems (Allan 2004) and consequently cause loss of biological diversity (Silveira 2001, Benstead et al. 2003). However, considering the large number of factors that can influence the structure of aquatic communities in response to different land uses, predicting and preventing loss of species in these systems is difficult (Wang et al. 2001, Ourso and Frenzel 2003, Allan 2004).

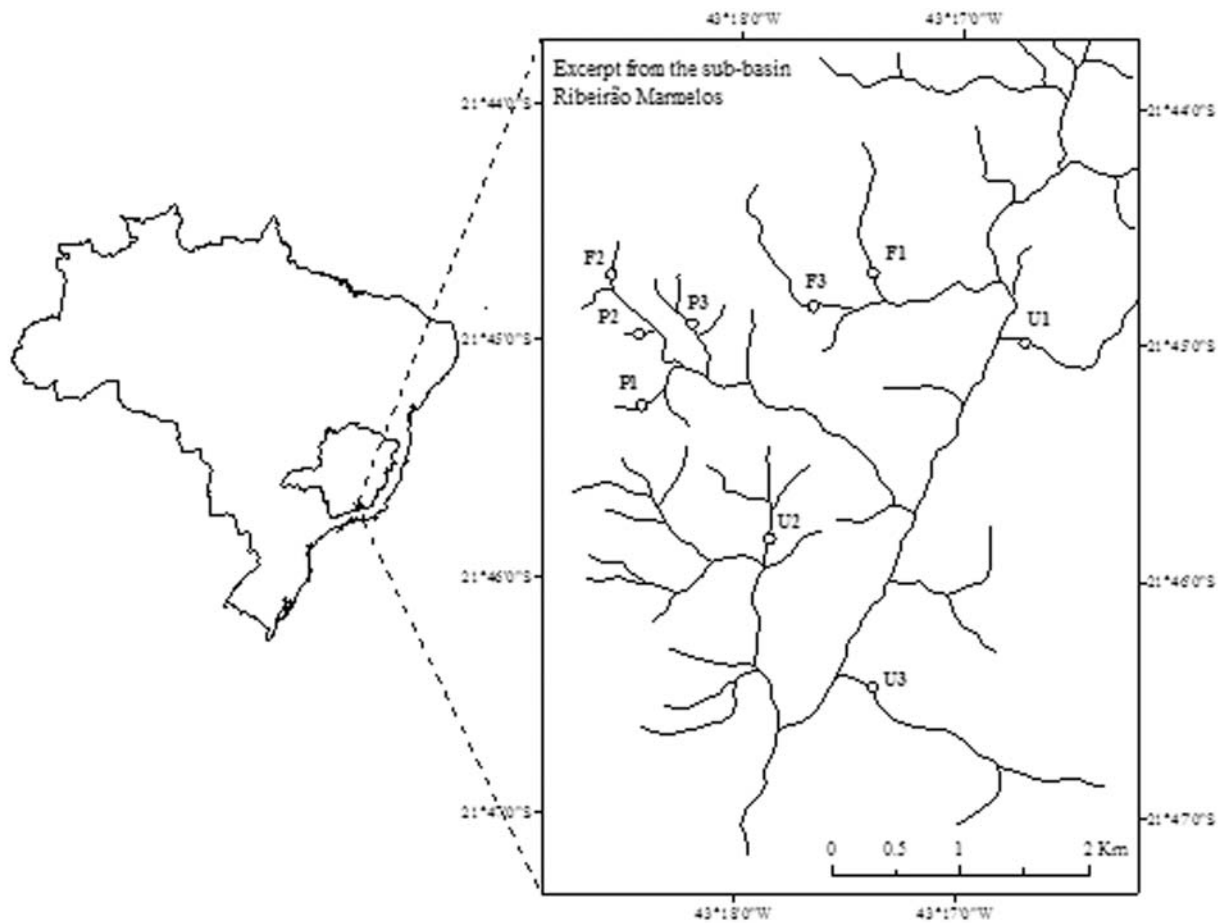
Riparian vegetation plays an important role in maintaining the ecological integrity of lotic environments (Tereza and Casatti 2010), because it acts as a physical barrier to sediments and other substances coming from the adjacent land (Gregory et al. 1991, Elmore 1992), besides regulating the water temperature (Benstead et al. 2003) and providing allochthonous material (Tereza and Casatti 2010). This material (stems, branches, fruits, seeds, leaves, and trunks) contributes to the formation of microhabitats that serve as shelter, breeding sites, and food for aquatic fauna (Passos et al. 2003, Schneider and Winemiller 2008). Additionally, trunks and branches block and/or guide the water flow, forming small pools, and riffles that increase the heterogeneity of habitats (Nessimian et al. 2008).

