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Effects of urbanization on stream benthic invertebrate communities in Central Amazon

Renato T. Martins[a,*], Sheyla R.M. Couceiro[b], Adriano S. Melo[c], Marcelo P. Moreira[d], Neusa Hamada[a]

[a] Programa de Pós-Graduação em Entomologia, Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia – INPA, Av. André Araújo, 2936, CP 478, CEP 69067-375, Manaus, AM, Brazil
[b] Instituto de Ciências e Tecnologia das Águas, Universidade Federal do Oeste do Pará. Rua Vera Paz s/n, Salé, CEP 68035-110, Santarém, PA, Brazil
[c] Departamento de Ecologia, ICB, Universidade Federal de Goiás, CP 131, CEP 74001-970, Goiânia, GO, Brazil
[d] Programa Geopolítica da Conservação, Fundação Vitória Amazônica, Rua Estrela D'Alva 146, Aleixo, CEP 69060-093, Manaus, AM, Brazil

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ABSTRACT

Urbanization and its physical and chemical effects on aquatic environments influence invertebrate communities negatively. Yet, it is not clear how urbanization affects inter-annual variation of invertebrate assemblages in streams. We 1) evaluated urbanization effects on the ecological conditions (biotic and abiotic) of streams in Manaus and 2) analyzed invertebrate community variation over time (between 2003 and 2010). Data on abiotic variables and invertebrates from 2003 were obtained from a previous study. In 2010 we sampled abiotic variables and invertebrate communities in the same low-order urban streams sampled in 2003 (n = 40). We recorded high values of total nitrogen, total phosphorous, deforestation, total impervious area (TIA), water temperature, pH, and electrical conductivity in the most urbanized streams, as compared to the least-impacted ones. In contrast, the least-impacted streams had high dissolved oxygen concentrations. Water quality was poorer in 2010 than in 2003: oxygen concentration was lower and total nitrogen, total phosphorous, deforestation, and TIA significantly higher in 2010. We recorded higher inter-annual variation of abiotic variables in the most-impacted streams as compared to the least-impacted streams. EPT (%, Ephemeroptera, Plecoptera, and Trichoptera) and richness metrics decreased with urbanization. On the other hand, % OP (percent of Oligochaeta and Psychodidae) increased with urbanization. Observed and EPT richness and% OP increased between 2003 and 2010. On the other hand, rarefied richness decreased between years. Increases of observed and EPT richness between 2003 and 2010 were related to low inter-annual variability in streams conditions; however, differences of% OP and rarefied richness were not related to inter-annual variability in environmental conditions. The degree of urbanization did not explain the magnitude of the within-stream difference of invertebrate communities between 2003 and 2010. The increased effects of urbanization represented by the abiotic variables sampled and the reduction of invertebrate richness and increased dominance of tolerant taxa indicate that public policy is not enough to protect or mitigate human impacts on the urban water systems under study.

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1. Introduction

Urbanization is a major threat to aquatic ecosystems (Ramírez et al., 2009; Wallace et al., 2013 Wallace et al., 2013) and developed countries have adopted various policies to reduce the impacts on these ecosystems (Booth et al., 2004). In developing countries, however, urban population growth has often not been accompanied by efficient public policies, resulting in degradation of aquatic ecosystems (Ramírez et al., 2009; Couceiro and Hamada, 2011 Couceiro and Hamada, 2011). In urban areas, the main impacts on aquatic environments has been related to the decrease of soil permeability, removal of riparian vegetation and to the increase of domestic and industrial wastewater input (Paul and Meyer, 2001; Walsh et al., 2005; Fig. 1).

Decrease in soil permeability increases superficial runoff and may increase the input of contaminants to streams (e.g., organic compounds and heavy metals, Schueler, 1994; Walsh et al., 2005). This pollutant input causes changes in physical and chemical water

* Corresponding author.
E-mail address: martinsrt@gmail.com (R.T. Martins).

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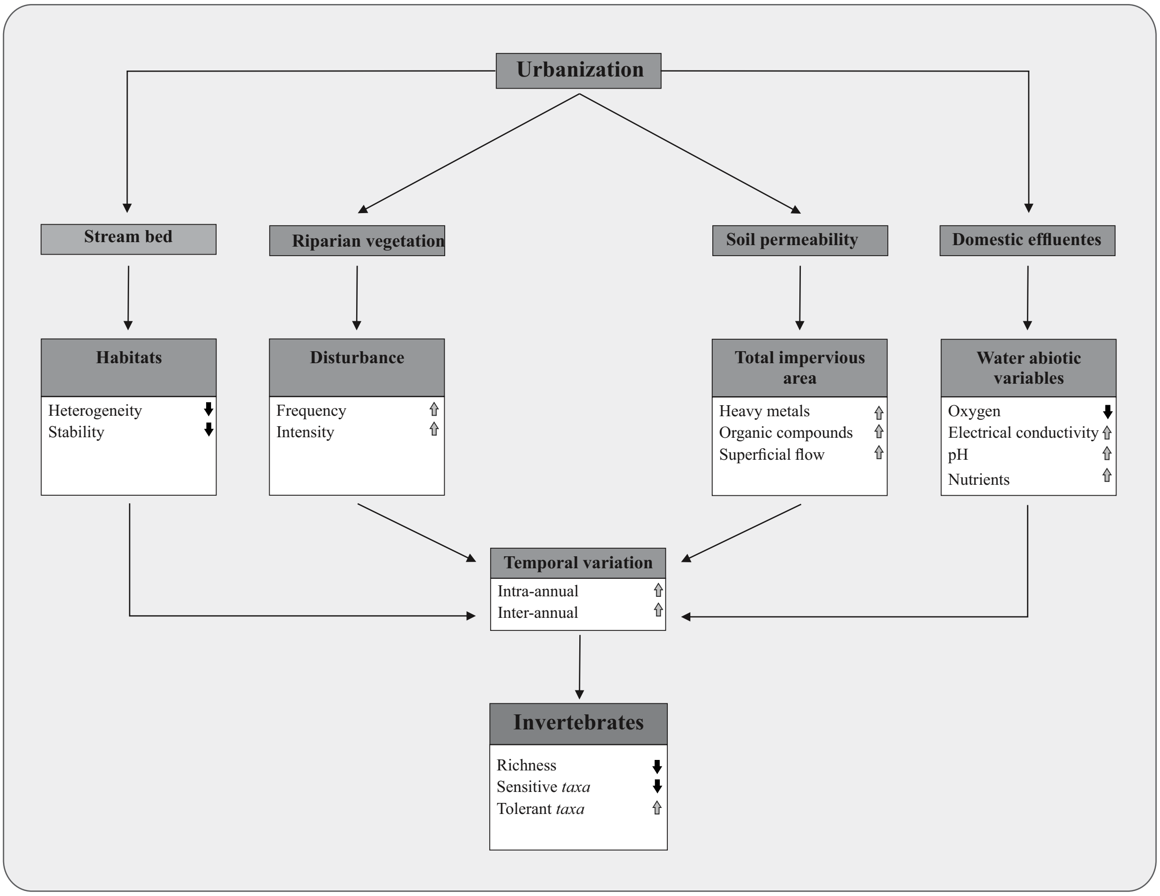

Fig. 1. Conceptual model of urbanization effects on invertebrates in aquatic ecosystems.

characteristics, especially due to increased turbidity, electrical conductivity, nutrient concentration and decreased dissolved oxygen (Wenger et al., 2009).

Riparian vegetation removal can result in increased bank erosion and, consequently, in the siltation of stream beds (Couceiro et al., 2010). Lack of canopy allows more light and results in increased water temperature, which decreases oxygen availability (Paul and Meyer, 2001; Callisto et al., 2012). In addition, riparian vegetation removal reduces the input of allochthonous organic matter in aquatic ecosystems and results in the decrease of habitats and food availability for organisms that depend on this resource (Sanchez-Arguello et al., 2010).

Domestic sewage pollution results in high nitrogen and phosphorus concentrations in streams, due to inputs of human wastes and detergents (Halstead et al., 2014). Increase of nutrients in aquatic systems favors high abundance and activities of micro-organisms (e.g., coliform bacteria and nitrifying bacteria) and results in high respiration and reduced dissolved oxygen (Walsh et al., 2005; Rosa et al., 2014).

