



Contrasting taxonomical and functional responses of stream invertebrates across space and time in a Neotropical basin

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With 4 figures, 6 tables and 1 appendix

Abstract: The structure of the EPT group (Ephemeroptera, Plecoptera and Trichoptera) reflects changes operating on the underlying freshwater system. We study the structure of the EPT assemblages inhabiting a Neotropical basin with mountain rain forests. Taxonomic richness and functional diversity are two relevant indicators of that structure. We measured real biological traits rather than using trait classifications arising from the Northern hemisphere, allowing us to more accurately calculate functional diversity metrics in these Neotropical streams. We also formalized a new approach to measuring functional dissimilarity. Our main scope is to know the effects of space (altitudinal zonation) and time (seasonality) on the behavior of both diversity indexes. We find complementarities between them, suggesting that the replacement in taxonomic composition is a process decoupled from the replacement in trait composition. While the taxonomic richness of sites in the dry season differs from the richness in upland sites sampled during spates, the functional diversity allows us to differentiate lowland sites sampled during spates from other sites. We hypothesize that functional diversity goes down in lowland sites because of the anthropogenic pressure on the riparian area. We additionally stress that the knowledge of functional diversity contributes to the better design of conservation strategies.

Key words: EPT community, functional dissimilarity index, coefficient of Rao, species richness.

Introduction

Species richness has been broadly used as a quantitative surrogate in the assessment of biodiversity of Neotropical stream invertebrates (i.e. Costa & Melo 2008, Henriques-Oliveira & Nessimian 2010, Ligeiro et al. 2010). However, it is important to consider that species traits differences matter for many important processes such as ecosystem functioning and assembly patterns (Diniz-Filho et al. 2011). Many studies on the relationship between biodiversity and ecosystem functioning have concluded that the ecological roles of the species are more informative than the simple

number of taxonomic items (Barnett et al. 2007). Functional diversity (FD) reflects the diversity of morphological, physiological and ecological traits within biological communities (Lepš et al. 2006, Petchey & Gaston 2006) and is considered to outperform other classical measures of diversity for understanding ecosystem functioning (Hooper et al. 2005).

In the context of stream ecology, several functional traits of benthic macroinvertebrates are used to explore the linkage between community and environment in addition to the anthropogenic degradation of ecosystem conditions (Dolédec et al. 1999, Lamouroux et al. 2004, Finn & Poff 2005). In this sense,

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many researchers have stressed the importance of environmental features in determining the trait-structure of freshwater macroinvertebrate assemblages, such as habitat structure, water chemistry and availability of trophic resources (Dolédec et al. 2006, Piscart et al. 2006, Mellado et al. 2008, Statzner & Bechê 2010). The species that thrive in the same micro-habitat are expected to share many biological/ecological characteristics according to the niche filtering concept (Franzen 2004, Statzner et al. 2004).

From a theoretical standpoint, FD decreases with increasing environmental constraints or stress (Mouillot et al. 2006), since a narrow spectrum of species fit to high constraints scenarios. More precisely, environmental factors could limit the presence of certain functional traits at certain sites and thus decrease the FD of local communities, as in sites under environmental pressure. Therefore, there should be a close correspondence between local habitat conditions and the profile of species traits.

The features shared by species are allegedly the traits responsible for overcoming the constraints. Since traits are proxies of ecosystem functionality, a number of indexes that summarize the observed variation in traits throughout the community have been proposed to assess functional diversity (Petchey & Gaston 2002, Botta-Dukát 2005, Ricotta 2005, de Bello et al. 2006, Laliberté & Legendre 2010). These indexes are based on the rationale that an assemblage characterized by species highly overlapped in their list of traits (i.e., species functionally similar) is less functionally diverse than an assemblage where species exhibit a different repository of traits. The Rao's quadratic entropy-Q index (Rao's Q) represents a standard

coefficient to quantify the FD of a given community. The Q index accounts for the average pairwise functional similarity between items randomly drawn from the community under analysis. It is a generalization of the Simpson's index of diversity and depends on the relative abundances of species. It can be coupled with various measures of similarity between species across the pool of traits that characterize them (Botta-Dukát 2005, Ricotta 2005, Lepš et al. 2006, Petchey & Gaston 2006, Gallardo et al. 2009).