Pools and riffles are typical mesohabitats of lotic ecosystems, differing mainly in water velocity (Jowett 1993) and the relative proportion and particle size of substrate components (Fidelis et al. 2008). As a consequence of these differences, riffles and pools can have distinct community compositions (Kobayashi and Kagaya, 2002) that show

different responses to land use. However, studies describing the effects of these physical differences on community structure are lacking. Furthermore, the information on the distribution and abundance of macroinvertebrates in these habitats is not consistent: some studies have found greater abundance in riffles (Bispo and Oliveira 2007, Rezende 2007) and others in pools (Lind et al. 2006), or have not observed significant difference between these habitats (Scullion et al. 2006).

Among the insects that inhabit lotic environments, the Ephemeroptera, Plecoptera, and Trichoptera (EPT) are common in low-order streams flowing through forests (Bispo and Oliveira 2007). In general, insects of these orders are sensitive to anthropic disturbances and hence are considered indicators of environmental quality (Rosenberg et al. 1993). For example, Pes (2001), studying small channels in the Amazon region, observed that some genera of Trichoptera are more abundant in open areas with moderate human alteration. In turn, according to Rosenberg et al. (1993) and Buss and Salles (2007), some genera of Ephemeroptera can respond differently to alterations in the physical structure and water quality of streams.

The effects of human activities on the EPT assemblages have been documented by various researchers, such as Fjeilheim and Raddum (1992), Bispo and Oliveira (2007), and Hepp et al. (2013), but few studies have been published assessing the influence of land use on these assemblages taking into account the differences between mesohabitats. We evaluated the structure of the EPT fauna associated with pools and riffles of streams with respect to different land use patterns (forest, pasture, and urban). Because of higher water oxygenation and substrate heterogeneity, we expected to observe greater richness and abundance



Source: IBGE 2010, PIF PDDU, 2000; DATUM Córrego Alegre

Fig. 1. Streams studied in the sub-basin of the Marmelos River, southeastern Brazil. Forest (F), pasture (P), and urban (U) areas.

Table 1. Order, coordinates, habitat integrity index (HII), and category of the nine streams investigated in the sub-basin of the Marmelos River, southeastern Brazil

Location	Order	Coordinates	HII	Category
1	1	21° 44'40" S; 43° 18'35" W	0.96	Forest
2	1	21° 44'45" S; 43° 17'23" W	0.9	Forest
3	1	21° 44'15" S; 43° 17'42" W	0.98	Forest
4	1	21° 45'40" S; 43° 18'24" W	0.47	Pasture
5	1	21° 44'58" S; 43° 18'27" W	0.47	Pasture
6	1	21° 44'54" S; 43° 18'15" W	0.51	Pasture
7	1	21° 45'2,8" S; 43° 16'36" W	0.24	Urban
8	3	21° 46'1,2" S; 43° 17'55" W	0.22	Urban
9	2	21° 46'25" S; 43° 17'28" W	0.24	Urban

of EPT in riffles than pools of forested streams, as well as higher sensitivity of riffles in detecting impacts from different land uses.

Materials and Methods

Study Areas. The study was carried out in nine streams belonging to the sub-basin of the Marmelos River, located in the state of Minas Gerais, southeastern Brazil (Fig. 1). Three streams are within an Atlantic Forest fragment, three are in a pasture area, and three are in an urban area. According to the document from the Brazilian Ministry of the Environment entitled "Assessment and priority actions to preserve Atlantic Forest and Southern Plains biodiversity," the study region is classified as being under high anthropic pressure, with status

of extreme biological importance for preservation of invertebrates (Ministry of Environment, Biodiversity and Forest Department [MMA/SBF] 2002).

We used the habitat integrity index to assess the various aspects of the landscape surrounding of the streams (Table 1). This index accounts for differences on a scale of 0–1 (values closer to 1 represent environments with the highest levels of integrity) and takes into consideration the preservation state and width of the riparian vegetation strip, land use pattern, stream bed and water retention features, presence of riffles and pools, sediments in the channel, structure, and stability of banks, aquatic vegetation, and detritus (Nessimian et al. 2008).