Urbanization generally affects aquatic organisms negatively, particularly invertebrates due to their limited mobility, sensitivity to habitat quality and dependence on allochthonous resources (Rosenberg and Resh, 1993). Thus, urbanization often results in a decrease in species richness by exclusion of sensitive taxa to environmental changes and an increase in abundance of taxa tolerant to impacts (Couceiro et al., 2007a, 2012; Feio et al., 2013; see Fig. 1).

The natural variation of fauna over time has a key role in the development of biomonitoring programs as it can mask the effects of environmental impacts (Mazor et al., 2009; Álvarez-Cabria et al., 2010). The relationship between inter-annual variation of communities and gradients of natural or anthropogenic impacts is not always linear. Reference streams that have high stability, heterogeneous habitats and low variation of abiotic variables over time, generally have communities with low inter-annual variability (Robinson et al., 2000; Mykrä et al., 2011). Thus, one could expect less annual variation of invertebrate fauna in reference streams as compared to impacted streams (Maul et al., 2004; Feio et al., 2010, 2015); however, this does not always occur. Huttunen et al. (2012) recorded similar temporal faunal variation in reference and human-disturbed sites in two catchments in Finland. This low variability of invertebrate communities in impacted streams was due to the simplified assemblage composed of only a few tolerant species in a homogenous stream habitat.

Tropical forests have been losing area to pasture and agriculture and, to a lesser degree, to growing cities. Although the area occupied by cities is far smaller than that of pasture and agriculture, the impacts caused by cities tend to be much more severe. Cities in developing countries tend to grow by occupation of the peripheral areas or by following roads (Laurance et al., 2001; Cohen, 2004). In most cases this growth occurs in a disorderly fashion by occupation of public or private areas, and the new neighborhoods have poor sanitary facilities. Consequently, streams in these areas are profoundly impacted due to deforestation and organic enrichment by domestic effluents (Couceiro et al., 2007a,b). After populations establish themselves in the newly occupied areas, it is expected that political pressure will drive urban improvements such as collection of domestic wastewater and establishment of green areas. Accordingly, one could expect an improvement of stream conditions and, consequently, a partial restoration of the original biota. However, stream conditions may deteriorate even more if population density in new neighborhoods continues to increase and this is coupled with absence of public policies to mitigate the impacts.

With data from the communities of aquatic invertebrates collected in 2003, Couceiro et al. (2007a) evaluated the environmental quality of urban streams in Manaus, a city in central Amazonia. Their study showed that 80% of the 65 streams studied were degraded by deforestation of riparian vegetation and/or organic pollution (Couceiro et al., 2007a,b). One might therefore expect improvement of urban facilities in neighborhoods that are more than 10 years old, mitigating the severe initial effects of urbanization. We used data from Couceiro et al. (2007a) and collected new samples in 2010 to evaluate urbanization effects on invertebrate communities and abiotic variables over the seven-year period from 2003 to 2010. Specifically, we tested the hypotheses that 1) due to human population increase in Manaus between the studied years (2003: ∼1.5 million; 2010: ∼1.8 million) and the absence of extensive public policies to mitigate the effects of this population growth, the ecological quality (biotic and abiotic) of streams in 2010 should have decreased; and 2) because non-impacted streams are generally more heterogeneous and feature low inter-annual variation of abiotic variables (see Fig. 1), we expect to record high invertebrate community variation over time in the streams most impacted by urbanization.

2. Material and methods

2.1. Study area

The city of Manaus (Amazonas state) occupies 11,401 km^2, with ∼2% (229 km^2) of urban area (IBGE, 2010). The climate is classified as humid equatorial, with high mean annual rainfall (2286 mm) and a mean annual temperature of 27 °C. There is a rainy season from November to May (monthly precipitation: minimum = 141 mm; maximum = 514 mm) and a less-rainy season from June to October (monthly precipitation: minimum = 27 mm; maximum = 119 mm; INMET, 2013).

Due to the implementation of an industrial district in the 1970s, the last 40 years have seen a huge population growth in Manaus, from ∼300,000 inhabitants in 1970 to 1.8 million in 2010 (IBGE, 2010), making Manaus the most populous city in the Amazon region. This rapid population growth was mainly concentrated in urban areas (∼99% of the population) and resulted in disorderly occupation of stream banks (areas with low financial value and protected by law), with consequent removal of riparian vegetation, siltation, and domestic sewage input into aquatic environments and wetlands (Bentes, 2005; Couceiro et al., 2007a). Due to the presence of a dense river network in the urban area of Manaus, these human impacts reflect directly in people's quality of life, such as floods, smelly water and waterborne diseases (Miagostovich et al., 2008; Marengo et al., 2013).

Couceiro et al. (2007a) sampled 65 streams (first to fourth order) in three sub-basins of the Rio Negro (Mindu, Quarenta, and Tarumã). These streams have different levels of impacts (deforestation and/or organic pollution). We resampled 40 first or second order stream sites (Fig. 2) studied in 2003 by Couceiro et al. (2007a). Sites were selected according to following criteria: i) small steams (first and second orders), ii) streams with different degrees of urban impact; iii) accessibility (e.g. some streams studied previously were buried or piped) and, iv) similar number of streams in each sub-basin. In both years, samples were collected in the less-rainy season (average monthly rainfall: 2003 = 97 ± 23 mm; 2010 = 88 ± 46 mm).

2.2. Abiotic variables

We made *in situ* measurements of the following variables: pH (potentiometer WTW, model PH90), electrical conductivity (μS/cm; conductivimeter WTW, model LF90), dissolved oxygen (mg/L; oxymeter TSI, model 55), and water temperature (°C; oxymeter TSI, model 55). Water samples were collected to determine concentrations of total phosphorus (μmol/L) and total nitrogen (μmol/L) in the laboratory, using Valderrama's method (1981).

The deforested area and total impervious area (TIA) in each stream basin were obtained for 2003 and 2010 using Landsat TM 5 satellite images (orbital point 231/062). We used Envi 4.6 software and supervised classification by maximum likelihood to classify the images into "*terra firme*" (upland) forest, secondary growth vegetation, agriculture/pasture, exposed soil, urban cover, and water bodies. Classified images were compared with the Landsat images in RGB format to evaluate the quality of classification. For this analysis, we used the Convolution Median Filter (3 × 3). In the ArcGIS® 10.1 program, images in shape format were processed and categories classified erroneously were manually corrected. We also estimated deforested area and TIA in a circle (radius = 500 m) around each sampled stream. We considered the exposed soil fraction and urban cover as impervious surfaces (Chadwick et al., 2006). The former consisted of a small fraction (usually <5%), and part of this area likely consists of compressed and semi-impermeable soils.

2.3. Sample and identification of invertebrates

In each stream, we collected five sample units of bed sediments along a reach of 60 m. We used an aquatic D-net (570 cm^2, 1 mm^2 mesh) dragged 1 m for each sample unit (Couceiro et al., 2007a). In the laboratory, samples were washed under running water in a metal sieve (125 μm) and then preserved in hydrated ethanol (80%) until the invertebrates were sorted under a stereoscopic microscope.

Invertebrate identification was performed to the lowest taxonomic level possible using literature on the regional fauna (e.g. Hamada and Couceiro, 2003; Pes et al., 2005; Hamada and Ferreira-Keppler, 2012) and with help from specialists (see Acknowledgements). The taxonomic resolutions of samples obtained in 2003 and 2010 were similar. Thus, Chironomidae identified at the genus level in the study of Couceiro et al. (2007a) were reclassified into subfamilies to be comparable with samples collected in 2010. Data from dredge samples collected by Couceiro et al. (2007a) in 2003 were not used in this study.

2.4. Statistical analysis

2.4.1. Abiotic variables

We used a paired *t*-test to individually identify significant differences of abiotic variables between 2003 and 2010. Principal component analysis (PCA) of abiotic data was used to summarize stream conditions along the urbanization gradient. Prior to this analysis, all variables were standardized ([observed – mean]/standard deviation). To assess the overall inter-annual variability of abiotic data, we calculated the abiotic dissimilarity (Euclidean distance) between 2003 and 2010 for each stream and regressed them against the urbanization gradient obtained in the PCA. In our study, the urbanization gradient consisted of averaged of scores of samples in 2003 and 2010 on the first PCA axis. Because some streams may have improved and others degraded over the period, we repeated the analyses using scores of samples for each year separately. Using the Broken-stick model (Jackson, 1993) we determined that only the first PCA axis was significant for representing the variability in abiotic data.

