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# Landscape variables influence taxonomic and trait composition of insect assemblages in Neotropical savanna streams

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## Summary

1. Stream invertebrate assemblages are structured by environmental factors acting at multiple spatial scales. Identifying the spatial scale that most influences the species–environment relationships is a major goal of community ecology.
2. We evaluated the importance of catchment and site scales and associated environmental variables in shaping Ephemeroptera, Plecoptera and Trichoptera (EPT) assemblages in Neotropical savanna headwater streams.
3. Sampling sites were associated with 20 catchment-scale variables that depicted land cover and land use as well as natural geophysical variables such as altitude and climate. Site-scale habitat was characterised by 55 variables that described habitat hydromorphology, substrate, flow, canopy, in-stream cover and water quality. EPT traits were assessed using 28 categories of 7 biological traits, which represented the best available current knowledge for EPT in Neotropical savanna streams.
4. We analysed the relationships between the catchment- and site-scale habitat variables and the taxonomic and trait composition of insect assemblages using 1,760 samples collected in 160 stream sites.
5. Catchment- and site-scale variables both explained significant variation in EPT taxon and trait composition. Substrate, habitat hydromorphology and land use most influenced variation in taxonomic composition, whereas trait composition was mainly affected by land use. Catchment geographic position explained less assemblage variation.
6. To our knowledge, this study is the first assessment of the impact of catchment- and site-scale variables on the trait and taxon composition of stream insect assemblages in Neotropical savanna streams. It highlights the need for better regional biological knowledge of invertebrates to generate more general trait-based approaches in freshwater ecosystem conservation.

## KEYWORDS

aquatic insects, Ephemeroptera, Plecoptera and Trichoptera, land use, physical and chemical habitat, spatial scale

## 1 | INTRODUCTION

Streams are organised hierarchically in the landscape, within which environmental factors operate at a range of spatial scales (Frissell, Liss, Warren, & Hurley, 1986; Leps, Tonkin, Dahm, Haase, & Sundermann, 2015; Mykrä, Heino, & Muotka, 2007), directly or indirectly affecting the structure and composition of biological assemblages (Macedo et al., 2014; Sandin & Johnson, 2004; Townsend, Dolédec, Norris, Peacock, & Ar Buckley, 2003). Therefore, assessing spatial patterns is essential for a comprehensive understanding of the drivers that determine the structural and functional diversity of stream assemblages (Heino, Muotka, & Paavola, 2003; Hoeinghaus, Wine-miller, & Birnbaum, 2007; Liu et al., 2016; Macedo et al., 2014; Sandin & Johnson, 2004).

Site-scale physical and chemical habitat is determined by larger-scale processes (Leal et al., 2016), which hinders disentangling the roles of different environmental drivers in aquatic communities (Allan, 2004; Frissell et al., 1986). For example, catchment characteristics affect riparian zones, substrates and hydrological regimes, which in turn affect habitat availability and thereby influence the structure and composition of aquatic assemblages (Allan, 2004; Greenwood & Booker, 2016; Vannote, Minshall, Cummins, Sedell, & Cushing, 1980). Furthermore, geographical position may influence environmental factors that determine spatial patterns in assemblage structure and composition (Townsend et al., 2003).

Understanding the role of catchment- and site-scale environmental variables in determining stream invertebrate biodiversity is especially relevant in tropical freshwater ecosystems (e.g. Al-Shami et al., 2013; Tonkin, Arimoro, & Haase, 2016). Although these systems are among the most diverse on earth, they are highly threatened by a broad suite of stressors (Dudgeon et al., 2006; Strayer & Dudgeon, 2010; Taniwaki, Piggott, Ferraz, & Matthaai, 2016). The effects of human activities on tropical streams are poorly understood and the pace of stream deterioration exceeds the pace of scientific research to understand ecosystem responses (Ramírez, Pringle, & Wantzen, 2008). For example, the Neotropical savanna (Cerrado) is the second largest biome in South America, a biodiversity hotspot (Myers, Mittermeier, Mittermeier, Fonseca, & Kent, 2000), and one of the most threatened biomes in the world. However, little attention has been paid to the conservation of its freshwater ecosystems and biota (Klink & Machado, 2005; Strassburg et al., 2017). Habitat fragmentation and agriculture are the main threats to biodiversity conservation in this region (Carvalho, De Marco, & Ferreira, 2009; Hunke, Mueller, Schröder, & Zeilhofer, 2015; Overbeck et al., 2015). Although studied worldwide (e.g. Al-Shami et al., 2013; Beauchard, Gagneur, & Brosse, 2003; Bonada, Dolédec, & Statzner, 2012; Heino, Mykrä, Kotanen, & Muotka, 2007; Townsend et al., 2003), spatial patterns of aquatic assemblages are poorly considered in tropical savanna streams (Macedo et al., 2014).

In the Neotropics, there have been a few studies addressing the influence of environmental drivers at different scales on functional composition of phytoplankton (e.g. Machado, Teresa, Vieira, Huszar, & Nabout, 2016) and fishes (e.g., Leitão et al., 2017; Terra, Hughes,

& Araújo, 2016), but not invertebrates. Understanding how environmental pressures interact to affect functional composition of aquatic invertebrates at different scales and in different regions is therefore a major challenge for freshwater management in tropical regions and globally.

Most previous studies addressing species traits have been generally restricted to trophic guilds (e.g. Brasil, Juen, Batista, Pavan, & Cabette, 2014; De Castro et al., 2016; Ferreira et al., 2017). However, the Multiple Trait-Based (MTB) approach (Dolédec & Statzner, 2010; Menezes, Baird, & Soares, 2010) offers more mechanistic understanding than traditional taxonomy-based approaches for assessing relationships between stream assemblage composition and environmental variables. This is because multiple traits reflect multiple functional relationships between biota and environmental characteristics (Dolédec, Phillips, & Townsend, 2011; Feld & Hering, 2007; Townsend, Dolédec, & Scarsbrook, 1997). For example, Townsend et al. (1997) found that disturbance strengthened the pattern of preponderance of resilience/resistance traits in benthic insect communities. Feld and Hering (2007) observed that, in contrast to taxonomic structure, functional measures were more strongly related to hydromorphological gradients at various spatial scales. Dolédec, Phillips and Townsend (2011), assessing aquatic invertebrate trait and taxonomic response to land use, found that trait responses were consistent at the broad and catchment scales, with similar traits responding to land use at both scales. Functional trait information for Neotropical aquatic invertebrates has increased (Dedieu, Rhone, Vigouroux, & Céréghino, 2015; Milesi, Dolédec, & Melo, 2016; Reynaga & Santos, 2013; Tomanova, Moya, & Oberdorff, 2008; Tomanova & Usseglio-Polatera, 2007) and provides opportunity for MTB analyses to be used in determining their interactions with multiscale pressures. Nonetheless, this approach has not been attempted previously in the Neotropical savanna.