Environmental Variables. The samples were collected in August 2012, in the dry season because of the better distinction between the riffles and pools. In each stream, the environmental and biological variables were measured in five riffles and five pools, located approximately 15 m from each other, for a total of 90 samples (3 land use categories × 3 streams × 2 mesohabitats × 5 samples).

The plant cover was recorded by digital photographs (Fujifilm CCD, 5-mm lens). These images, with size of 4,000 × 3,000/12 megapixels, were converted into black and white and analyzed by the ImageJ free software (Rasband 2012). The result obtained was an average value in pixels, which varied from zero (absence of white areas) to 255 pixels (total entrance of luminosity). These values were converted into percentage.

The electrical conductivity and water temperature were measured with a Digimed DM-3 p meter; dissolved oxygen with an Instrutherm MO-900 oxygen meter; pH with a Digimed DM-22 pH meter; and turbidity with a Lutron TU-2016 digital turbidity meter. The water speed

was determined by the float method (Ramos and Oliveira 2003) and the depth with a meter stick. To determine the concentrations of nitrate (Crumpton et al. 1992), nitrite (Strickland and Parsons 1968), ammonium (American Public Health Association [APHA] 1995), and total phosphorous (Wetzel and Likens 2001), water samples were collected in 500-ml flasks in three riffles and three pools of each stream.

The substrate samples were passed through sieves with meshes of 2 mm, 1 mm, 500 μm , 250 μm , 106 μm , and 53 μm . The particulate organic matter was classified as coarse fraction (≥ 2 mm, CPOM) or fine fraction (≥ 1 mm, FPOM), whereas the inorganic sediment was classified as coarse sand (≥ 500 μm), medium sand (≥ 250 μm), fine sand (≥ 106 μm), and ultrafine sand (≥ 53 μm). For each sediment fraction, the concentration of organic matter was determined by burning a sample in a muffle furnace at 550°C for 4 h, with the total organic matter of the sediment being considered to be the sum of all these fractions.

Collection and Identification of Fauna. To obtain the EPT fauna, substrate samples (mixture of sand, litter, and stones) were collected from each mesohabitat with a Surber sampler (area of 0.04 m² and mesh of 210 μm) and fixed in 4% formaldehyde. The organisms were sorted under a stereoscopic microscope. Identification to the genus level was also performed with a stereoscopic microscope, and the Baetidae (Ephemeroptera) larvae were mounted on slides and identified to the genus level with an optical microscope. The following keys were used for identification: Bouchard (2004) and Salles (2006) for Ephemeroptera; Olifiers et al. (2004) and Lecci and Froehlich (2007) for Plecoptera; and Pes (2005), Pes et al. (2005), and Calor and Froehlich (2008) for Trichoptera. Afterward, the specimens were placed in glass jars containing 70° GL alcohol and maintained in the Benthic Invertebrates Laboratory of Juiz de Fora Federal University.

Data Analysis. To rank the streams and mesohabitats in relation to the environmental variables, we used principal component analysis (PCA) with the physical and chemical data, after standardization by the standard deviation. The number of principal components (PCs) was determined by considering eigenvalues greater than those generated by the broken stick method. The environmental variables that contributed most to the formation of the axes were selected by Pearson's correlation analysis ($r > 0.7$). The PCA was carried out with the PC-ORD 5.15 program (McCune and Mefford 2006).

The structure of the EPT assemblages in pools and riffles of the streams in the forest, pasture, and urban areas were analyzed regarding abundance, number of genera, Shannon–Wiener diversity (H'), and Simpson's dominance (D). After checking the homogeneity of the data (Levene test), we applied one-way analysis of variance to detect differences in the values of the attributes of the assemblages among streams with different land use. To verify differences between mesohabitats of streams, we applied the t -test (independent samples). These two analyses were performed with the Statistica Version 7 Program (Statsoft Inc. 2012).

To rank the streams and mesohabitats in relation to the composition and abundance of EPT, the abundance data were log transformed ($\log x + 1$) and submitted to detrended correspondence analysis (DCA) with the PC-ORD 5.15 program (McCune and Mefford 2006). The samples in which EPT genera were absent were not included in the analysis. The pools of urban stream 1 were not included in the analysis due to the absence of EPT specimens.