2.4.2. Aquatic invertebrates

In order to assess the ecological quality of streams in 2003 and 2010, we calculated indicator metrics based on measurements of composition (% EPT [Ephemeroptera, Plecoptera and Trichoptera]

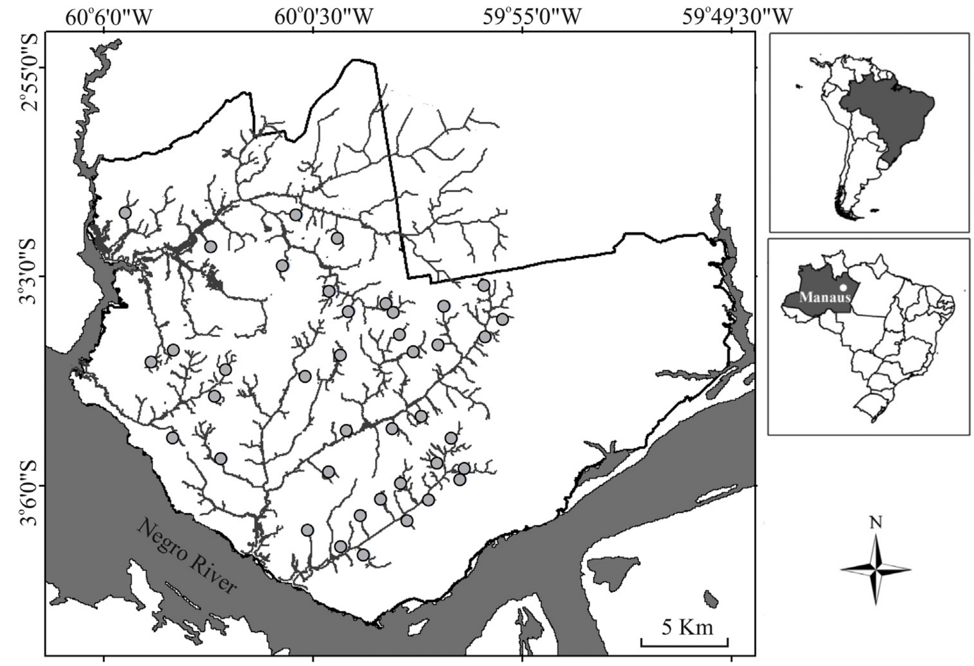

Fig. 2. Streams sampled (n = 40) in 2003 and 2010 in the city of Manaus, Amazonas, Brazil. The continuous line indicates the limit of the urban area of Manaus.

and% OP [Oligochaeta and Psychodidae]), and richness (observed richness, rarefied richness and EPT richness). These metrics usually respond to a disturbance gradient (Couceiro et al., 2012). Rarefied richness was calculated in order to exclude abundance effects (Gotelli and Colwell, 2001). To calculate rarefied richness, we used the lower value of the abundances found in 2003 and 2010 for each stream. Differences in each metric between 2003 and 2010 were tested separately using a paired *t*-test. The effects of urbanization gradient (data from 2003 and 2010) on invertebrate metrics were tested using regression models. We used a generalized linear model (GLM) and Quasibinomial distribution for composition (percent data), Normal distribution for the rarefied richness (continuous data) and the Poisson distribution for observed richness and EPT richness (count data). We also assessed the effects of temporal variability of abiotic data on variation of each metric (difference between 2010 and 2003 values). This analysis was performed only to metrics that significantly differ between years.

We used non-metric multidimensional scaling (NMDS) to assess invertebrate community similarities between 2003 and 2010 and along the urbanization gradient. We used presence-absence data (Sørensen distance) to evaluate changes in species composition and abundance data (Bray–Curtis dissimilarity, log[x + 1]) in order to evaluate species composition and relative abundances. To assess the inter-annual variability of invertebrate communities, we calculated Sørensen and Bray–Curtis dissimilarities between 2003 and 2010 for each stream. We regressed these dissimilarities against the urbanization gradient and the temporal variability of abiotic data. In addition, we repeated the linear regression between inter-annual variability of invertebrate communities and the urbanization gradient using scores of each year in the first PCA separately. All statistical analyses were performed in R software (R Core Team, 2012) using the vegan package (Oksanen et al., 2015).

3. Results

3.1. Abiotic variables

We observed a decrease in oxygen values from 2003 to 2010 ($t = 2.61$, $df = 39$, $p = 0.013$; Table A1). Total nitrogen ($t = -3.42$, $df = 39$, $p = 0.001$), total phosphorous ($t = -4.60$, $df = 39$, $p < 0.001$), deforestation ($t = -6.04$, $df = 39$, $p < 0.001$), and TIA ($t = -6.19$, $df = 39$, $p < 0.001$) were higher in 2010. Electrical conductivity ($t = -1.01$, $df = 39$, $p = 0.317$), water temperature ($t = 1.76$, $df = 39$, $p = 0.086$), and pH ($t = 1.84$, $df = 39$, $p = 0.074$) did not differ between years (Table A1).

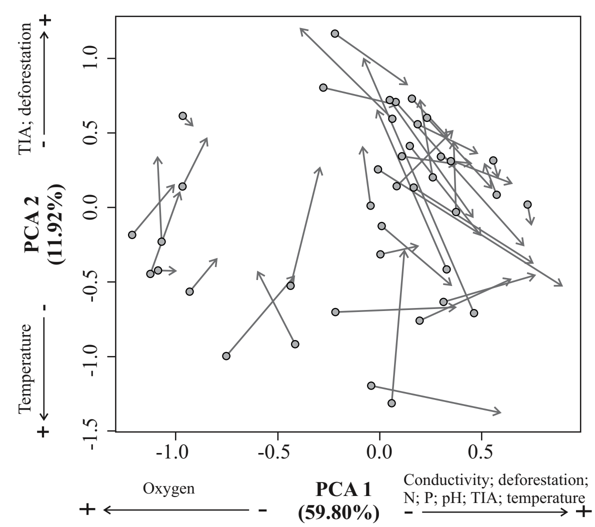

Fig. 3. Principal components analysis (PCA) of abiotic variables from 2003 and 2010 in urban streams in Manaus, Central Amazonia. The points of the arrows indicate the position of streams in 2010. Arrows indicate the variation in abiotic variables between sample years for the same stream. N = total nitrogen, P = total phosphorous, TIA = total impervious area.

The first PCA axis separated streams along an urbanization gradient with the least-impacted streams scoring at the left of the ordination (Fig. 3). Axis I of the PCA explained 59.8% of the total variation and was positively related to pH, electrical conductivity, total nitrogen, total phosphorus, temperature, deforested area, and TIA and negatively related to oxygen. Axis II of the PCA explained 11.9% of the total variation and was positively associated with TIA and deforestation values and negatively with water temperature. The urban gradient (first PCA axis) ranged from −1.21 to 0.73 and −1.09 to 0.90 in 2003 and 2010, respectively. Although there was not an increase in the amplitude of urbanization, it should be noted that streams tended to assume higher scores in 2010, indicating higher urbanization impact. We observed a positive relationship between the within-stream change in abiotic conditions between the years and the urbanization gradient using data of 2003 ($R^2 = 0.25$, $F_{1,38} = 13.81$, $p = 0.001$) or 2010 ($R^2 = 0.28$, $F_{1,38} = 16.54$, $p < 0.001$; see Appendix B). Similar results were obtained when we used urbanization as the pooled data of 2003 and 2010 in the first PCA axis ($R^2 = 0.30$, $F_{1,38} = 16.75$, $p < 0.001$). These regressions indicate that most-impacted streams tended to change more than the least-impacted streams in abiotic condition between 2003 and 2010.

3.2. Aquatic invertebrates

We collected 77,176 specimens (2003 = 38,358; 2010 = 38,818) distributed in 15 orders and 95 taxa (Table A2). Percent of Oligochaeta and Psychodidae ($t = -2.93$, $df = 39$, $p = 0.006$), and EPT richness ($t = -2.26$, $df = 39$, $p = 0.029$) increased from 2003 to 2010 (Table A3). Observed richness ($t = 2.40$, $df = 39$, $p = 0.021$) and rarefied richness ($t = 4.84$, $df = 39$, $p < 0.001$) decreased from 2003 to 2010. Percent of EPT (%; $t = 0.87$, $df = 39$, $p = 0.390$) did not differ between years ($t = 0.87$, $df = 39$, $p = 0.390$; Table A3).