Macroinvertebrates are important components of lotic environments. The group EPT is a subset composed by insects of the orders Ephemeroptera, Plecoptera and Trichoptera. According to Marchant et al. (1995), patterns found for EPT represent the same patterns found for the macroinvertebrate fauna as a whole; for this reason, studies could be restricted to that subset of fauna (Bispo & Oliveira 2007). Our main aim is to study the structural changes (revealed by diversity measures) of EPT assemblages across the spatial (altitudinal zonation) and temporal (seasonality of rains) dimensions of a Neotropical basin which is mainly occupied by mountain forests. Our main research questions focused on (i) the difference in the pattern of EPT diversity in space and time (ii) the comparison between taxonomic richness and FD in order to confirm if they represent either complementary or redundant measures of the responses of the EPT community to environmental factors causing variability. We also focused on (iii) the delineation of functional groups within the EPT group using the matrix of functional dissimilarity between taxa and on (iv) the formalization of a new quantitative procedure to measure functional dissimilarity taking into account fuzzy-

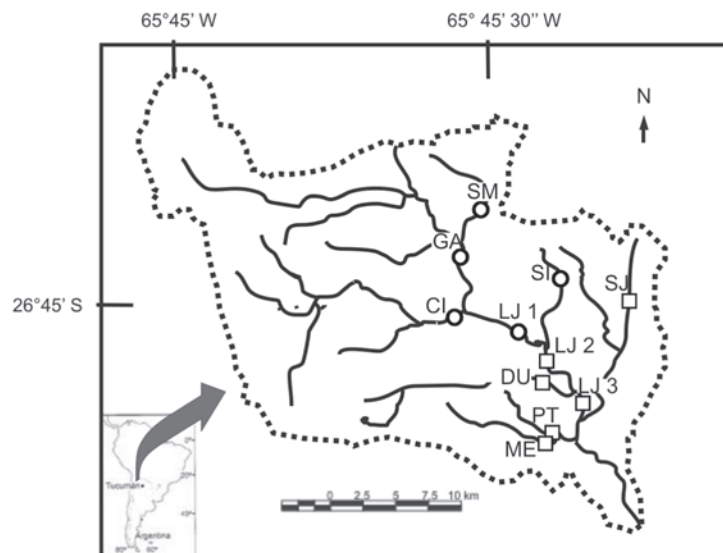


Fig. 1. Study area with sampling sites projected on the drainage network. The circles represent sites at high altitude (>960 m a.s.l.) whereas the squares represent sites at low altitude (\leq 960 m a.s.l.).

coded ordinal traits. To our knowledge, our paper is the first attempt to evaluate the configuration of FD based on EPT traits in a Neotropical mountainous basin. We stress the measurement of FD as a valuable approach to assess numerically the mechanisms behind ecosystem functioning.

Material and methods

Study area

The Lules River Basin (26° 36' S, 65° 45' W) is located at the Tucumán province in Northwestern Argentina. It is a subtropical Andean basin with an area of 820.25 km² and drains the moist mountainous forest called "Las Yungas". The climate is subtropical with monsoon rainfall and a rainy season rang-

ing from November to April. For a complete hydrogeographic characterization of the zone see Mesa (2006). Figure 1 displays the sampling sites over the main river network of this watershed. Geographical co-ordinates are shown in Table 1. Five sampling sites (SM, GA, CI, SI and LJ1) were selected in the upper portion of the Lules River characterized by deep creeks and deep intermountain valleys. Riparian vegetation consists of native species of the subtropical mountainous forest (Sirombra & Mesa 2010). Running waters supply human consumption and livestock (cattle, sheep and horses). A further six sites (LJ2, DU, LJ3, PT, ME and SJ) were scattered in the lower portion of the basin where an incipient process of water erosion, lateral erosion of valleys and landslides are more common. The riparian areas of lowland sites show anthropogenic pressures such as agricultural land, overgrazing and urbanization. Following Reynaga & Dos Santos (2012), the terrain height of 960 m a.s.l. has been used as the altitudinal threshold to separate lowland from upland sites.