To verify whether there were differences in the composition of the EPT assemblage among the different stream categories, we applied the nonparametric permutation test (MRPP) with the PC-ORD 5.15 program (McCune and Mefford 2006), whereas to detect differences between pools and riffles of streams in the same category, we employed analysis of similarity (ANOSIM), with the R program (R Development Core Team 2013). We measured the similarity percentage (SIMPER) with the fauna abundance data to evaluate which taxa were mainly responsible for the differences between the mesohabitats for each stream category, using the PAST 2.17 program (Hammer et al. 2013).

To compare the response of the EPT assemblages of riffles and pools across land use categories, we employed two metrics, precision and sensitivity. The precision was calculated as the squared correlation (coefficient of determination, adjusted R^2) between the observed and adjusted values, measured by the variance explained by the model. In turn, the sensitivity of the EPT assemblages to land use was calculated as the magnitude of the change (slope of the line, b) of a predictable stress–response relation (e.g., Carlson et al. 2013). The values of adjusted R^2 and b were obtained by simple regression between the first PCA axis (independent variables) and the abundance, number of genera, dominance, Shannon diversity (H'), first DCA axis, and second DCA axis (dependent variables), using the Statistica version 7 program (Statsoft Inc. 2012).

Results

Environmental Variables. The first PCA axis distinguished the forest and urban streams, whereas the pasture streams were in an intermediate position in the ordination. This axis was positively related to the plant cover, dissolved oxygen, and the forest and pasture streams and negatively to NO_2 , NH_4 , NO_3 , and the urban streams. The second axis distinguished pools and riffles. This axis was positively related to water speed and riffles and negatively to ultrafine sand and pools (Fig. 2).

Biotic Variables. We found 706 immature EPT specimens in forest streams, 855 in pasture streams, and 226 in urban streams ($F(2,15) = 0.66$; $P = 0.529$). The richness observed in forest streams was 37 genera, 21 in pasture streams, and 7 in urban streams ($F(2,15) = 20.05$; $P < 0.001$). The Shannon–Wiener diversity was 2.52 in forest streams, 2.08 in pasture streams, and 0.97 in urban streams ($F(2,15) = 31.00$; $P < 0.001$). Finally, the Simpson dominance was 0.14 in forest streams, 0.16 in pasture streams, and 0.47 in urban streams ($F(2,15) = 28.50$; $P < 0.001$). With respect to mesohabitats, the diversity was greatest in riffles of the forest streams, whereas the abundance and richness were highest in this habitat in the urban streams (Table 2).

In the DCA, the first axis distinguished the forest streams from those in the pasture and urban areas, whereas the second axis distinguished the riffles and pools (Fig. 3).

The composition of the EPT taxa differed among the land use categories (MRPP: $T = -14.063$; $A = 0.072$; $P < 0.001$) and between the pools and riffles of forest streams (ANOSIM: $R = 0.402$; $P = 0.001$) and of pasture streams (ANOSIM: $R = 0.464$; $P = 0.001$). In the mesohabitats of the urban streams, there was no difference in the composition of EPT taxa (ANOSIM: $R = 0.112$; $P = 0.125$). According to the similarity percentage (SIMPER), *Phylloicus* and *Tripsectides* were representative of pools in forest streams, *Oxyethira* of pools in pasture streams, and *Smicridea* and *Americabaetis* of riffles in pasture streams (Table 3).

Response to Land Use. The sensitivity (slope) and precision (R^2) of the metrics of the EPT assemblage when analyzed together were similar between pools and riffles (Fig. 4). When analyzed separately, the diversity was correlated with the first PCA axis in the two mesohabitats, whereas richness was only correlated in riffles (Table 4).

Discussion

Disturbances from human removal of riparian vegetation can lead to alterations in the longitudinal flow profile and homogeneity of habitats within aquatic systems, causing reduced diversity of the faunal composition between distinct habitats (Roy et al. 2003, Hepp et al. 2013). The greater diversity and richness of the EPT genera in the forest streams can be explained by the higher oxygenation of the water, greater organic matter accumulation (coarse and fine), and higher percentage of plant cover in relation to the pasture and urban streams. EPT species depend on plant material from streamside vegetation to build their cocoons and/or as food and shelter (Uieda and Kikuchi 1995,