Ephemeroptera, Plecoptera and Trichoptera (EPT) comprise diverse aquatic assemblages in headwater streams (Bispo, Oliveira, Bini, & Sousa, 2006; Vinson & Hawkins, 1998), and they have important roles in nutrient cycling and energy transfer (Ferreira et al., 2014; Graça, 2001). In addition, the EPT are commonly used for assessing the biological condition of stream ecosystems in North America (e.g. Lenat, 1988; Stoddard et al., 2008), South America (e.g. Chen et al., 2017; Pereira, Souza, Baptista, Oliveira, & Buss, 2016), Europe (e.g. Hering, Feld, Moog, & Ofenbock, 2006), Asia (e.g. Chen et al., 2014) and Australia (e.g. Chessman, Thurtell, & Royal, 2006). In this study, we investigated the response of taxonomic and trait composition of EPT assemblages to several physical and chemical variables at catchment and site scales and geographic position in Neotropical savanna headwater streams. We sought to answer three questions: (1) Which of those two scales most accounts for the relationships between landscape variables and EPT assemblages in terms of taxonomy and traits? (2) Are these relationships the same for both natural and human-modified environmental variables? (3) To what degree does geographic position (longitude/latitude) explain EPT assemblage distribution?

## 2 | METHODS

### 2.1 | Study area

The study area was located in the Neotropical savanna of south-eastern Brazil (the Cerrado), which has two well-defined seasons: a wet season from October to April (rainfall 100–330 mm/month), and a dry season from May to September (rainfall 10–55 mm/month), with 1,600-mm mean annual rainfall (Ferreira et al., 2017). We sampled wadeable first- to third-order streams (average width =  $3.4 \pm 1.9$  m, average depth =  $0.25 \pm 0.12$  m) belonging to four different hydrological units (HU): São Francisco, Rio Grande, Paranaíba and Araguari (Figure 1). In each HU, we sampled a drainage area within 35 km upstream of each hydropower dam: Três Marias, Volta Grande, São Simão and Nova Ponte, respectively, in each of these HUs. We collected samples in a given HU in a given year over the period between 2010 and 2013 at the end of the dry season (September).

### 2.2 | Site selection

In each HU, we randomly selected 40 perennial stream sites, totaling 160 sites for the four HUs. Site selection followed the generalised random tessellation stratified sampling design developed for the U.S. EPA's national Wadeable Stream Assessment (Olsen & Peck, 2008; Stevens & Olsen, 2004). In this approach, a master sample frame is first established using a digital hydrographical map (1:100,000 scale) and then the sample sites are selected via a hierarchical, spatially weighted criterion (Stevens & Olsen, 2004). This procedure ensures a balanced selection of sites across the range of stream orders and geographic location, besides enabling the selection of sites along different disturbance levels (Macedo et al., 2014).

### 2.3 | Environmental variables

Environmental variables acting at varying spatial scales within HUs were classified into three groups: catchment scale, site scale, and geographic position (Figure 2, Table S1). A total of ~250 catchment- and site-scale variables were initially measured. We eliminated those variables that had more than 90% zero values, low variability and high correlation with each other ( $r > |.8|$ ). For those variables that correlated with each other, we retained the most ecologically meaningful ones. Because we initially produced many environmental variables (c. 250), we defined criteria to select the most important variables based on the literature. Those steps yielded a final set of 75 variables (Macedo et al., 2014).

Catchment-scale variables included land use and land cover variables as well as natural geophysical variables: rainfall, altitude, drainage area, elevation and slope (range, average and standard deviation). Catchment total annual rainfall time series were obtained from the Brazilian National Water Agency (ANA, 2014). Using GIS software, we extracted geophysical variables from 160 catchments, which were manually delineated to the entire catchment area ( $\text{km}^2$ )

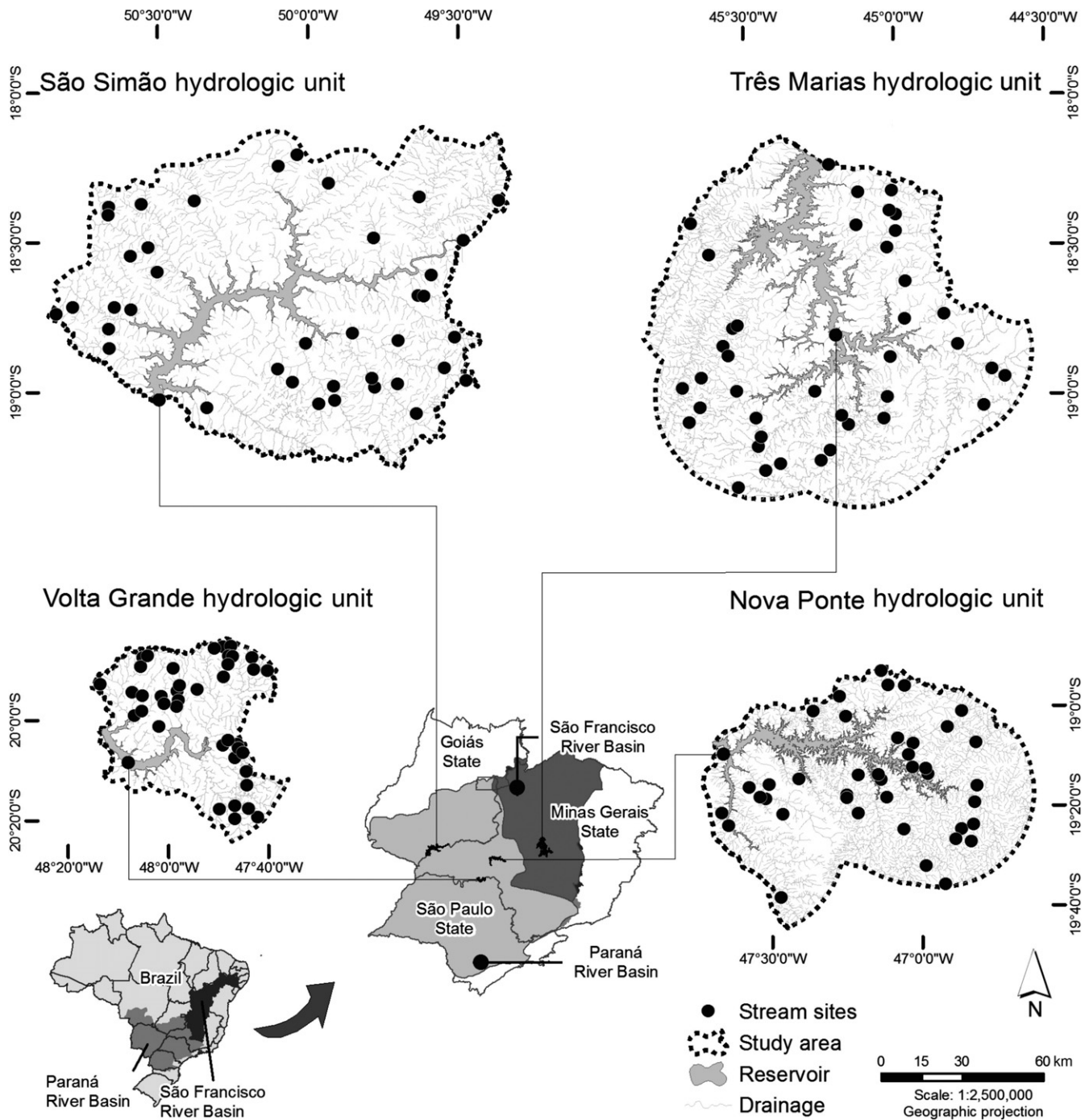
for each site. Basin elevation data (range, mean and standard deviation) were extracted from Shuttle Radar Topographic Mission – SRTM imagery (USGS, 2005) – and catchment slope was calculated from the maximum rate of change in elevation in every grid cell based on SRTM elevation raster.