Percent of EPT was negatively related to urbanization (all samples collected in 2003 and 2010; $R^2 = 0.23$, $F_{1,78} = 60.26$, $p < 0.001$; Fig. 4A). On the contrary, percent of Oligochaeta and Psychodidae (% OP) was positively associated with the urbanization gradient ($R^2 = 0.41$, $F_{1,78} = 49.30$, $p < 0.001$; Fig. 4B). Regarding non-proportional data, observed richness ($R^2 = 0.61$, $F_{1,78} = 219.10$; $p < 0.001$), rarefied richness ($R^2 = 0.57$, $F_{1,78} = 104.20$; $p < 0.001$) and EPT richness ($R^2 = 0.55$, $F_{1,78} = 355.98$; $p < 0.001$) were negatively associated with urbanization (Fig. 4C–E).

Inter-annual change in% OP (2010-2003) were not associated to temporal variability of abiotic data ($R^2 = 0.05$, $F_{1,38} = 2.87$; $p = 0.098$; Appendix C). Similarly, differences in rarefied richness in 2010 in relation to 2003 were not associated to changes in environmental conditions ($R^2 = 0.06$, $F_{1,38} = 3.515$; $p = 0.069$). In contrast, streams with higher observed richness in 2010 in relation to 2003 were less variable in abiotic conditions ($R^2 = 0.24$, $F_{1,38} = 13.10$; $p = 0.001$). The same trend was observed for EPT richness ($R^2 = 0.23$, $F_{1,38} = 12.95$; $p = 0.001$).

We recorded distinct communities along the urbanization gradient using both Sørensen and Bray-Curtis dissimilarities in the NMDS ordinations (Fig. 5). In addition, it was possible to identify a clear pattern in the distribution of taxa along the urbanization gradients in both 2003 and 2010 (Fig. 6). Most-impacted streams were associated with communities dominated by Oligochaeta, and Psychodidae (Fig. 4B). Least-impacted streams were associated with Elmidae, Orthocladiinae and EPT taxa (Figs. 4 A; 6).

We did not record significant differences in the magnitude of the within-site inter-annual variability of invertebrate communities along the urbanization gradient (see Appendix D). This means that the least-impacted streams changed as much as the most-impacted streams in terms of species composition and the relative abundances in the 2003–2010 period.

4. Discussion

4.1. Abiotic variables

We recorded a decrease in environmental conditions of streams after seven years since the original study. Increase of urbanization pressure on streams was related to oxygen decrease and nitrogen, phosphorus, deforestation and TIA increases. Within-stream inter-annual variation of abiotic variables was higher in the most-impacted streams in relation to the least-impacted streams. High proportion (%) of EPT and richness (EPT, observed and rarefied) were related to low urbanization effects. On the other hand, high values of tolerant taxa (Oligochaeta and Psychodidae; %) were related to high urbanization values. However, the high temporal variability in environmental conditions of the most impacted streams did not result to high variability in composition of invertebrate communities.

In impacted streams, environmental conditions tended to be less stable than the non-impacted streams, principally due to high domestic sewage input and lack of riparian vegetation. Thus, impacted streams are more likely to have large variations over time (Townsend et al., 1987; Davies et al., 2010). In our study, the inter-annual variation of abiotic variables in the streams was influenced mainly by changes in dissolved oxygen concentration and in variables often associated with urbanization (total nitrogen, total phosphorus, deforested area, and TIA) (Couceiro et al., 2007a; Feio et al., 2013).

4.2. Aquatic invertebrates and environmental quality

We observed a decrease in richness metrics along the urbanization gradient due to the exclusion of sensitive taxa. Several studies have reported a negative relationship between urbanization and number of taxa, mainly due to exclusion of taxa in the orders Ephemeroptera, Plecoptera, and Trichoptera (Walsh et al., 2007; Evans-White et al., 2009; Yuan, 2010; Couceiro et al., 2012; Weissinger et al., 2012). Moreover, we recorded change in community structure along urbanization gradient. Orthocladiinae was common in the least-impacted streams. The genera in this chironomid subfamily are usually sensitive to urbanization, are intolerant to high concentrations of nutrients, and require high concentrations of oxygen (Hicham and Lotfi, 2007). They are observed in high abundance in environments that are not impacted (Marques et al., 1999; Oliveira et al., 2010). Other taxa recorded in the least-impacted streams are frequently sampled in the Brazilian Amazon and are related to environments with well-oxygenated water and the presence of riparian vegetation (Walker, 1994; Cleto-Filho and Walker, 2001; Couceiro et al., 2010, 2011; Martins et al., 2015). In addition, some of these taxa use allochthonous leaves as a food source (e.g., *Phylloicus* and *Triplectides*) or habitat (e.g., Palaemonidae).

EPT richness were higher in 2010 and this resulted mostly of increases in the least-impacted streams and was probably related to the higher abundance in these streams as compared to 2003 (mean abundance: 2003 = 401.29 ± 445.08; 2010 = 1082.29 ± 652.21) and not to the improvement of environmental quality. In fact, we detected a decrease in rarefied richness from 2003 to 2010 and this may be related to increase of urbanization pressure in these streams, principally increases in deforestation, TIA and nitrogen. In fact, aquatic environments in protected areas in the city of Manaus (e.g., Reserva Ducke) have been affected negatively by increased urbanization (Ferreira et al., 2012).

We recorded an increase in relative abundance of tolerant organisms (e.g., Oligochaeta and Psychodidae) in 2010 in most streams. These taxa are frequently associated with environments impacted by urbanization (Cleto-Filho and Walker, 2001; Walsh et al., 2005; Tang et al., 2009). Oligochaeta (mainly Tubificinae) have hemoglobin (Flores-Tena and Martmnez-Tabche, 2001), whereas Psychodidae have a respiratory siphon and obtain oxygen directly from the atmosphere (Fausto et al., 1998). These adaptations allow organisms in these groups to remain in impacted streams and reach high abundance because of high food availability (Haase and Nolte, 2008; Martins et al., 2008; Lopes et al., 2015). Oligochaeta was