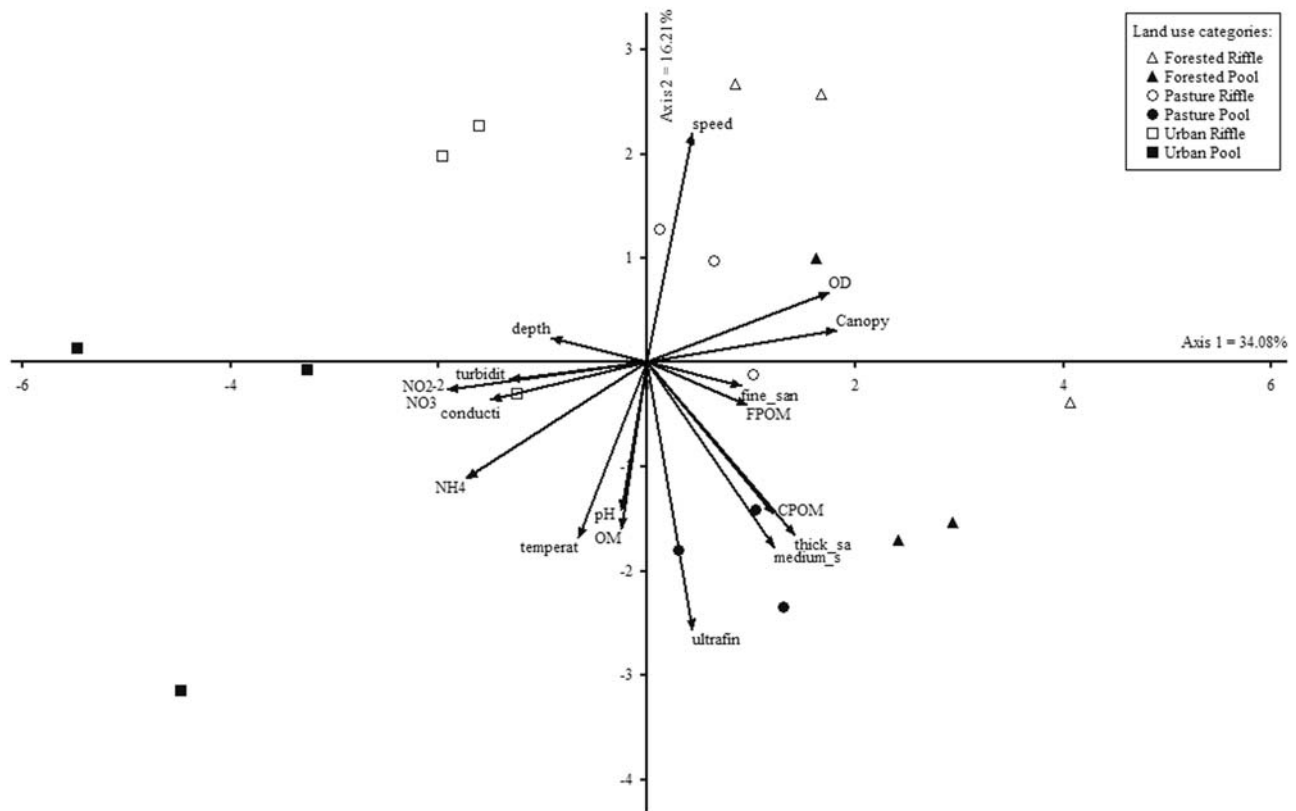


Fig. 2. PCA considering the environmental variables in the pools and riffles of the streams in forest, pasture, and urban areas, in the sub-basin of the Marmelos River, southeastern Brazil. Conducti: conductivity; Turbidit: turbidity; OD: dissolved oxygen; NO₃: nitrate; NO₂: nitrite; NH₄: ammonium; thick_sa: thick sand; medium_s: medium sand; fine_san: fine sand; ultrafin: ultrafine sand.

Table 2. t-test with the values of abundance, richness, Shannon–Wiener diversity, and Simpson dominance of the EPT fauna in pools and riffles of streams in forest, pasture, and urban streams of the sub-basin of the Marmelos River, southeastern Brazil

	Forested			Pasture			Urban		
	P	R	t	P	R	t	P	R	t
Abundance	274	432	0.97	52	803	2.28	4	222	3.66**
Richness	22	30	2.58	9	18	2.53	3	6	3.02**
Shannon	1.7	2.5	6.07*	1.7	1.9	0.10	1	0.9	0.75
Dominance	0.3	0.14	6.27**	0.3	0.2	1.18	0.4	0.54	0.22

R, riffles; P, pools.