We assessed catchment land use and land cover for each site by interpreting high-resolution satellite images (0.6–5 m spatial resolution, Google Earth data; Google, 2014) in conjunction with Landsat multispectral satellite images (R4G3B2 false colour band combination). The high-resolution images provided information about the shape and texture of the elements, and the multispectral images allowed distinguishing vegetation leaf structure. We identified four natural savanna cover types (woodland savanna, grassy-woody savanna, parkland savanna and wetland palm swamp) (IBGE, 2012) and four human-induced land uses (pasture, agriculture, eucalyptus forest and urban areas) in the 160 catchments. We also calculated the total percentage of natural land cover by summing the preceding four natural land covers. In addition, to further characterise potential anthropogenic influences on the sites, we measured Euclidian distance to cities and to paved highways (km) and we calculated the density of households (houses/ $\text{km}^2$ ) in each catchment.

Physical habitat at the site scale was characterised using the U.S. EPA field methods (Hughes & Peck, 2008; Peck et al., 2006) adapted to Neotropical savanna headwater streams by Callisto, Hughes, Lopes, and Castro (2014). The length of each sampled site was 40 times its mean wetted width, with a minimum length of 150 m. Each reach was divided into 11 equally spaced transects. At each site, we characterised the physical habitat by 10 channel hydromorphological variables (e.g. thalweg depth, bank angle, channel sinuosity; see Table S1); 12 substrate variables (e.g. % of boulders, % of sand, % of total organic matter, % of large wood); 6 flow variables (e.g. velocity, % of glides, % of pools); 11 riparian canopy cover variables (e.g. herbaceous cover, exposed soil, ground cover) and 8 in-stream habitat variables (e.g. aquatic macrophytes, large woody debris). All site variables were calculated according to Kaufmann, Levine, Robison, Seeliger, and Peck (1999), who described concepts and analytical procedures for calculating metrics based on data generated from the physical habitat field protocols. We also measured temperature, electrical conductivity, pH, turbidity and total dissolved solids (TDS) in situ using a multi-parameter probe (YSI, 650 MDS, model 6920). In the laboratory, we determined total alkalinity, total nitrogen and dissolved oxygen concentrations from preserved water following Standard Methods (APHA, 2005). Water samples and parameters were always obtained at each site at the same time of day (around 10 a.m.).

### 2.4 | Benthic macroinvertebrate sampling

We sampled benthic macroinvertebrates at all 160 sites with a D-frame kick net (30 cm aperture, 500  $\mu\text{m}$  mesh). Eleven sub-sample units (0.09  $\text{m}^2$  quadrat) were taken per site, one per transect (see above), generating one composite sample for each site and totalling an area of ~1  $\text{m}^2$  per site. Thus, stream sites were our replicates.



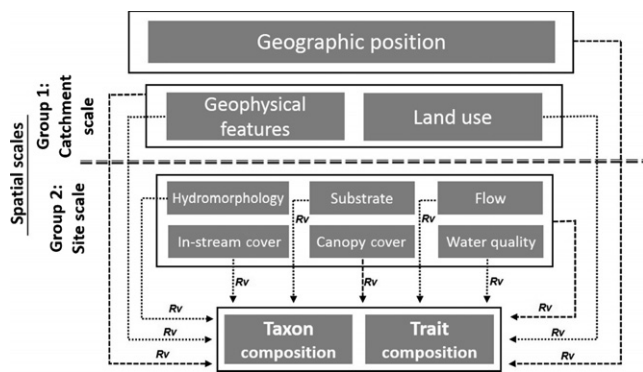
**FIGURE 1** Locations of wadeable stream sites ( $n = 160$ ) in four hydrological units in the Neotropical savanna

The sample units were obtained by following a systematic zigzag trajectory along the reach to avoid bias in habitat selection (Hughes & Peck, 2008; Peck et al., 2006). Immediately after collection, samples were placed in individual plastic buckets and preserved with 4% formalin. In the laboratory, macroinvertebrates were sorted, and EPT individuals were identified to genus by means of taxonomic keys (Dominguez, Molineri, Pescador, Hubbard, & Nieto, 2006; Mugnai, Nessimian, & Baptista, 2010; Pes, Hamada, & Nessimian, 2005) and counted under a  $80\times$  magnification stereomicroscope.

## 2.5 | Biological traits

We used trait information that was available exclusively for Neotropical macroinvertebrates (Baptista et al., 2006; Dedieu et al., 2015; Reynaga & Santos, 2012; Tomanova & Usseglio-Polatera, 2007). Biological trait categories described EPT genus profiles in terms of resilience or resistance ability (including morphology and life cycle) and feeding habits. Feeding habits and body size potentially reflect stream functional aspects (e.g., nutrient cycling, biomass





**FIGURE 2** Hierarchical organisation used in this study to quantify the relationship between geographic position, environmental variables and the taxonomic and trait composition of Ephemeroptera, Plecoptera and Trichoptera assemblages. Environmental variables (see Table S1) were classified into catchment-scale variables, which comprise geophysical features and land use, and site-scale variables, which depicted habitat hydromorphology, substrate, flow, in-stream cover, riparian canopy cover and water quality. *Rv*-coefficients were computed for each set of environmental variables to assess their respective contribution to the variability in the aquatic invertebrate taxonomic or trait composition

accumulation), and other morphological and behavioural traits were potentially linked to physical habitat constraints (Tomanova & Usseglio-Polatera, 2007). We produced a trait database comprising 7 biological traits and 28 trait categories (Table 1). The affinity of each taxon for each category within a trait was described using a fuzzy coding approach (Chevenet, Dolédec, & Chessel, 1994). The score for each taxon belonging to each trait category ranged from 0 to 3, with 0 indicating no affinity with the category, 1 indicating weak affinity, 2 indicating moderately strong affinity and 3 indicating strong affinity. This coding methodology helps to compensate for different types and levels of information available, and the inclusion of within-species trait variation associated with the natural variation in populations (Chevenet et al., 1994; Menezes et al., 2010). To determine the body size of each EPT genus, we measured the body lengths (from head to the end of the abdomen) of ~10% of all EPT individuals sampled in the 160 sites. These measurements were then classified into six categories according to the distribution of sizes in a histogram, where the breaks were defined so that the categories had a normal distribution (Table 1).

Affinity scores were standardised so that their sum for a given taxon and a given trait equalled 1. As recommended by Gayraud et al. (2003), we described the trait composition of assemblages by multiplying the frequency of each category per trait by the  $\ln$ -transformed abundances of taxa in a site. The resulting trait-by-sites table, which contained the abundance of each category per trait in each site, was further analysed.