Table 1. Environmental characterization of the study sites. The dash (–) separates chronologically the samples taken across the low water (w) and the high water (W) periods corresponding to: September 2005 | September 2007 and March 2006 | March 2007, respectively.

		Altitude (m a.s.l.)	Discharge (m ³ s ⁻¹)	Temperature (°C)	Conductivity (µS/cm)	pH	Streamwidth (m)	Code
San Miguel (26° 40' 59.99" S, 65° 31' 12.97" W)	W	1330	0.49–0.06	18–17	32.5–144.8	6.1–6	3.6–1.6	SM
	w	1330	0.005–0.011	20–19	166–163.2	8.3–7	1.4–2.8	
Garabatal (26° 43' 39.72" S, 65° 31' 9.98" W)	W	1278	2.11–1.03	16–21	76.4–105.8	6.1–6	10.8–7.7	GA
	w	1278	0.39–0.50	19–15	114–115.37	7.8–6	1.4–6.4	
La Ciénaga (26° 44' 52.98" S, 65° 30' 45.97" W)	W	1105	1.39	20	78.6	6	7.40	CI
	w	1105	0.43–0.49	18–15	90–86.9	7.7–7	4.57–3.4	
Siambón (26° 43' 12" S, 65° 26' 42" W)	W	1080	0.17–0.16	13–21	93.6–134	6.1–6	5.4–3.7	SI
	w	1080	0.02–0.03	18–19	232–209	8.2–6	2.5–2.8	
Las Juntas 1 (26° 45' 29.99" S, 65° 29' 30.98" W)	W	1069	2.64	18	104.3	6	10.80	LJ1
	w	1069	0.87–1.47	18–13	124–120.4	7.9–7	12.3–7.4	
Las Juntas 2 (26° 46' 9.98" S, 65° 28' 19.99" W)	W	960	4.27	21	108.4	6	12.70	LJ2
	w	960	0.96–0.89	15–15	135–128.6	7.9–7	12.7–9.6	
Las Juntas 3 (26° 50' 42" S, 65° 26' 1.97" W)	W	698	5.42	25	29.2	7	15.60	LJ3
	w	698	0.97–1.85	19–18	600–483	8.1–8	4.6–9.5	
Duraznillo (26° 48' 24.01" S, 65° 27' 55" W)	W	942	0.22–0.26	20–22	220–127	6.1–6	12.6–6.4	DU
	w	942	0.04–0.04	20–19	657–568	9.1–7	11.1–3	
Potrero de las Tablas (26° 51' 18" S, 65° 25' 55" W)	W	685	0.99–0.71	19–25	110–344	7.1–7	4.2–7	PT
	w	685	0.30–0.15	20–19	200–207	8.8–8	4–5.6	
Membrillo (26° 51' 22.97" S, 65° 25' 52.97" W)	W	686	0.15–0.13	23–26	304–573	7.1–6	5.2–5.6	ME
	w	686	0.04–0.04	20–21	931–809	8.6–8	5.4–5.6	
San Javier (26° 46' 26" S, 65° 23' 23" W)	W	908	0.1–0.24	19–20	276–229	7.1–7	3.6–3.6	SJ
	w	908	0.08–0.06	16–20	373–270	7.8–6	4.1–4.2	

Data collection

Macroinvertebrate sampling was performed every six months between 2005 and 2007 covering both high (March) and low waters (September). Benthic samplings for sites {CI, LJ1, LJ2} and {LJ3} during March 2006 and March 2007, respectively, could not be carried out because of the high discharges recorded at those times (Table 1). Three Surber replicates (area 0.09 m², mesh size 300 µm) were taken from riffles at each sampling site. Fauna were preserved in 4% formalin and transferred to the laboratory for further processing and identification. All macroinvertebrates from orders Ephemeroptera, Plecoptera and Trichoptera (EPT) were removed and identified to the lowest taxonomical level of resolution consulting regional specialized keys (Dominguez & Fernández 2009).