* $P < 0.01$.

** $P < 0.05$.

Crisci-Bispo et al. 2007, Cortezzi et al. 2009). In these streams studied, *Phylloicus* and *Triplectides* were the most abundant genera. *Phylloicus* larvae shred leaves to build shelters (Prather 2003) and *Triplectides* larvae normally use wood chips for shelter (Crisci-Bispo et al. 2004). This result shows the importance of substantial inputs of plant material in these streams in comparison with the pasture and urban streams.

The streams flowing through pastures presented the highest total EPT abundance. This result corroborates the findings of Azrina et al. (2006), who reported that the loss of sensitive taxa in altered environments, accompanied by an increase of more tolerant ones, can result in greater total abundance of organisms compared with forest environments, which generally present richer and more diverse fauna, as also observed in our study. In these streams, nymphs of the genus *Americabaetis* were found in high abundance. They are less sensitive to environmental impacts (Callisto et al. 2001, Buss and Salles 2007, Souza et al. 2011), allowing them to use a variety of habitats, including disturbed sites (Siegloch et al. 2008). Likewise, *Oxyethira* and

Smicridea, also observed in high abundance in pasture streams, might have benefited from the opening of the dossel, which favors growth of diatoms and green algae, the main food of larvae of this genus (Wiggins 1996, Oliveira and Froehlich 1996, Pes et al. 2008).

Contrary to our initial hypothesis of a greater distinction between the composition of the assemblages between riffles and pools in the forest streams, we found a similar distinction between these two mesohabitats in the pasture streams. Normally, the formation of riffles and pools is associated with the presence of riparian vegetation, because these plants stabilize the stream banks and prevent the excessive entry of sediments, helping to maintain the channel's morphology (Poff et al. 1997) and hence the configuration of the habitats. In the pasture streams studied, the presence of riparian vegetation at some points along the banks might have helped prevent the entry of fine sedimentary material caused by the cattle's trampling. Besides this, the pasture areas through which these streams flow are not heavily grazed and still contain some trees and bushes, ameliorating the impacts. This is reflected in the PCA results, which indicated that the pasture streams had intermediate conditions in relation to the two other environments. Wasson et al. (2010) also found that the combination of pasture and small groves counteracted the pressures from tilled areas, so preservation of such areas can be an effective measure to mitigate the impacts of farming on watercourses.

The association of *Smicridea* with riffles can be explained because this habitat receives more fine particles in suspension, favoring organisms of this genus, which have filter-collector feeding habits (Cummins and Klug 1979, Oliveira and Froehlich 1996, Wallace and Webster 1996, Flint et al. 1999). On the other hand, *Phylloicus* was associated with pools, as reported by other authors (Flint et al. 1999, Wantzen and Wagner 2006). This habitat favors the presence of these immature organisms because they accumulate coarse plant matter for shelter and food (Baptista et al. 2001).