## 2.6 | Data analysis

To obtain scores for sites based on taxon composition, we performed principal components analysis (PCA) on the  $\ln(x + 1)$ -

**TABLE 1** Traits and their categories and codes used for 73 EPT genera collected in Neotropical savanna streams

Traits	Code	Categories
Body Size	SIZE_<1.5	<1.5
	SIZE_1.5_2.5	1.5–2.5
	SIZE_2.5_3.5	2.5–3.5
	SIZE_3.5_5	3.5–5.0
	SIZE_5_10	5.0–10.0
	SIZE_large	>10
Potential number of cycles per year	CY_<1y	< or = 1
	CY_>1y	>1
Feeding Habits	FG_collector	Collector-Gatherer
	FG_shredder	Shredder
	FG_scraper	Scraper
	FG_filterer	Collector-Filterer
	FG_predator	Predator
Locomotion	LO_burrower	Burrower
	LO_climber	Climber
	LO_sprawler	Sprawler
	LO_clinger	Clinger
	LO_swimmer	Swimmer
Body flexibility	FL_low	<10
	FL_inter	>10–45
	FL_high	>45
Body form	FO_streamlined	Streamlined
	FO_flattened	Flattened
	FO_cylindrical	Cylindrical
	FO_spherical	Spherical
Relation to substrate	RS_free	Free Living
	RS_silknet	Silk net builders
	RS_case	Case builder

transformed abundance of taxa, and to obtain scores for sites based on trait composition, we used a fuzzy principal components analysis (FPCA) on the table that contained the  $\ln(x + 1)$ -transformed trait–category abundances in each site (Chevenet et al., 1994). To test for homogeneity among HUs, which would allow using all 160 sites together, we compared the observed variance across the sites in each HU and the distribution of simulated variances obtained after 999 permutations of the rows (sites) of the taxon (or trait) composition table. For taxon and trait composition, the tests indicated a very weak separation of sites grouped by HU (explained variance = 0.094 and 0.078, simulated- $p = .001$ , for taxa and traits respectively). We thus considered the totality of sites in further analyses.

Continuous environmental variables were log-transformed and proportional variables were arcsine-squared root transformed to meet normality of distributions. To identify and produce smaller sets of the most representative variables, we performed PCA on catchment- and site-scale variables as a whole, and for separate sets (i.e. geophysical features, land use, habitat hydromorphology, substrate,

flow, riparian canopy cover, in-stream cover, water physical and chemical, Table S1). We further investigated the relationships between geographical locations and the above environmental variables and taxon (or trait) composition of EPT assemblages by using co-inertia analyses (Dolédéc & Chessel, 1994; Dray, Chessel, & Thioulouse, 2003). This analysis is an eigenvector technique that matches two datasets (in this case, environmental variables and invertebrate data) in a symmetric way. The correlation between each set of environmental variables and EPT taxon or trait composition was measured with the  $R_v$ -coefficient (Figure 2), which is a multidimensional equivalent of the ordinary correlation coefficient between two variables (Robert & Escoufier, 1976). We performed Monte-Carlo tests to evaluate the statistical significance of  $R_v$ -coefficients by comparing the observed  $R_v$ -coefficient to the distribution of 999 replicated matches of the two tables (after the random permutations of their rows, i.e. sites). In addition, to assess the importance of the site and catchment scales in comparison to geographical position in determining EPT taxon (or trait) composition, we used a procedure proposed in Townsend et al. (2003). In this approach, we let  $E_1$  and  $E_2$  denote the two environment tables at the site and/or the catchment scales, and considered  $F$  the EPT taxon (or trait) composition table. We tested the null hypothesis  $R_v(E_1, F) = R_v(E_2, F)$  against the alternative  $R_v(E_1, F) > R_v(E_2, F)$  and we performed bootstraps on the statistics  $t = R_v(E_1, F) - R_v(E_2, F)$  using 999 replicates. We assessed the statistical significance of the observed  $R_v$ -coefficient difference by comparing it to the distribution of the simulated values. Per cent changes between  $R_v$ -coefficients were obtained by  $[(R_v(E_1, F) - R_v(E_2, F)) / (R_v(E_1, F))] \times 100$ .

Finally, given that the variability within a table is related to the number of variables, which differed between taxonomic and trait composition (73 genera versus 28 trait categories), we simulated taxonomic composition tables by randomly selecting 28 genera (i.e. the same number as trait categories) and recalculated the  $R_v$ -coefficient for each set of environments with this reduced taxon composition. The distribution of 999 simulated variances was then compared with the observed variance obtained from the analysis of the entire taxonomic and trait composition tables (see, e.g. Bonada, Dolédéc, & Stutzner, 2007). All analyses were conducted in R (R Core Development Team, 2015) with the *ade4* package (Chessel, Dufour, & Thioulouse, 2004).

### 3 | RESULTS

#### 3.1 | Taxonomy

We collected 53,698 EPT individuals distributed in 20 families and 73 genera. Four sites had no EPT and were excluded from further analysis (hereafter  $n = 156$  sites). Co-inertia analysis showed a significant relationship between EPT taxon composition and catchment-scale variables ( $R_v = .247$ ,  $p = .001$ ; Table 2). The first two axes incorporated 95.5% of the environmental variability and 55% of the EPT variability (Table 2). The most significant variables along the first co-inertia axis included the percentage of parkland and natural cover

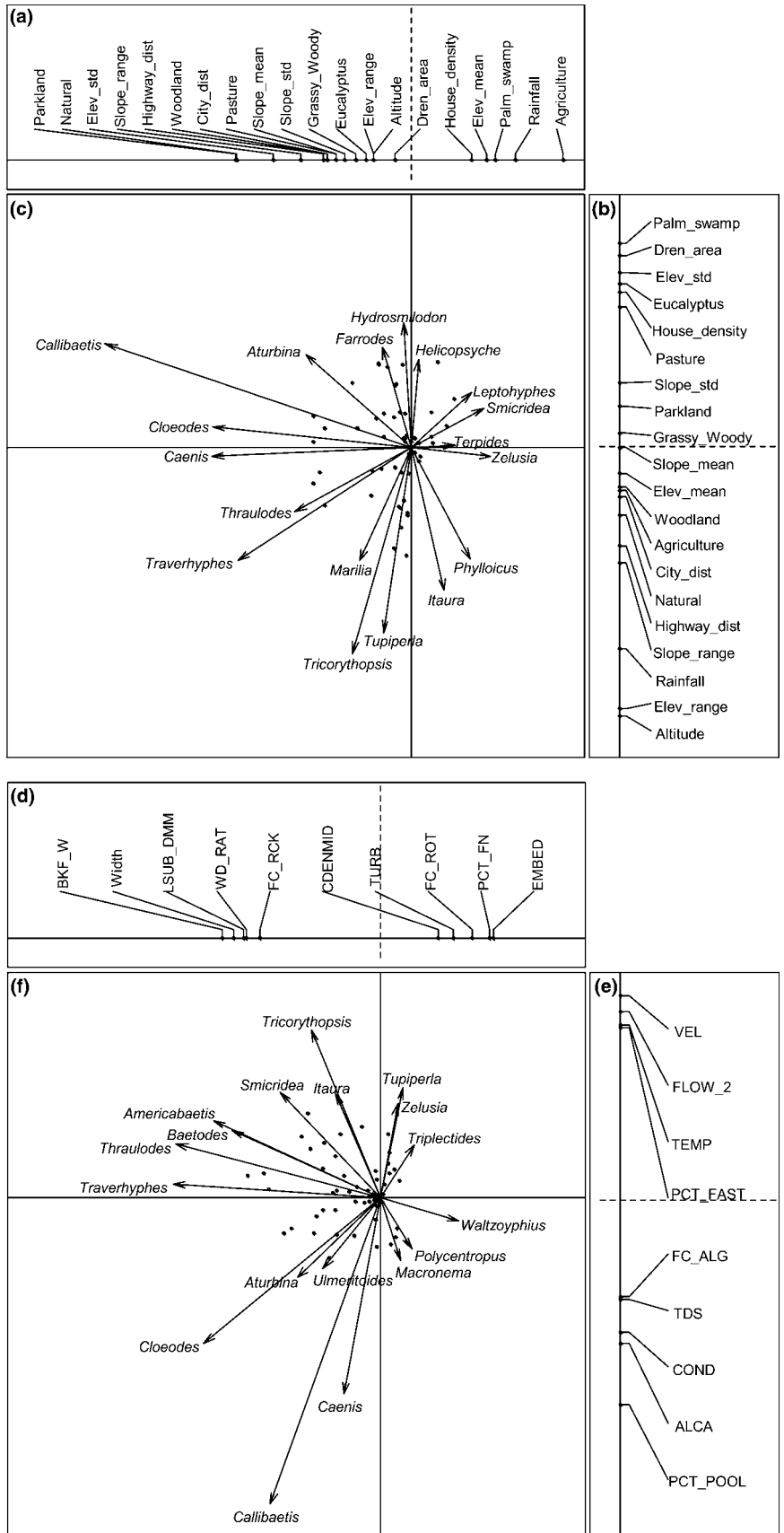
**TABLE 2** Results of co-inertia analyses performed between geographic position, catchment- and site-scale variables and (a) taxonomic and (b) trait composition of EPT assemblages.  $R_v$ : correlation coefficient between tables,  $p$ : simulated probability equal to the frequency of random values higher than the observed  $R_v$ -coefficient, %: percentage of variance in EPT taxonomic (a) or trait (b) composition explained by the first two axes of a co-inertia analysis