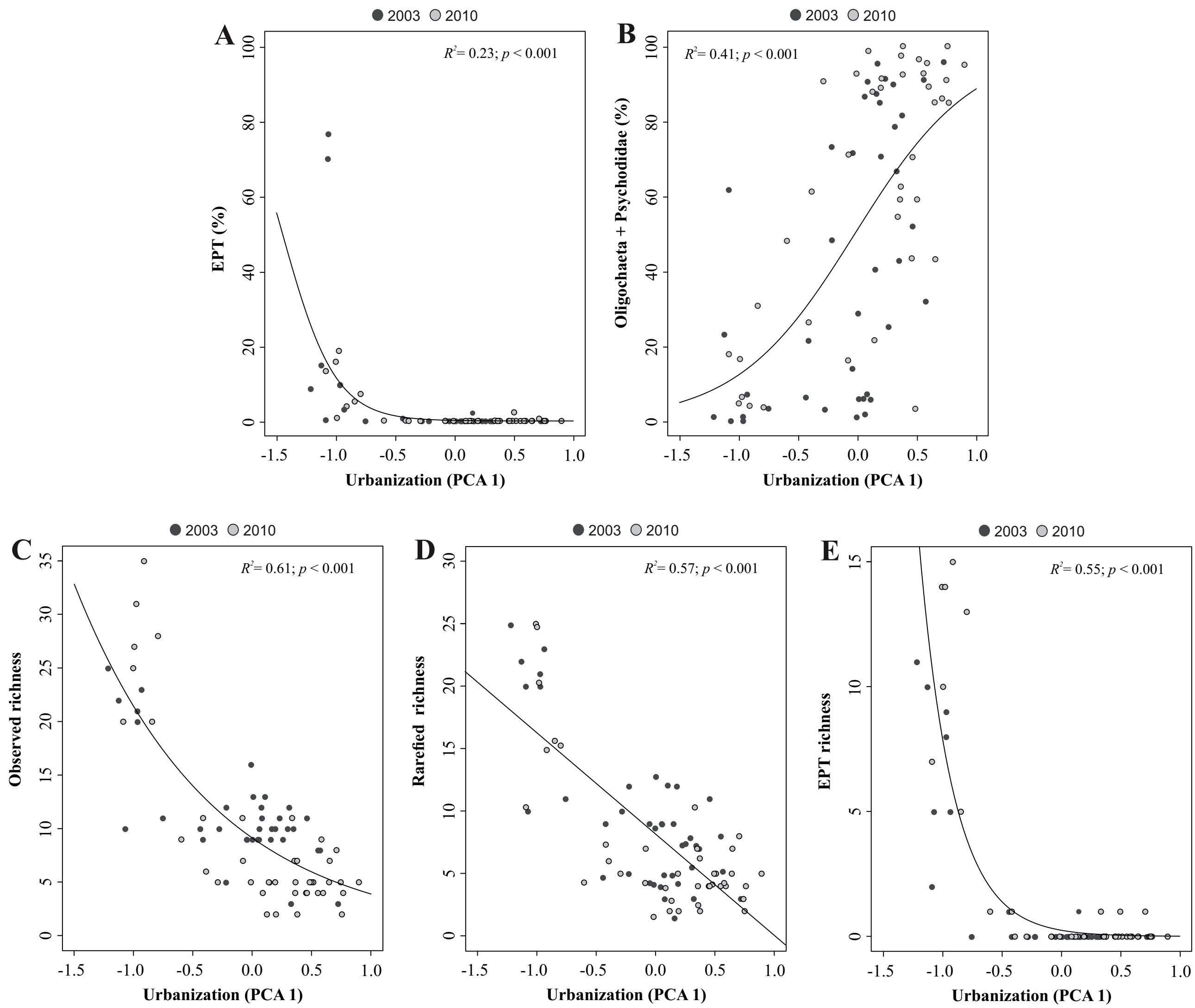

Fig. 4. Relationship between invertebrate metrics and urbanization gradient using sampled data from 2003 and 2010 in Manaus, Central Amazonia. We used generalized linear model (GLM) to count data (Poisson distribution) and percent data (Quasibinomial distribution). To rarefied richness (continuous data) was used linear regression (Normal distribution). EPT = Ephemeroptera, Plecoptera and Trichoptera.

present in most samples along the urbanization gradient, therefore, the simple occurrence of these taxa does not indicate environmental degradation. Instead, it is their high dominance that indicates urbanization effects on streams.

4.3. Aquatic invertebrates and inter-annual variation

Contrary to our hypothesis, within-site inter-annual variation of invertebrate communities did not increase along the urbanization gradient. High variability in communities in the least-impacted stream may occur due to a high number of taxa recorded only in one of the sampled years (2003: n = 14; 2010: n = 24; 40% of the taxa in the present study). The abundance of taxa that occurred in only one year ranged from 1 to 34 individuals. Of these, ~70% were recorded in low abundance (up to five individuals) and in 1–2 streams. On the other hand, in most-impacted streams, high community inter-annual variability is related to the change in relative abundance of the dominant taxa, because the number of Oligochaeta individuals increased by ~2.2 times and Psychodidae decreased by ~3.5 times from 2003 to 2010 (Table A2).

Increase of inter-annual variability of fauna may indicate mild environmental impact on the ecosystem, because high community variation is often associated with replacement or exclusion of taxa (Odum et al., 1979; Underwood, 1991). In contrast, non-impacted streams and those highly-impacted may present low inter-annual variation of fauna, although due to different reasons. Highly-impacted streams presented low richness and high environmental variation. In this case, low inter-annual variation of fauna may be due to the restricted number of stress-tolerant species present. They should present wide tolerance to environmental change and thus remain unchanged in face of environmental variability. Non-impacted streams presented high richness and low environmental variation. This high invertebrate community variability in the least-impacted streams seems to be related to rare species and, thus, to the difficulty of estimating their abundance and frequency of occurrence. However, it could be speculated that this high variation is associated with increases in deforestation (from 16.0% to 30.5%), TIA (from 13.4% to 28.0%), and nitrogen (from 3.3 μmol/L to 4.2 μmol/L) average values, which are metrics associated to increased pressure of urbanization. However, because temporal studies are scarce in the tropical region (and especially in the Amazon), it is not possible to claim that the observed inter-annual variability is related to the increase in human impacts without understanding the community's inter-annual variation in

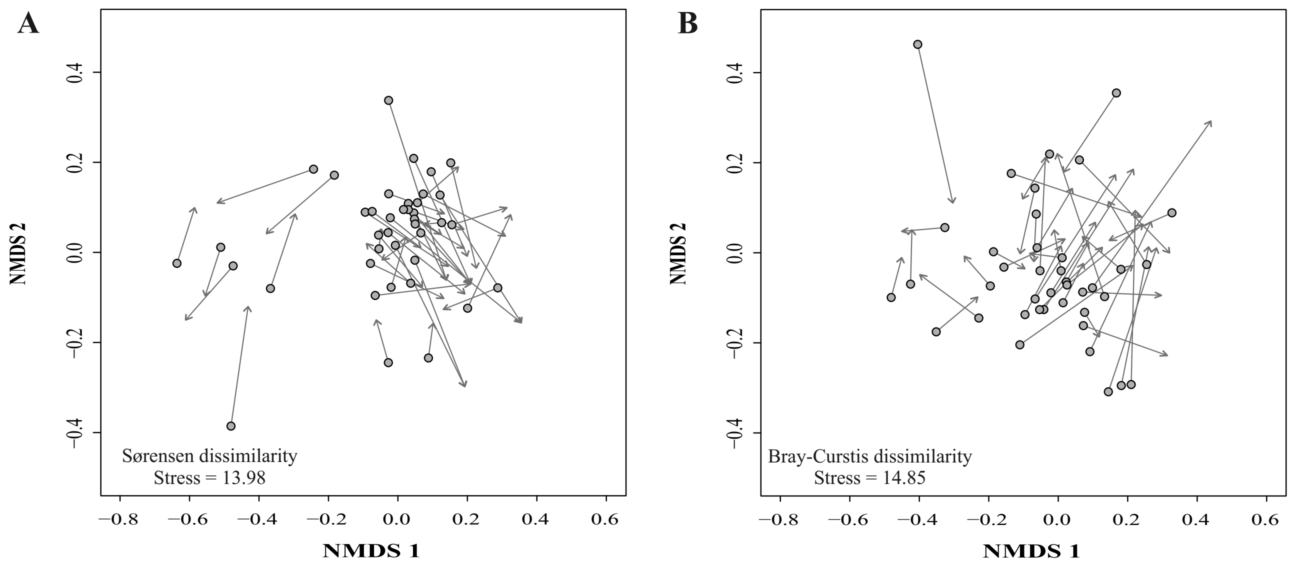

Fig. 5. Non-metric multidimensional scaling of invertebrate communities (Sørensen [A] and Bray-Curtis [B] distance) from 2003 and 2010 in streams in Manaus, Central Amazonia. The points of the arrows indicate the position of streams in 2010. Arrows indicate the variation in invertebrate communities between sample years for the same stream.