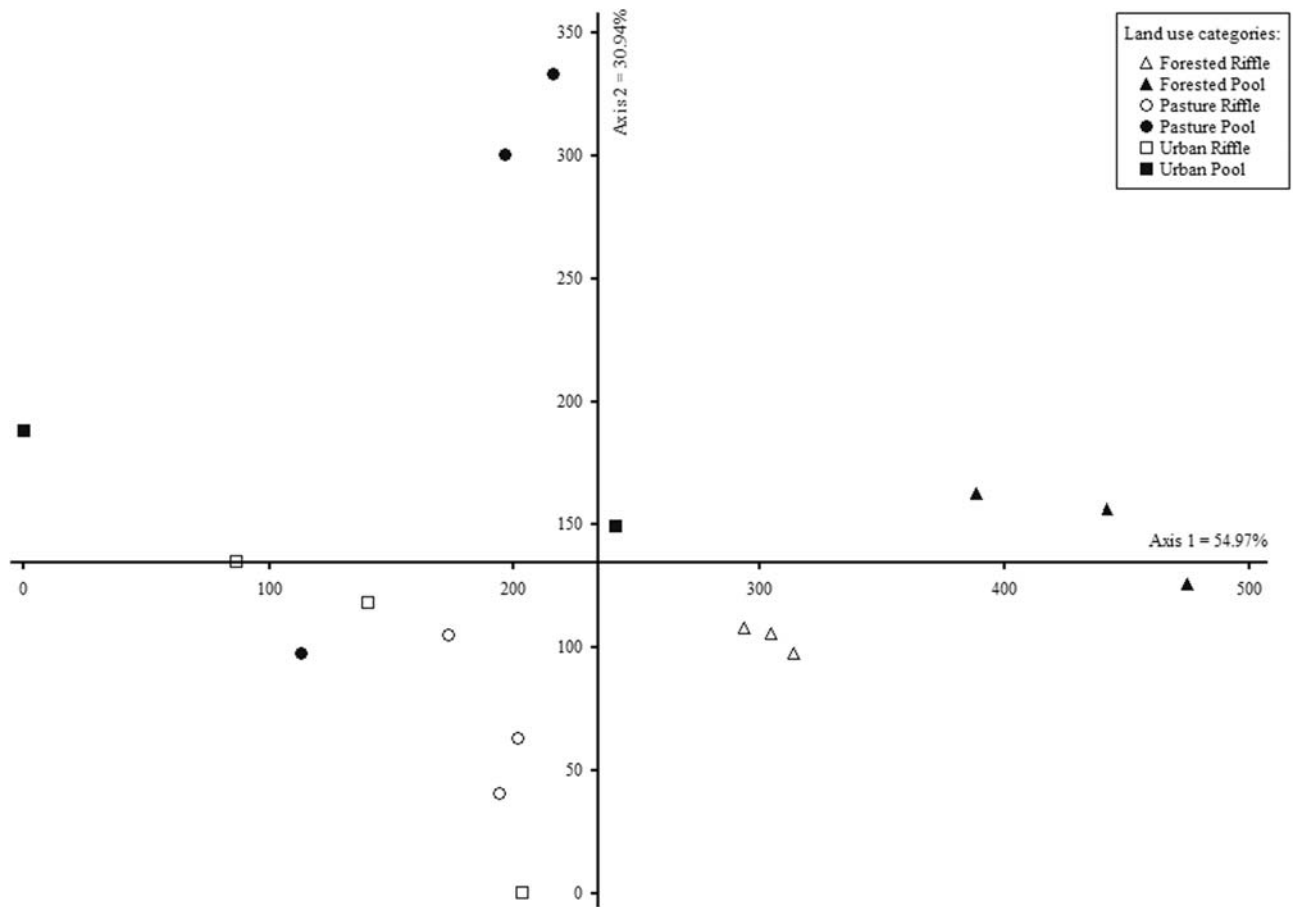


Fig. 3. DCA considering the abundance of EPT genera in pools and riffles of streams in forest, pasture, and urban areas, in the sub-basin of the Marmelos River, southeastern Brazil.

Table 3. SIMPER analysis with listing of only the 10 most abundant EPT taxa in pools and riffles of streams in forest, pasture, and urban areas of the sub-basin of the Marmelos River, southeastern Brazil

Taxon	Cumulative %	Mean abundance		
		Forest	Pasture	Urban
Comparison of pool habitats (average dissimilarity = 88.87)				
<i>Phylloicus</i>	25.21	10.1	0	0
<i>Triplectides</i>	36.95	0.6	0.333	0.067
<i>Oxyethira</i>	46.99	0	1.2	0
<i>Caenis</i>	53.78	0.467	1.13	0
<i>Farrodes</i>	59.54	2.6	0	0
<i>Helicopsyche</i>	64.25	0.667	0	0
<i>Hydroptilla</i>	68.35	0	0.2	0.133
<i>Americabaetis</i>	72.36	0.067	0	0.133
<i>Apobaetis</i>	75.97	0.267	0	0
<i>Hagenulopsis</i>	79.44	1.93	0	0
Comparison of riffle habitats (average dissimilarity = 88.87)				
<i>Smicridea</i>	26.97	9.33	9.67	4.33
<i>Americabaetis</i>	42.5	1.73	15.1	0.8
<i>Hydroptilla</i>	56.47	0.133	10.2	9.27
<i>Traveryphe</i>	62.54	2.27	3.53	0
<i>Phylloicus</i>	68.35	3.07	0.067	0
<i>leptohyphes</i>	72.91	2.67	0	0
<i>Anacroneuria</i>	76.81	2.4	0.533	0
<i>Oxyethira</i>	79.36	0.067	7.53	0.2
<i>Helicopsyche</i>	81.8	1.27	0	0
<i>Farrodes</i>	84.16	1.13	0.867	0