	$R_v$	$p$	%
(a)			
Geographic position	.140	.001	43.7
Catchment	.247	.001	55.1
Site	.320	.001	84.9
(b)			
Geographic position	.067	.001	69.1
Catchment	.133	.001	80.7
Site	.118	.001	92.7

as opposed to percentage of agricultural lands (Figure 3a). Along the second axis, prominent variables included altitude, catchment elevation range and rainfall as opposed to percentage of palm swamp, drainage area and elevation standard deviation (Figure 3b). EPT taxonomic richness was significantly related to the first co-inertia axis ( $R^2 = .068$ ,  $p < .001$ ). Taxa most responding to the environmental patterns described by the first co-inertia axis included Ephemeroptera, such as *Callibaetis*, *Cloeodes*, *Caenis* and *Traverhyphes* (Figure 3c). Taxa less sensitive to land use included Trichoptera (*Smicridea*) and Ephemeroptera (*Leptohyphes* and *Zelus*). Along the second co-inertia axis, taxa colonising higher elevation streams included Plecoptera (*Tupiperla*) and Ephemeroptera (*Tricorythopsis*). In contrast, lower elevation streams supported Ephemeroptera such as *Hydrosmilodon* and *Farrodes* and Trichoptera such as *Helicopsyche* (Figure 3c).

At the site scale, the relation between EPT taxon composition and environmental variables was slightly stronger than for catchment-scale variables ( $R_v = .320$ ,  $p = .001$ ; Table 2). The first two co-inertia axes represented up to 85.9% of the environmental variability and 84.9% of the EPT variability (Table 2). The most important site-scale variables along the first co-inertia axis included mean bankfull and wetted width, substrate diameter and mean width/depth ratio as opposed to embeddedness, percentage of fines, percentage of areal cover in live trees and turbidity (Figure 3d). The most significant variables along the second co-inertia axis included mean water velocity, and percentage of pools, rapids and glides (Figure 3e). The first co-inertia axis separated sites with high EPT diversity from those having lower diversity ( $R^2 = .268$ ,  $p < .001$ ) with *Traverhyphes*, *Thraulodes*, *Cloeodes* as opposed to *Waltzoyphius*. Taxa most related to slow waters were the Ephemeroptera *Callibaetis*, *Caenis* and *Cloeodes*, as opposed to *Tricorythopsis*, *Tupiperla*, *Smicridea* and *Itaura* at higher flow velocities (Figure 3f).

The patterns observed above were significantly related ( $R_v = .140$ ,  $p < .001$ ; Table 2) to geographic position: an East-West



**FIGURE 3** First two axes of a co-inertia analysis depicting the relationship between the taxonomic composition of Ephemeroptera, Plecoptera and Trichoptera assemblages and (a–c) catchment-scale variables and (d–f) site-scale variables. (a) and (b) show the prominent catchment-scale variables along the first and second co-inertia axis respectively. (d) and (e) show the prominent site-scale variables along the first and second co-inertia axis respectively. (c) and (f) show the taxa loadings associated with the response of taxa to catchment-scale and site-scale variables respectively. Dots represent taxa with low loadings whose names are not shown for clarity (see Table S1 for variable acronyms)



gradient along the first co-inertia axis and a North-South gradient along the second co-inertia axis. However, the extracted variance was substantially lower than that obtained from catchment- and site-scale variables (Table 2).

### 3.2 | Biological traits

Co-inertia analysis showed a significant relationship between EPT trait composition and catchment-scale variables ( $R_v = .133$ ,  $p = .001$ ). The first two axes accounted for 90.3% of environmental variability and 80.7% of the trait variability (Table 2). Like EPT taxon composition, environmental variables were not equally important in accounting for trait composition. Along the first co-inertia axis, the percentage of natural cover, highway distance and percentage of woodland and parkland were opposed to house density, percentage of agriculture and palm swamp (Figure 4a). The most important variables along the second co-inertia axis included rainfall, elevation range, altitude and percentage of agriculture as opposed to percentage of palm swamp, percentage of eucalyptus and standard deviation of elevation (Figure 4b). The fauna responding to this gradient along the first co-inertia axis were free-living univoltine organisms with streamlined body form and/or intermediate body flexibility as opposed to silk net-builder organisms, having cylindrical body form and/or being multi-voltine. Along the second co-inertia axis, sprawlers, shredders, case builders and organisms with low body flexibility ( $<10^\circ$ ) and/or cylindrical body forms were opposed to scrapers and organisms with high body flexibility ( $>45^\circ$ ) (Figure 4c).

The correlation between the trait composition of EPT assemblages and site-scale variables was slightly weaker than at the catchment scale ( $R_v = .118$ ,  $p = .001$ ). The first two axes accounted for 62.8% of the environmental variability and 92.7% of the trait variability (Table 2). The most important site-scale variables along the first co-inertia axis included relative bed stability, percentage of coarse gravel, mean substrate diameter and percentage of fine gravel as opposed to water turbidity, percentage of fines, conductivity, embeddedness and total dissolved solids (Figure 4d). Along the second co-inertia axis, the most important variables were mean water velocity, discharge, temperature and percentage of riffles opposed to percentage of pools, mean exposed soil, mean riparian canopy cover, mean thalweg depth and reach length (Figure 4e). Along this first co-inertia axis, sprawlers and univoltine organisms, with low body flexibility ( $<10^\circ$ ) and/or small body size, were opposed to swimmers and multi-voltine organisms with high body flexibility ( $>45^\circ$ ). Along the second co-inertia axis, silk net builders and organisms with cylindrical body form were opposed to scrapers and free-living organisms with streamlined body shape and intermediate body flexibility (Figure 4f).

Like taxonomic composition, the patterns observed above were significantly related ( $R_v = .067$ ,  $p < .001$ ; Table 2) to geographic position: an East-West gradient along the first co-inertia axis and a North-South gradient along the second co-inertia axis (not shown). However, the extracted variance was markedly lower than that obtained from catchment- and site-scale variables (Table 2).