Traits of taxa

We defined ten biological traits split into levels or modalities (Table 2). The selected traits are thought to reflect limiting aspects of stream environmental conditions such as food resource, oxygen availability and physical constraints to settlement imposed by the characteristics of substrate and flow. Presence and shape of the gills, body size, shape and flexibility, and hardness of the exoskeleton were morphological traits obtained by direct observation of the collected material. Behavioral traits such as mobility and/or attachment to substratum were identified by field observations and specialist consultations. Trophic information was determined using gut content analysis and behavioral observations (Reynaga 2009, Reynaga & Rueda Martín 2010). The trait corresponding to physiological toler-

Table 2. Biological traits and modalities for the taxa considered in the present study.

Trait	Modality	Code
Maximum body size (mm)	< 5	< 5
	5–10	5–10
	10–15	10–15
	15–20	15–20
	> 20	> 20
Body form	Cylindrical	cyl
	Streamlined	str
Body flexibility (degrees)	None (< 10)	flnone
	Low (10–45)	flow
	High (> 45)	flhigh
Hardness exoskeleton	Low	hlow
	Moderate (sclerotized or case of silk)	hmod
	High	hhigh
Feeding habits	Collector-gatherer	cg
	Collector-filterer	cf
	Schredder	sch
	Scraper	scr
	Predator	pre
Gills	Absent	abs
	abdominal and thoracic	abd+thor
	abdominal or thoracic	abd/thor
Gills shape	circular	cir
	oval	ov
	elongated	elo
	Absent	gabs
Locomotion	Surface swimmer	sw
	Crawler	craw
	Burrower	burr
Specific adaptation to flow constraints	Silt gland	silt
	Mineral material-case	mmc
	Tarsal or Anal hooks	hook
Tolerance to oxygen deficit (based on scores of BMWP ⁺)	Low	tlow
	Moderate	tmod
	High	thigh

ance to oxygen deficit was inferred from the BMWP (Biological Monitoring Working Party) scores modified for the region (Domínguez & Fernández 1998). The affinity of taxa towards the trait modalities was coded through a fuzzy system of coding (Chevenet et al. 1994). Assigned scores of trait affinity ranged from 0 (= null) and 1 (= maximal) depending on the degree of relationship between the taxon and the trait modality under consideration.

Functional diversity

The FD was calculated on the basis of the Rao's Q (Ricotta 2005, Lepš et al. 2006) as:

$$\text{Rao's } Q = \sum_{i=1}^s \sum_{j=1}^s d_{ij} p_i p_j$$

where d_{ij} expresses the dissimilarity between the pair of taxa i and j . The above formula implies that the dissimilarity in trait space is summed over all pairs of taxa and weighted by the respective relative abundances. The coefficient d_{ij} ranges from 0 (when the two species show the same pool of traits) to 1 (when the two species have completely different traits). All traits were treated as semi-quantitative variables and scaled between 0 and 1, to standardize trait dimensions (see Botta-Dukat 2005, Lepš et al. 2006). Compared to other indices of FD, the Rao's Q includes information about the abundance of species which is a relevant parameter to consider the effects of species and trait composition on several ecosystem processes (e.g. Petchey & Gaston 2006, Ricotta & Moretti 2008).