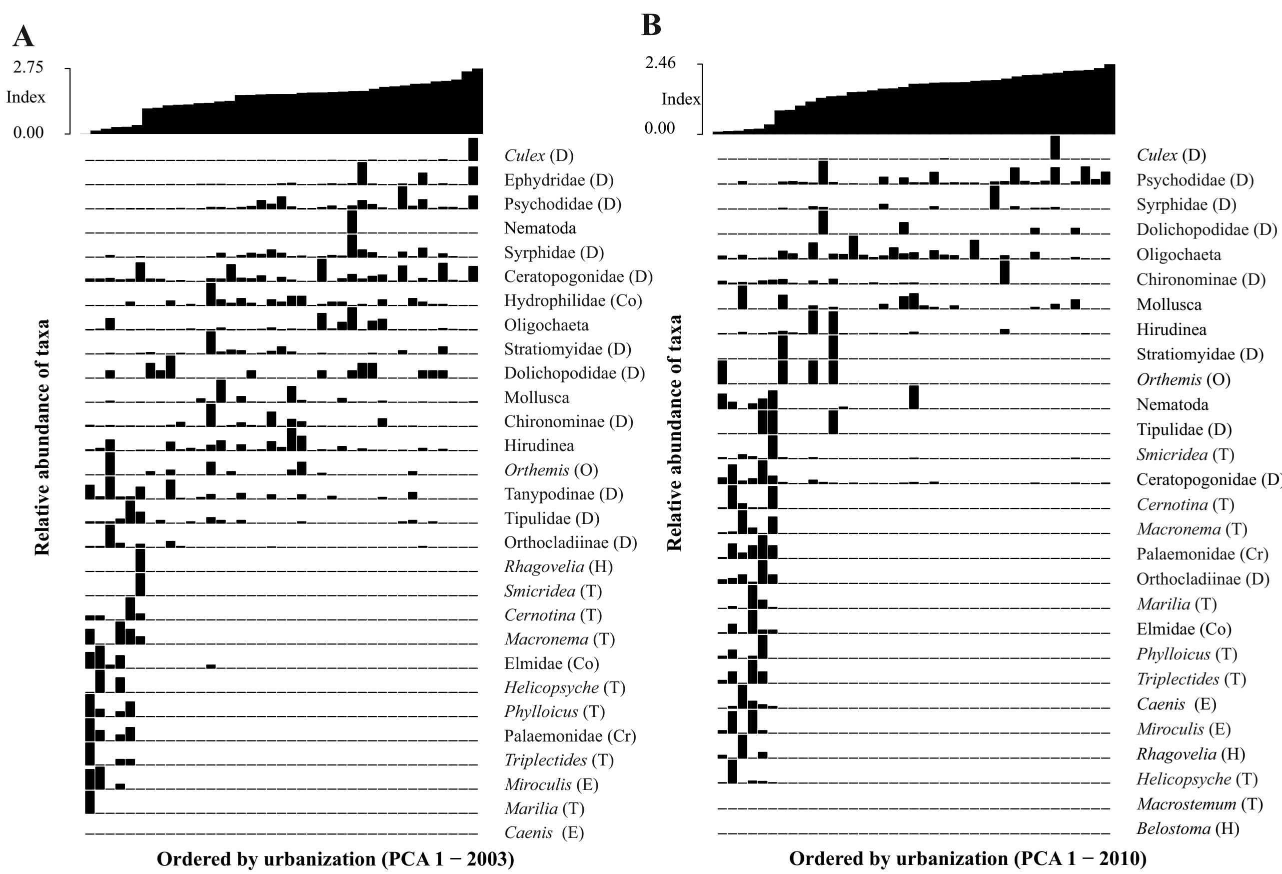

Fig. 6. Taxa abundance ordered by urbanization gradients from 2003 (A) and 2010 (B) in streams of Manaus, Central Amazonia. Code in parenthesis is the main group of the taxon: Co = Coleoptera; Cr = Crustacea; D = Diptera; E = Ephemeroptera; H = Hemiptera; O = Odonata; T = Trichoptera.

non-impacted environments (e.g. by flow variability; Scarsbrook et al., 2000; Mazor et al., 2009). Thus, future studies in this region should include a greater number of sampling years in order to better understand the temporal variation of these organisms and to provide greater accuracy in the use of aquatic invertebrates in environmental assessments.

We recorded an increased impact of urbanization on streams in Manaus from 2003 to 2010, mainly due to the increase of deforestation (e.g., total impervious area) and domestic sewage input (e.g., N and P). These increased impacts resulted in a decrease of sensitive taxa (e.g., EPT) and invertebrate rarefied richness and an increase of tolerant organisms (particularly Oligochaeta). Although least-impacted streams were affected by urbanization impacts, they are located in preserved areas which generally mitigate the negative impact. Thus, efficient public policy need to be designed to prevent the intensification of the effects of urbanization on Amazonian aquatic ecosystems.

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Appendix A.

Table A1
Minimum (Min), maximum (Max), mean (standard deviation) values and the results of paired *t*-tests of abiotic variables in streams (n = 40) in 2003 and 2010 in Manaus (Central Amazonia). TIA = total impervious area.

Abiotic variables	2003			2010			Paired *t*-test		
	Min	Max	Mean	Min	Max	Mean	gl	t	*p*
Electrical conductivity (μS/cm)	5.67	435.33	207.03 ± 130.25	9.06	391.33	222.59 ± 121.39	39	−1.01	0.317
Nitrogen (μmol/L)	0.21	170.56	33.71 ± 35.57	0.30	127.80	62.10 ± 43.04	39	−3.42	0.001
Oxygen (mg/L)	0.80	8.87	3.36 ± 2.01	0.28	6.77	2.64 ± 1.93	39	2.61	0.013
pH	4.83	7.83	6.47 ± 0.71	4.43	7.26	6.27 ± 0.71	39	1.84	0.074
Phosphorus (μmol/L)	0.40	13.13	4.56 ± 3.87	0.17	34.60	11.55 ± 10.83	39	−4.60	<0.001
Water temperature (°C)	25.00	33.00	28.91 ± 2.06	24.03	33.43	28.39 ± 2.01	39	1.76	0.086
Deforestation (%)	3.87	100.00	68.27 ± 32.65	12.64	100.00	76.81 ± 28.02	39	−6.04	<0.001
TIA (%)	2.60	100.00	62.90 ± 35.02	6.92	100.00	75.33 ± 29.14	39	−6.19	<0.001

Table A2
Minimum (Min), maximum (Max) and mean (standard deviation) values and the total abundance of invertebrates in streams (n = 40) in 2003 and 2010 in Manaus (Central Amazonia).

Order	Family	Taxa	2003				2010			
			Min	Max	Mean	Total	Min	Max	Mean	Total
Blattaria			0	0	0.00 ± 0.00	0	0	3	0.08 ± 0.47	3
Coleoptera	Curculionidae		0	1	0.03 ± 0.16	1	0	0	0.00 ± 0.00	0
	Dryopidae		0	1	0.03 ± 0.16	1	0	0	0.00 ± 0.00	0
	Dytiscidae		0	2	0.10 ± 0.38	4	0	7	0.18 ± 1.11	7
	Elmidae		0	7	0.53 ± 1.52	21	0	35	1.58 ± 5.94	63
	Gyrinidae		0	1	0.03 ± 0.16	1	0	6	0.15 ± 0.95	6
	Hydrophilidae		0	19	2.28 ± 3.59	91	0	0	0.00 ± 0.00	0
	Scirtidae		0	4	0.20 ± 0.72	8	0	3	0.08 ± 0.47	3
	Limnichidae		0	0	0.00 ± 0.00	0	0	5	0.13 ± 0.79	5
Diptera	Calliphoridae	*Lucilia*	0	1	0.05 ± 0.22	2	0	0	0.00 ± 0.00	0
	Cecidomyidae		0	7	0.20 ± 1.11	8	0	0	0.00 ± 0.00	0
	Ceratopogonidae		0	83	16.33 ± 22.54	653	0	79	6.93 ± 16.48	277
	Chironomidae	Chironominae	0	6872	570.50 ± 1348.89	22820	0	9415	537.78 ± 1536.02	21511
	Chironomidae	Tanypodinae	0	98	11.75 ± 23.09	470	0	137	13.48 ± 31.00	539
	Chironomidae	Orthocladiinae	0	49	2.18 ± 8.05	87	0	37	2.03 ± 6.60	81
	Culicidae	*Culex*	0	1120	29.93 ± 176.92	1197	0	178	4.60 ± 28.13	184
	Dolichopodidae		0	3	0.43 ± 0.75	17	0	4	0.20 ± 0.72	8
	Drosophilidae		0	6	0.23 ± 1.00	9	0	0	0.00 ± 0.00	0
	Empididae		0	1	0.05 ± 0.22	2	0	1	0.03 ± 0.16	1
	Ephydridae		0	122	8.40 ± 25.82	336	0	27	3.00 ± 5.51	120
	Psychodidae		0	1022	122.73 ± 212.99	4909	0	221	35.68 ± 55.09	1427
	Simuliidae		0	0	0.00 ± 0.00	0	0	11	0.28 ± 1.74	11
	Stratiomyidae		0	34	2.25 ± 5.83	90	0	1	0.05 ± 0.22	2
	Syrphidae		0	39	3.75 ± 7.07	150	0	29	1.28 ± 4.67	51
	Tabanidae		0	7	0.25 ± 1.15	10	0	1	0.03 ± 0.16	1
	Tipulidae		0	16	1.15 ± 2.84	46	0	2	0.13 ± 0.40	5
Ephemeroptera	Baetidae	*Callibaetis*	0	1	0.05 ± 0.22	2	0	3	0.08 ± 0.47	3
		Waltzoyphius	0	0	0.00 ± 0.00	0	0	3	0.15 ± 0.66	6
		Zelusia	0	1	0.03 ± 0.16	1	0	1	0.03 ± 0.16	1
	Caenidae	*Brasilocaenis*	0	2	0.10 ± 0.38	4	0	5	0.18 ± 0.84	7
		Caenis	0	0	0.00 ± 0.00	0	0	13	0.55 ± 2.15	22
	Coryphoridae	*Coryphorus*	0	1	0.03 ± 0.16	1	0	0	0.00 ± 0.00	0
	Euthyplociidae	*Campylocia*	0	5	0.23 ± 0.89	9	0	2	0.05 ± 0.32	2
	Leptohyphidae	*Amanahyphes*	0	0	0.00 ± 0.00	0	0	3	0.10 ± 0.50	4