We found that the richness and diversity metrics were the most effective to detect the impacts of land use, an observation not reported by Carlson et al. (2013), who found greater sensitivity and precision only in the taxonomic composition. This result may have occurred in our study due to the marked differences found in the richness and diversity of the EPT assemblage among the three stream categories. In particular, the riffles showed a strong response of richness ($R^2 = 0.80$) and diversity ($R^2 = 0.66$) in relation to environmental alterations, whereas in the pools, only diversity was a sensitive metric. This result can be explained by the fact the riffle habitats shelter a higher number of EPT taxa, and the diversity of the assemblage was similar between the two mesohabitats. Similar to this result, Roy et al. (2003) also reported greater sensitivity of riffle metrics with respect to the entire community of invertebrates. This indicates the importance of paying attention to the characteristics of pools and riffles in areas with distinct environmental characteristics to optimize biomonitoring programs.

The results of this study demonstrate that urban land use causes differences in the structure of the EPT assemblages and compromised the distinction of riffles and pools. The absence of riparian vegetation impairs the natural soil retention mechanisms, resulting in changes in water flow and loss of habitats within the stream, directly affecting the structure of the EPT fauna. On the other hand, preservation of part of the natural streamside vegetation in pasture areas can mitigate the negative impacts of animal grazing, at least when this is less intense. Furthermore, the analyses demonstrated that the EPT assemblage metrics of riffles and pools respond differently to distinct land uses, indicating that the EPT richness and diversity in riffles are more robust to determine the impacts of removal of riparian vegetation and the input from domestic sewage. Therefore, we suggest that stream

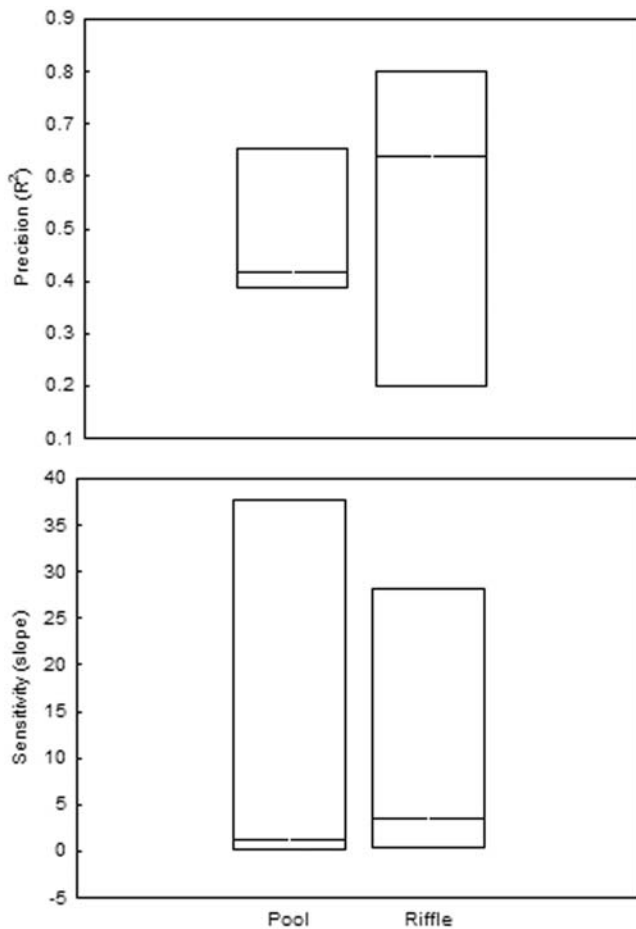


Fig. 4. Box-plot showing the sensitivity and precision of the six metrics for pools and riffles of streams in forest, pasture, and urban areas of the sub-basin of the Marmelos River, southeastern Brazil.

Table 4. Simple regression between the six response variables of the EPT community and the first PCA axis for pools and riffles of streams in forest, pasture, and urban areas of the sub-basin of the Marmelos River, southeastern Brazil

	Pools			Riffles		
	R^2	Slope	P	R^2	Slope	P
Taxa richness	0.39	1.26	0.058	0.8	3.50	0.001
Shanon diversity	0.65	0.16	0.001	0.64	0.37	0.006
DCA1	0.42	37.86	0.049	0.20	28.09	0.128

biomonitoring programs should evaluate riffles and pools separately when using EPT organisms, as these taxa may show different responses on the mesohabitat scale.

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