### 3.3 | Catchment versus site scale: Taxa and traits

Different environmental variables most influenced different taxa and trait variables. The taxa that were most influenced by catchment-scale variables, those most correlated with the first co-inertia axis, were *Aturbina*, *Tricorythopsis*, *Smicridea*, *Callibaetis*, whereas taxa most influenced by site-scale variables were *Traverhypes*, *Itaura* and *Cloodes* (Figure 5a). Traits most influenced by catchment-scale variables were shredders and organisms with cylindrical or streamlined body form, and small body size, whereas traits most influenced by site-scale variables were silk net builders, filterers and organisms with more than one reproductive cycle per year and large body size (Figure 5b).

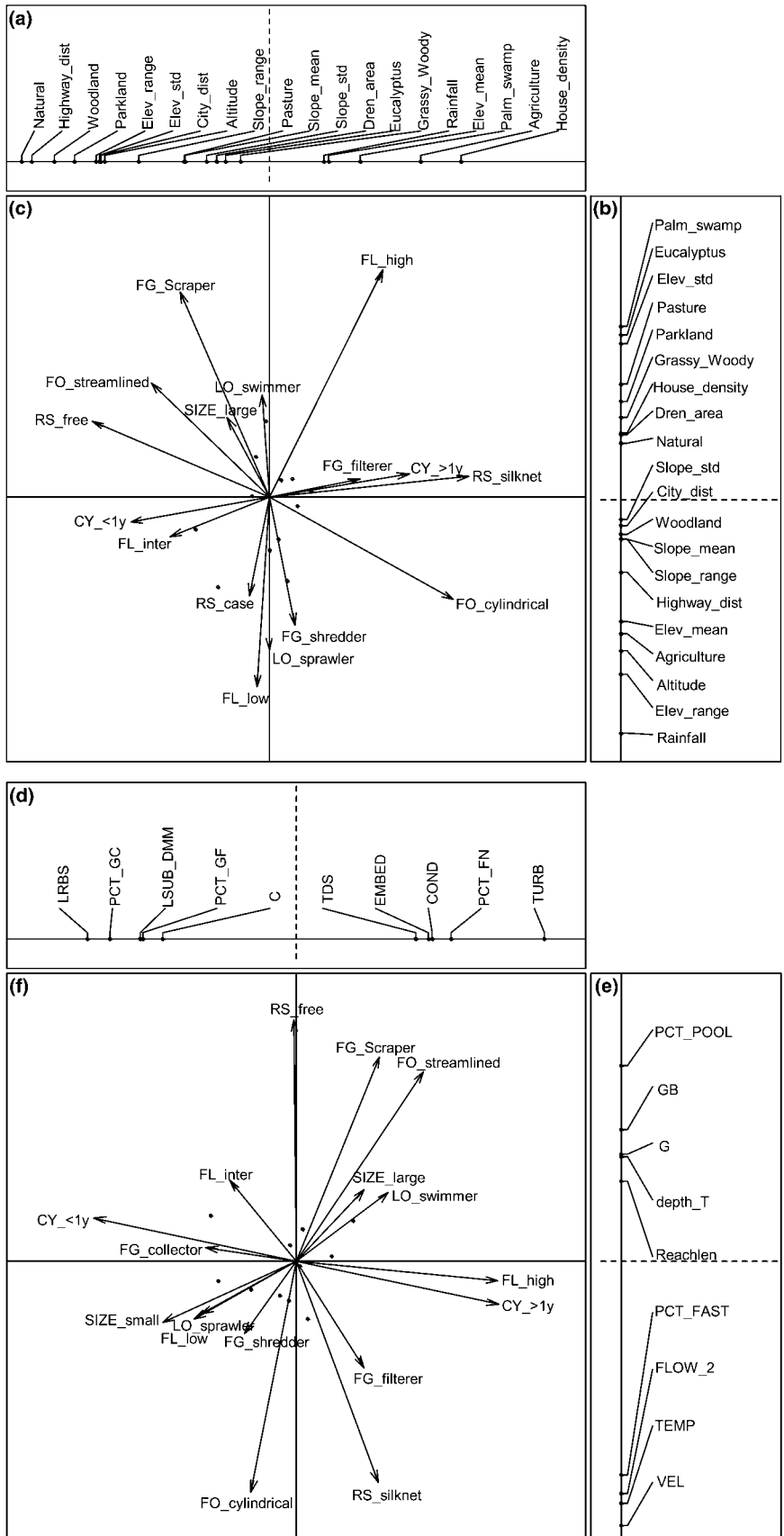
### 3.4 | Subsets of variables: Taxa versus traits

We performed co-inertia analyses between each subset of environmental variables and taxon (or trait) composition. Substrate, hydro-morphology and land use were most correlated with EPT taxon composition (Figure 6a, Table S2), whereas land use, flow and water quality were most correlated with EPT trait composition (Figure 6b, Table S2). Overall, the taxonomic structure of EPT assemblages exhibited stronger relationships with all groups of environmental variables than the functional trait composition ( $R_v = .181 \pm .05$  and  $R_v = .076 \pm .03$  for taxonomic and trait composition, respectively). Simulating the variance explained by each group of environmental variables for taxon composition by randomly selecting 28 genera (corresponding to the number of trait categories) confirmed that the higher variability in taxonomic data was not responsible for the difference in overall  $R_v$ -coefficients observed for taxon in comparison to trait composition (Figure S1).

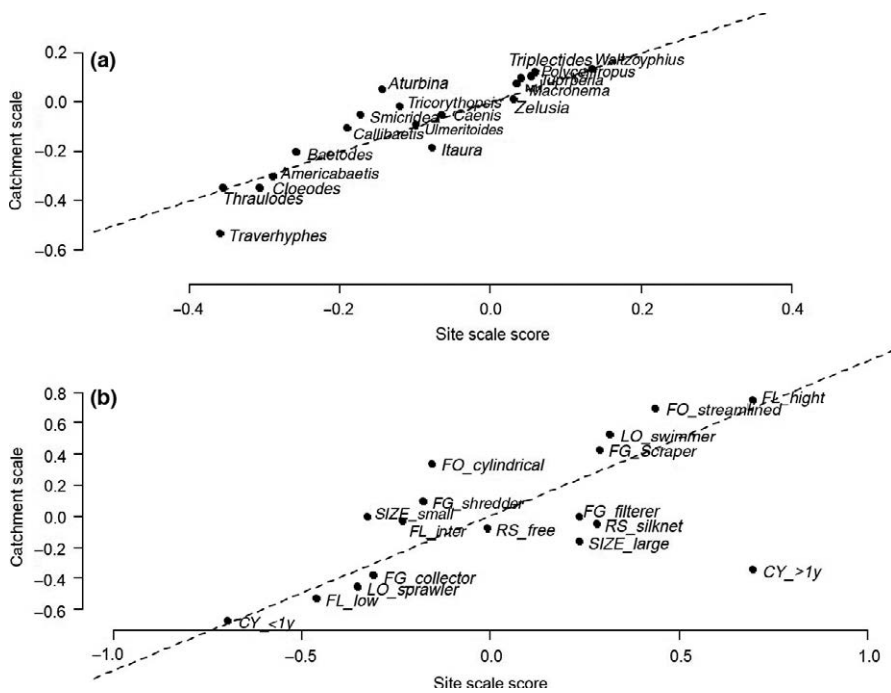
### 3.5 | Comparative importance of geographic position and catchment- and site-scale variables

Considering EPT taxonomic composition, the differences in  $R_v$ -coefficients between the catchment- and site-scale variables as a whole were not significantly different from zero, suggesting that both groups equally influenced EPT taxon composition (Table 3). In this analysis, geographic position was less influential than catchment- and site-scale variables. Significant positive  $R_v$ -coefficient differences occurred between the geophysical features and site-scale variables describing riparian canopy cover (60.3% higher), in-stream cover (22.6%) and water quality (8.2%) (Table 3). In addition, significant positive  $R_v$ -coefficients differences occurred between land use and site-scale variables describing riparian canopy cover (65.1%), in-stream habitat cover (31.6%), water quality (19.4%) and flow (18.1%), suggesting that land use had a greater influence on taxon composition than site-scale variables (Table 3).