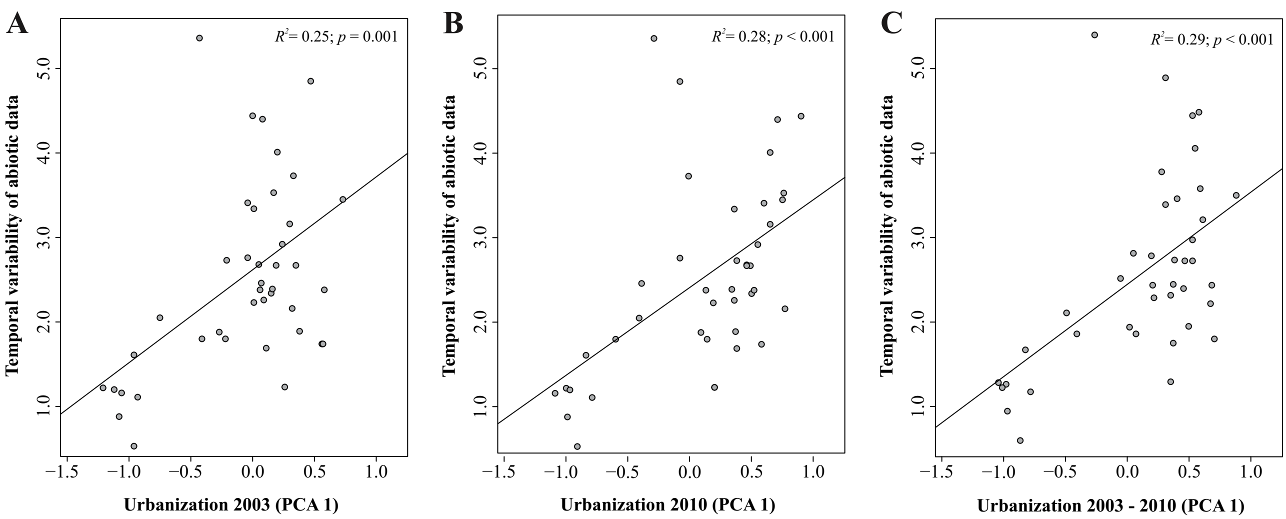
Table A2 (*Continued*)

Order	Family	Taxa	2003				2010			
			Min	Max	Mean	Total	Min	Max	Mean	Total
		Leptohyphidae sp.	0	3	0.18 ± 0.68	7	0	0	0.00 ± 0.00	0
	Leptophlebiidae	*Askola*	0	0	0.00 ± 0.00	0	0	1	0.03 ± 0.16	1
		Farrodes	0	0	0.00 ± 0.00	0	0	3	0.10 ± 0.50	4
		Hagenulopsis	0	0	0.00 ± 0.00	0	0	1	0.03 ± 0.16	1
		Microphlebia	0	2	0.08 ± 0.35	3	0	0	0.00 ± 0.00	0
		Miroculis	0	9	0.48 ± 1.89	19	0	18	0.98 ± 3.87	39
		Simothraulopsis	0	2	0.05 ± 0.32	2	0	3	0.08 ± 0.47	3
	Belostomatidae	*Belostoma*	0	24	0.93 ± 3.89	37	0	0	0.00 ± 0.00	0
	Gerridae	Gerridae sp.	0	1	0.03 ± 0.16	1	0	0	0.00 ± 0.00	0
		Brachymetra	0	0	0.00 ± 0.00	0	0	1	0.03 ± 0.16	1
	Naucoridae	Naucoridae sp.	0	1	0.03 ± 0.16	1	0	0	0.00 ± 0.00	0
	Nepidae	*Ranatra*	0	2	0.08 ± 0.35	3	0	0	0.00 ± 0.00	0
	Veliidae	Veliidae sp.	0	18	0.45 ± 2.85	18	0	0	0.00 ± 0.00	0
		Rhagovelia	0	1	0.03 ± 0.16	1	0	16	0.58 ± 2.60	23
		Stridulivelia	0	2	0.08 ± 0.35	3	0	2	0.08 ± 0.35	3
Lepidoptera	Pyralidae	Pyralidae	0	1	0.05 ± 0.22	2	0	1	0.05 ± 0.22	2
Megaloptera	Sialidae	*Protosialis*	0	8	0.23 ± 1.27	9	0	0	0.00 ± 0.00	0
	Corydalidae	*Corydalus*	0	0	0.00 ± 0.00	0	0	1	0.03 ± 0.16	1
Odonata	Aeshnidae	Aeshnidae sp.	0	1	0.03 ± 0.16	1	0	0	0.00 ± 0.00	0
	Calopterygidae	*Hetaerina*	0	2	0.08 ± 0.35	3	0	3	0.20 ± 0.65	8
	Coenagrionidae	*Argia*	0	0	0.00 ± 0.00	0	0	2	0.15 ± 0.53	6
	Cordulidae	*Aeschnosoma*	0	4	0.10 ± 0.63	4	0	1	0.03 ± 0.16	1
	Gomphidae	*Agriogomphus/Ebegomphus*	0	0	0.00 ± 0.00	0	0	1	0.05 ± 0.22	2
		Phyllocycla	0	2	0.05 ± 0.32	2	0	2	0.05 ± 0.32	2
		Progomphus	0	1	0.05 ± 0.22	2	0	4	0.18 ± 0.71	7
		Zonophora	0	0	0.00 ± 0.00	0	0	4	0.13 ± 0.65	5
	Libellulidae	*Elga*	0	0	0.00 ± 0.00	0	0	2	0.05 ± 0.32	2
		Erythodiplax	0	3	0.13 ± 0.52	5	0	0	0.00 ± 0.00	0
		Gynothemis	0	0	0.00 ± 0.00	0	0	1	0.03 ± 0.16	1
		Orthemis	0	14	1.08 ± 2.81	43	0	1	0.10 ± 0.30	4
	Megapodagrionidae	*Heteragrion*	0	0	0.00 ± 0.00	0	0	1	0.03 ± 0.16	1
	Perilestidae	*Perilestes*	0	0	0.00 ± 0.00	0	0	1	0.05 ± 0.22	2
	Protoneuridae	*Epipleoneura*	0	0	0.00 ± 0.00	0	0	3	0.08 ± 0.47	3
		Protoneuridae sp.	0	0	0.00 ± 0.00	0	0	17	0.50 ± 2.70	20
Plecoptera	Perlidae	Perlidae sp.	0	1	0.03 ± 0.16	1	0	0	0.00 ± 0.00	0
		Enderleina	0	1	0.03 ± 0.16	1	0	0	0.00 ± 0.00	0
		Macrogynoplax	0	1	0.03 ± 0.16	1	0	3	0.08 ± 0.47	3
Trichoptera	Calamoceratidae	*Phylloicus*	0	9	0.53 ± 1.75	21	0	11	0.58 ± 2.04	23
	Ecnomidae	*Austrotinoides*	0	2	0.10 ± 0.38	4	0	0	0.00 ± 0.00	0
	Glossosomatidae	*Protoptila*	0	0	0.00 ± 0.00	0	0	3	0.08 ± 0.47	3
		Mortoniella	0	0	0.00 ± 0.00	0	0	17	0.43 ± 2.69	17
	Helicopsychidae	*Helicopsyche*	0	13	0.45 ± 2.11	18	0	55	1.68 ± 8.71	67
	Hydropsychidae	*Leptonema*	0	1	0.03 ± 0.16	1	0	1	0.05 ± 0.22	2
		Macronema	0	3	0.20 ± 0.65	8	0	29	1.50 ± 5.65	60
		Macrostemum	0	0	0.00 ± 0.00	0	0	29	0.88 ± 4.60	35
		Smicridea	0	1	0.03 ± 0.16	1	0	46	1.65 ± 7.34	66
	Hydroptilidae	*Neotrichia*	0	0	0.00 ± 0.00	0	0	2	0.10 ± 0.38	4
		Hydroptilidae sp.	0	0	0.00 ± 0.00	0	0	1	0.03 ± 0.16	1
	Leptoceridae	*Nectopsyche*	0	2	0.05 ± 0.32	2	0	5	0.25 ± 0.87	10
		Triplectides	0	4	0.18 ± 0.68	7	0	8	0.43 ± 1.52	17
		Oecetis	0	3	0.08 ± 0.47	3	0	2	0.10 ± 0.44	4
	Odontoceridae	*Marilia*	0	4	0.20 ± 0.88	8	0	25	0.93 ± 4.17	37
	Polycentropodidae	*Cernotina*	0	11	0.45 ± 1.83	18	0	28	1.73 ± 6.11	69
		Cyrnellus	0	7	0.28 ± 1.26	11	0	0	0.00 ± 0.00	0
Decapoda	Palaemonidae		0	15	0.88 ± 2.95	35	0	25	1.98 ± 5.46	79
Hirudinea			0	49	6.15 ± 10.50	246	0	287	18.20 ± 62.19	728
Mollusca			0	132	8.80 ± 25.60	352	0	15	1.58 ± 3.36	63
Nematoda			0	1	0.03 ± 0.16	1	0	14	1.25 ± 3.21	50
Oligochaeta			0	1632	162.58 ± 361.82	6503	0	2002	324.68 ± 465.98	12,987