Operatively, the computation of Rao's Q necessitates a procedure for calculating the dissimilarity (or 1 – similarity) between the repertoires of traits exhibited by the taxa. Many of the traits portrayed by aquatic insects can be treated as different levels of an ordinal variable (e.g. hardness of exoskeleton). We propose here a novel numerical procedure to calculate the dissimilarity between two taxonomic items when there are fuzzy-coded ordinal variables. Let X be an ordinal variable provided of k attributes or modalities. The affinity of each item towards those attributes is characterized through a vector of length k with entries between 0 and 1. For a fuzzy-coded ordinal variable, the scores of affinity sum to the unity across its levels. Affinities can be inferred from the relative frequency of occurrence of each attribute in the set of individuals comprising the item under consideration. Now, let us consider two taxonomic items, called p and q , which are characterized through the respective vectors of affinities $\mathbf{p} = [p_1, p_2, \dots, p_k]$ and $\mathbf{q} = [q_1, q_2, \dots, q_k]$ where $\sum_{i=1,k} p_i = 1 = \sum_{i=1,k} q_i$. To assess the dissimilarity between both vectors, the conventional procedure accounts for the overlap between homologous elements of the involved vectors (comparison in parallel). Nevertheless, this procedure is flawed because it disregards the dispersion of affinities at adjacent levels of the variable. In fact, there are cases with little (or no) overlap between homologous scores despite the neighboring entries yielding a very close cumulative score. For instance, the vectors $\mathbf{a} = [0.4, 0.2, 0.1, 0.1, 0.2]$, $\mathbf{b} = [0.2, 0.4, 0, 0.3, 0]$ and $\mathbf{c} = [0.1, 0.1, 0.1, 0.6, 0.1]$ are considered as equally similar (total overlap equals 0.5) by the conventional procedure (comparison of vector entries in parallel). However, when we focus on the distribution of affinities at the immediacy of each entry, we can observe that vector \mathbf{a} is clearly closer to \mathbf{b} than to \mathbf{c} because the nearby entries yield closer cumulative affinities. The proposed measure of dissimilarity (or 1 – similarity) is implemented in the R script available at the Appendix 1.

Table 3. EPT collected in the sampled streams. %F: Frequency of occurrence (n = 120).

Taxa	%F	Code
Insecta		
Ephemeroptera		
Baetidae		
<i>Americabaetis</i> sp.	3.9	Ame
<i>Baetodes huaico</i>	34.5	Baeh
<i>Baetodes</i> sp.	1.4	Baesp
<i>Camelobaetidium penai</i>	9.0	Came
<i>Nanomis galera</i>	2.4	Nano
<i>Varipes</i> sp.	0.3	Var
Caenidae		
<i>Caenis</i> sp.	1.5	Cae
Leptohyphidae		
<i>Haplohyphes baritu</i>	0.2	Hapl
<i>Leptohyphes eximius</i>	6.1	Lepto
<i>Tricorythodes popayanicus</i>	1.4	Trico
Leptophlebiidae		
<i>Farrodes</i> sp.	< 0.1	Farro
<i>Thraulodes</i> sp.	4.4	Thrasp
<i>Thraulodes consortis</i>	0.1	Thracon
<i>Thraulodes cochunaensis</i>	0.1	Thracoch
Plecoptera		
Perlidae		
<i>Anacroneuria</i> sp.	2.2	Ana
Trichoptera		
Glossosomatidae		
<i>Mexitrichia</i> sp.	0.1	Mexi
<i>Mortoniella</i> sp.	1.4	Mort
<i>Protoptila</i> sp.	< 0.1	Prot
Glossosomatidae	0.2	Glos
Helicopsychidae		
<i>Helicopsyche</i> sp.	0.8	Heli
Hydroptilidae		
<i>Hydroptila</i> sp.	0.4	Hydrofsp
<i>Hydroptilidae</i>	8.3	Hydrop
<i>Ithytrichia</i>	0.1	Ithy
<i>Metrichia</i> sp.	11.3	Metr
<i>Oxyethira</i> sp.	0.1	Oxy
<i>Neotrichia</i> sp.	0.2	Neo
Hydropsychidae		
<i>Smicridea</i> sp.	8.7	Smi
Leptoceridae		
<i>Nectopsyche</i> sp.	< 0.1	Nect
<i>Oecetis</i> sp.	0.1	Oece
Odontoceridae		
<i>Marilia</i> sp.	0.1	Mar
Philopotamidae		
<i>Chimarra</i> sp.	< 0.1	Chi
Polycentropodidae		
<i>Polycentropus joergenseni</i>	< 0.1	Poly
Hydrobiosidae		
<i>Atopsyche</i> sp.	0.6	Atop
<i>Cailloma</i> sp.	< 0.1	Cai
Hydrobiosidae	< 0.1	Hydrob

Statistical analyses and graphics

The structure of data concerning to traits of taxa was represented through a complete linkage dendrogram coupled with a complete profile of traits across the involved taxa. This dendrogram arranges the taxa hierarchically in function of the pairwise dissimilarity scores. Dissimilarity was calculated as indicated above. Complete linkage is an agglomerative or clustering method that merges groups together based on the most dissimilar pair of elements under comparison.