Considering the trait composition of EPT assemblages, the significant positive differences in  $R_v$ -coefficients between catchment and site-scale variables as a whole indicated that catchment-scale variables were slightly more influential than site-scale variables (11.2%



**FIGURE 4** First two axes of a co-inertia analysis depicting the relationships between the *trait* composition of Ephemeroptera, Plecoptera and Trichoptera assemblages and (a–c) catchment-scale variables and (d–f) site-scale variables. (a) and (b) show the prominent catchment-scale variables along the first and second co-inertia axis respectively. (d) and (e) show the prominent site-scale variables along the first and second co-inertia axis respectively. (c) and (f) show the taxa loadings associated with the variation in trait composition in response to catchment-scale and site-scale variables respectively. Dots represent taxa with low loadings whose names are not shown for clarity (see Table S1 for variable acronyms)



**FIGURE 5** Influence of catchment and site scales on both (a) taxa and (b) traits most correlated with the first co-inertia axis. Each axis has the same variance. Taxa or traits above the dotted line are more influenced by catchment-scale variables and those below the line are more related to site-scale variables.

higher; Table 4). Similar to taxonomic composition, geographic position was less influential than catchment- and site-scale variables for traits. Significant positive  $R_v$ -coefficients differences occurred between geophysical variables and site-scale variables describing in-stream cover (59% higher), hydromorphology (50%) and riparian canopy cover (39.7%) (Table 4). Significant positive  $R_v$ -coefficients differences occurred between land use and all site-scale variables suggesting a greater influence of land use (between 33.8% and 75.5%) on EPT trait composition.

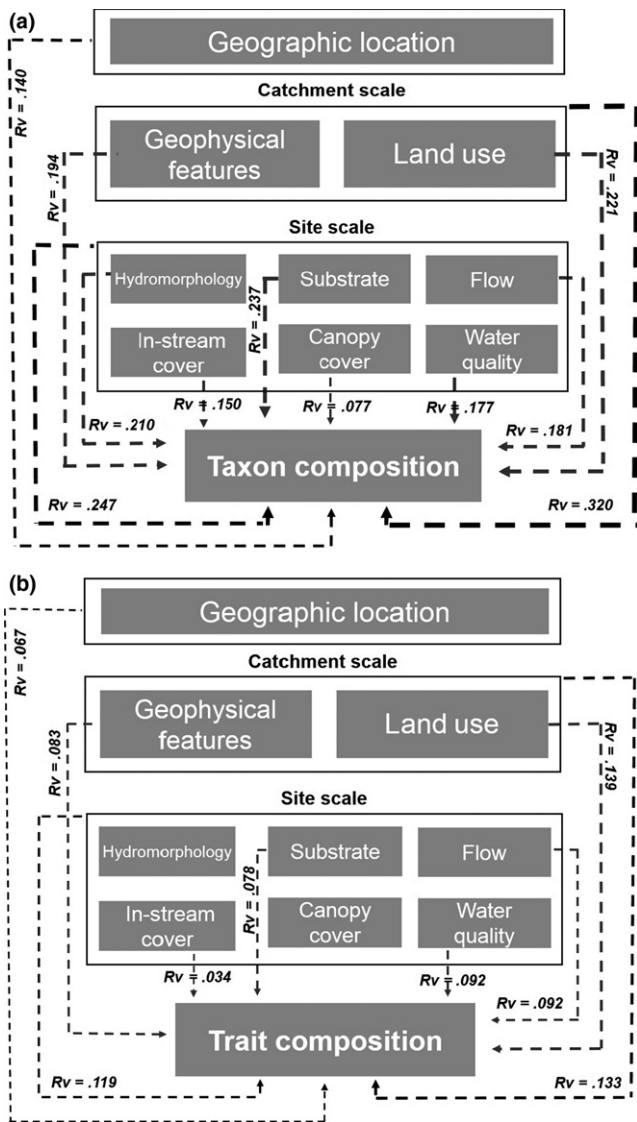
## 4 | DISCUSSION

Our aim was to assess the influence of environmental variables at varying spatial scales on taxonomic and trait composition of EPT assemblages found in Neotropical savanna headwater streams. Both catchment- and site-scale variable groups equally influenced EPT taxon composition, and geographic position was less influential than both. Considering EPT trait composition, catchment-scale variables were more influential than site-scale variables, and geographic location was also less influential than the two groups. Substrate, hydromorphology and land use were prominent in explaining the variation in EPT taxonomic composition, whereas land use mainly explained the variation in EPT trait composition.

Traits such as relation to substrate and potential number of reproductive cycles per year best discriminated EPT assemblages. Substrate conditions are known to constrain invertebrate distribution and abundance in streams (Rabeni, Doisy, & Zweig, 2005; Wood & Armitage, 1997). Fine sediments have direct effects on benthic habitats and are key stressors for benthic invertebrates (Bryce, Lomnický, & Kaufmann, 2010), being a result of increased agricultural runoff, elimination of riparian vegetation, and channelisation of streams

(Jones et al., 2012; Wood & Armitage, 1997). Corroborating Rabeni et al. (2005), we also observed that sprawlers were particularly intolerant to increased fine sediment and embeddedness. Assemblages with more multi-voltine organisms generally have a higher resilience to frequent disturbance. Our results corroborate the findings of Dolédec, et al. (2006) who observed that the number of cycles per year of New Zealand stream invertebrates increased with the intensity of land use.