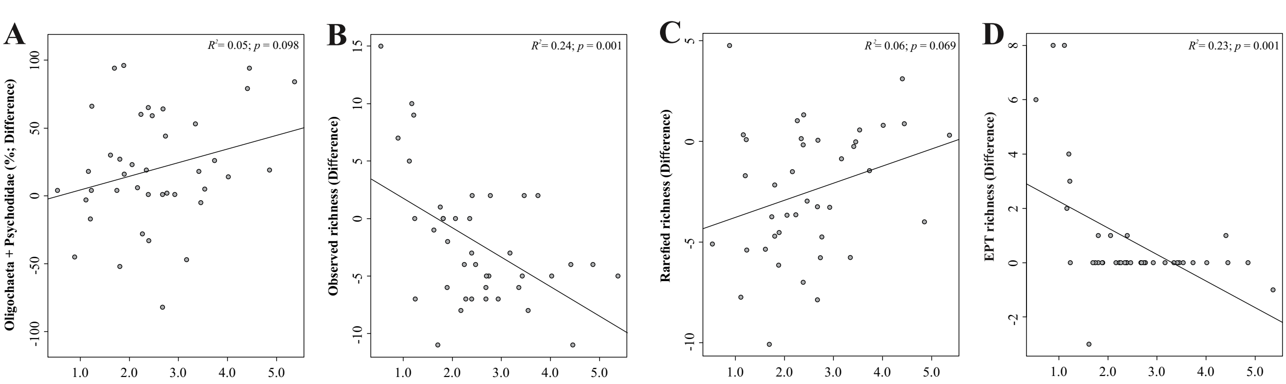
Table A3
Minimum (Min), maximum (Max) and mean (standard deviation) values and the results of paired *t*-tests of invertebrate metrics in streams (n = 40) in 2003 and 2010 in Manaus (Central Amazonia). EPT = Ephemeroptera, Plecoptera and Trichoptera.

Abiotic variables	2003			2010			Paired *t*-test		
	Min	Max	Mean	Min	Max	Mean	gl	t	*p*
EPT (%)	0.00	66.67	2.89 ± 10.87	0.00	18.67	1.70 ± 4.42	39	0.87	0.390
Oligochaeta + Psychodidae (%)	0.00	95.83	42.35 ± 36.08	3.20	100.00	61.97 ± 34.26	39	−2.93	0.006
Observed richness	3.00	25.00	11.40 ± 5.13	2.00	35.00	9.25 ± 8.66	39	2.40	0.021
Rarefied richness	1.44	24.92	9.43 ± 6.03	1.54	25.00	6.94 ± 5.88	39	4.84	<0.001
EPT richness	0.00	11.00	1.30 ± 3.01	0.00	15.00	2.08 ± 4.51	39	−2.26	0.029

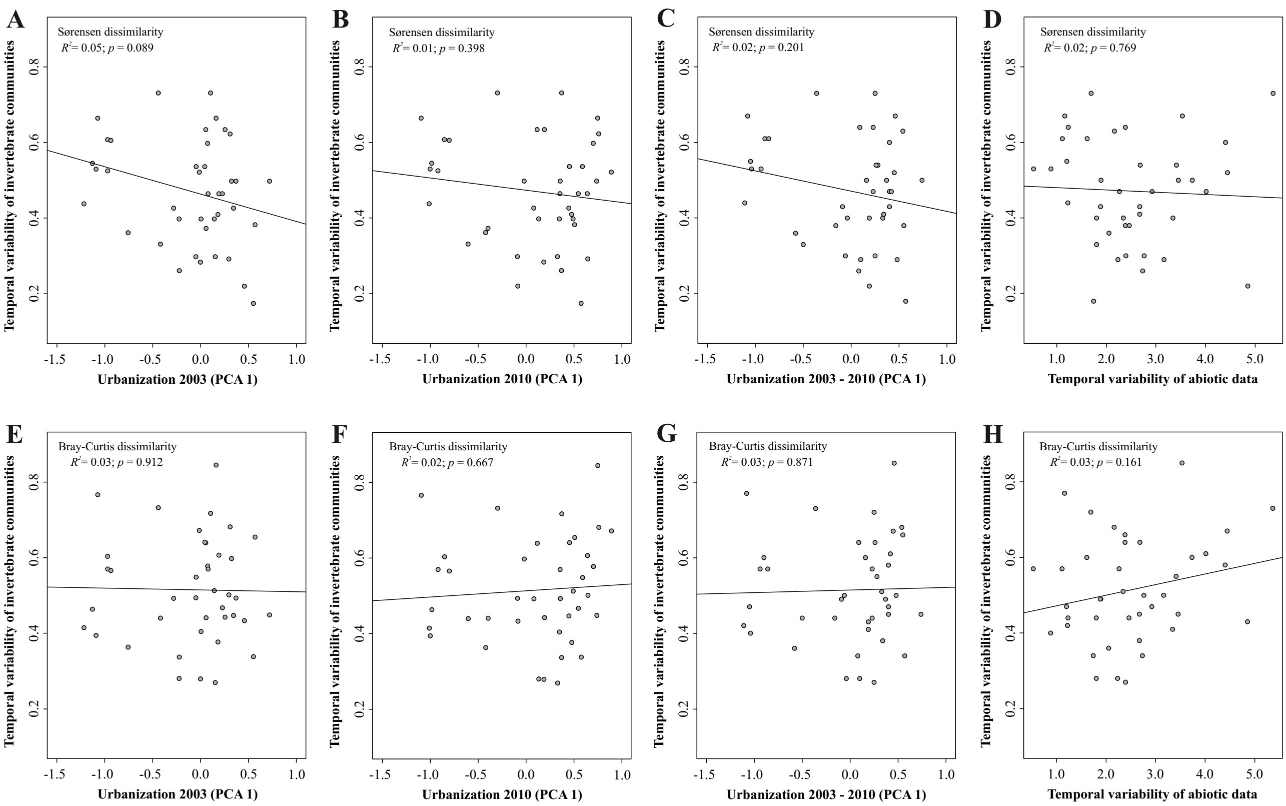
Appendix B. Relationship between temporal variation of abiotic variables and urbanization gradient using sampled data from 2003 and 2010 in Manaus, Central Amazonia. Urbanization was defined as the first axis of the principal components analysis (PCA) using abiotic data from 2003 (A), 2010 (B) and average from 2003 and 2010 (C).



Appendix C. Relationship between differences of invertebrate metrics and temporal variability of abiotic data using sampled data from 2003 and 2010 in Manaus, Central Amazonia. Temporal variability of abiotic data was defined as dissimilarity (Euclidean distance) between 2003 and 2010 for each stream. EPT = Ephemeroptera, Plecoptera and Trichoptera.



Appendix D. Relationship between temporal variation of invertebrate communities and the urbanization gradient using sampled data from 2003 and 2010 in Manaus, Central Amazonia. We used Sørensen (A-C) and Bray-Curtis (D-E) dissimilarities to estimate temporal variation of fauna. Urbanization was defined as the first axis of the principal components analysis (PCA) using data from 2003 (A, E), 2010 (B, F), average of both years (C, G), or temporal variability of abiotic data (D, H). Temporal variability of abiotic data was defined as dissimilarity (Euclidean distance) between 2003 and 2010 for each stream.



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