Environmental variables at both catchment and site scales explained significant amounts of variation in EPT taxon and trait composition. Although the relationship between catchment-scale variables and EPT taxon composition was generally higher than that observed between site-scale variables and EPT taxon composition (Figure 6a), the overall difference between them was not significant. Hydromorphology, substrate and land use accounted for most of the variation in EPT taxonomic composition. Hydromorphological characteristics such as stream size and mean wetted and bankfull width and depth particularly influenced the taxonomic composition of EPT assemblages. Stream size is the primary determinant of the amount of lotic habitat in a locality and modifications in channel dimensions change the quantity and quality of aquatic habitat (Kaufmann et al., 1999; Ligeiro et al., 2013). Furthermore, hydromorphological alterations are considered major stressors of lotic ecosystems and their biology (Vaughan et al., 2009), although the links between river ecology and hydromorphology are still incomplete (Feld, de Bello, & Dolédec, 2014). In addition, percentage of fines, substrate diameter and relative bed stability were important variables to the taxonomic composition of EPT assemblages. Bottom characteristics are often cited as major parameters that control macroinvertebrate composition (Jones et al., 2012; Rabeni et al., 2005). Substrate size influences the hydraulic roughness and consequently the range of water velocities in a stream channel. It also influences the size range of



**FIGURE 6** Relationships between the environmental variables and (a) taxon and (b) trait composition of EPT assemblages. Arrow width is proportional to the  $Rv$ -coefficient. Arrows not shown means that the correlation is not significant

interstices that provide living space and cover for aquatic assemblages (Kaufmann et al., 1999). Decreases in the mean substrate size and increases in the percentage of fine sediments may destabilise channels and indicate changes in the rates of upland erosion and sediment supply (Bryce et al., 2010; Dietrich, Kirchner, Ikeda, & Iseya, 1989). In addition, substrate heterogeneity (i.e. proportion of different sediment types, sizes or textures) allows more species to coexist and positively affects macroinvertebrate assemblage structure, acting as an abiotic filter that selects for a set of functionally different organisms (Milesi et al., 2016). In an earlier study conducted in the Nova Ponte and Três Marias HUs, Ferreira et al. (2014) pointed out the importance of site-scale physical habitat in the distribution of EPT richness and concluded that channel morphology (width and depth), riparian structure, substrate composition and water quality were important for structuring macroinvertebrate

**TABLE 3** Results from the bootstraps performed on the differences between  $Rv$ -coefficients resulting from the comparison between EPT *taxonomic* composition and geographic position, and catchment- and site-scale variables.  $p$ : probability that the observed difference [ $Rv(E_1,F) - Rv(E_2,F)$ ] is significantly positive (n.s. = non-significant)

	Observed	$p$
Site – Geographic position	0.180	.001
Catchment – Geographic position	0.107	.001
Site – Catchment	0.073	n.s.
Land use – Canopy cover	0.144	.001
Geophysical features – Canopy cover	0.117	.001
Geophysical features – In-stream cover	0.044	.002
Land use – Water quality	0.043	.003
Land use – In-stream cover	0.070	.001
Land use – Flow	0.040	.012
Geophysical features – Water quality	0.016	.026
Geophysical features – Land use	0.027	n.s.
Geophysical features – Flow	0.013	n.s.
Land Use – Hydromorphology	0.010	n.s.
Geophysical features – Hydromorphology	-0.017	n.s.
Land Use – Substrate	-0.017	n.s.
Geophysical features – Substrate	-0.043	n.s.

**TABLE 4** Results from the bootstraps performed on the differences between  $Rv$ -coefficients resulting from the comparison between EPT *trait* composition and geographic position, and catchment- and site-scale variables.  $p$ : probability that the observed difference [ $Rv(E_1,F) - Rv(E_2,F)$ ] is significantly positive (n.s. = non-significant)

	Observed	$p$
Catchment – Geographic position	0.066	.001
Site – Geographic position	0.051	.020
Catchment – Site	0.015	.005
Land use – In-stream cover	0.105	.001
Land use – Morphology	0.098	.001
Land use – Canopy cover	0.089	.001
Land use – Substrate	0.061	.002
Geophysical features – In-stream cover	0.049	.001
Land use – Flow	0.047	.004
Land use – Water quality	0.047	.011
Geophysical features – Hydromorphology	0.042	.002
Geophysical features – Riparian canopy cover	0.033	.010
Geophysical features – Substrate	0.006	n.s.
Geophysical features – Flow	-0.008	n.s.
Geophysical features – Water quality	-0.009	n.s.
Geophysical features – Land use	-0.056	n.s.

richness in headwater streams. The percentage of natural cover influenced both the taxonomic and trait composition of EPT assemblages, the relation for traits being weaker than for taxa.

Modifications of natural vegetation land cover can cause multiple influencing factors, including increased nutrients in the water, fine sediments in the streambed, chemical contamination, loss of habitat heterogeneity and increased light availability and aquatic macrophyte growth through the clearing of riparian vegetation (Allan, 2004; Leitão et al., 2017).

Catchment-scale variables, mainly land use, were more influential on the trait composition of EPT assemblages in comparison with site-scale variables. In general, taxonomic structure should be more strongly affected by regional processes, whereas functional structure should be mostly shaped by site-scale characteristics (Hoeinghaus et al., 2007) because of their greater proximity and interaction with the organisms. However, a mismatch between patterns of taxonomic and functional structure may also be related to spatial scales. Large-scale processes shape the pool of species in a local assemblage. Sites may be located within a single regional species pool, then all species and functional trait combinations may colonise each site, and taxonomic and functional structure should exhibit similar patterns along major environmental gradients (Heino et al., 2007). The multiple spatial scales regulating assemblage structure are related to multiple environmental filters, where each spatial scale acts as an environmental filter and only species possessing a specific set of traits pass the filter at each scale and become established in a community (Poff, 1997; Tonn, 1990). In fact, land use influenced assemblages in our study through its effects on canopy cover, water quality, in-stream cover, flow and hydromorphology. Such a high influence of land use induced stressors on the functional trait composition of stream macroinvertebrate assemblages supports the findings of previous studies (Dolédec et al., 2011; Richards et al., 1997).

We also observed that the correlations with environmental variables were generally lower for trait than for taxon composition of EPT assemblages at both spatial scales. In contrast, in New Zealand streams subjected to intensive land use, both taxonomy and trait approaches were able to discriminate land use practices, but the trait approach accounted for more variance between different land uses (Dolédec et al., 2006). However, this study may not be comparable to ours because the intensity of land use and the taxa differ. Nonetheless taxa that naturally fluctuate with environmental conditions and geography may have similar traits, thus smoothing the response of traits to environmental variation at larger scales. This higher spatial stability of traits is potentially linked to the physical harshness of the stream conditions, which act as a strong abiotic filter on biological traits and involves trait convergence in stream biota (e.g. Poff, 1997; Statzner et al., 2004).

Similar to the findings of Townsend et al. (2003), geographic position accounted for less assemblage variation than catchment- and site-scale variables for both taxa and traits. This suggests three alternative hypotheses. (1) Different species have evolved in an ecologically divergent way in the different locations not ignoring that geography and environmental variables may be correlated. (2) EPT evolutionary history may have constrained the colonisation of various locations. (3) Weak dispersal capabilities of Neotropical savanna

EPT have prevented them from overcoming geographic barriers (see Townsend et al., 2003). However, our current lack of biological knowledge of Cerrado EPT taxa prevents us from confirming one or more of those hypotheses. Townsend et al. (2003) also observed a lower relationship between geographical location and invertebrate assemblage composition in comparison to catchment- and site-scale variables, possibly due to a correlation between geography and those environmental conditions.

To our knowledge, this study is the first that considers environmental variables at catchment and site scales to explain both invertebrate taxonomic and functional composition in tropical savanna streams. Our results demonstrate four key issues regarding those streams: (1) Aquatic invertebrate taxonomic composition is mainly structured by land use, substrate and hydromorphology. (2) Land use filters specific traits in EPT assemblages. (3) It is essential to increase our biological knowledge of invertebrates if we want to include the multiple-trait based approach in water resource conservation. (4) Identification of variables driving the taxonomic and functional structure of macroinvertebrate assemblages determines the scale at which efforts for protection and/or rehabilitation would best be directed to improve and/or maintain environmental and ecological condition.

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