Our main scope is to compare the values of taxonomic richness against those of FD. In tight correspondence with this, we conducted correlation analyses and explored graphically the spatio-temporal responses yielded by both indexes using a bubble map. For that, on each sampling site of the drainage network we projected a circle with size proportional to the respective average value of diversity. This plot allows us to visualize the combined pattern of spatial and temporal variability for the taxonomic richness as well as for the FD.

Table 4. Scores of FD and species richness across the study area. Sampling sites are grouped into four spatio-temporal categories resulting from the cross-product between the levels of flow regime (W and w for high and low waters, respectively) and the levels of altitude (A and a for upland and lowland respectively). Sampling dates have been appended to the label of the respective site (M and S for March and September, respectively).

Flow regime	Altitude	Level of spatio-temporal factor	Sampling point	Rao's Q	Mean (\pm SD)	Taxonomic richness	Mean (\pm SD)
High waters	High altitude	WA	CI_M07	0.1029	0.1014 (\pm 0.0169)	12	13.3000 (\pm 3.9051)
		WA	GA_M06	0.0835			
		WA	GA_M07	0.1145			
		WA	LJ1_M07	0.1117			
		WA	LJ2_M07	0.0897			
		WA	SI_M06	0.1121			
		WA	SI_M07	0.1139			
		WA	SM_M06	0.1158			
		WA	SM_M07	0.0688			
	Low altitude	Wa	LJ3_M07	0.0578	0.058 (\pm 0.0258)	17	15.0000 (\pm 2.5495)
		Wa	ME_M06	0.0968			
		Wa	ME_M07	0.0261			
		Wa	DU_M06	0.0571			
		Wa	DU_M07	0.0310			
		Wa	PT_M06	0.0582			
		Wa	PT_M07	0.0688			
		Wa	SJ_M06	0.0938			
		Wa	SJ_M07	0.0324			
Low waters	High altitude	wA	CI_S05	0.0493	0.1006 (\pm 0.0335)	16	17.0000 (\pm 2.7961)
		wA	CI_S06	0.0451			
		wA	GA_S05	0.1090			
		wA	GA_S06	0.0715			
		wA	LJ1_S05	0.1238			
		wA	LJ1_S06	0.0793			
		wA	LJ2_S05	0.1350			
		wA	LJ2_S06	0.1297			
		wA	SI_S05	0.1422			
		wA	SI_S06	0.1263			
		wA	SM_S05	0.1105			
		wA	SM_S06	0.0858			
	Low altitude	wa	LJ3_S05	0.1023	0.0969 (\pm 0.0213)	21	17.7000 (\pm 2.9078)
		wa	LJ3_S06	0.0998			
		wa	ME_S05	0.1148			
		wa	ME_S06	0.1239			
		wa	DU_S05	0.1106			
		wa	DU_S06	0.1129			
wa	PT_S05	0.0969	17				
wa	PT_S06	0.0563	18				
wa	SJ_S05	0.0752	20				
wa	SJ_S06	0.0763	17				

In the task of formally testing for no differences in the means of diversity measures in function of a single spatio-temporal factor across the study area, two different one-way ANalysis Of VAriances (ANOVAs) were performed with taxonomic richness and FD acting as dependent variables, separately. The predictive spatio-temporal factor has four levels that come from combining the pair of spatial levels (lowland and upland) with the pair of temporal levels (low and high waters) associated to the sampling units. After detecting some differences in the levels of the factor, pairwise comparisons were conducted under the Tukey's Honest Significant Difference (HSD) technique. Since to use the ANOVA method requires that assumptions about the normality and homoscedasticity of residuals be satisfied (Montgomery 2001), we tested both conditions via standard procedures including both tests and exploratory graphics (QQ plot, residual-fitted plot, boxplots, Shapiro-Wilk normality test and Bartlett's test of homogeneity of variances). Finally, we produced a graphical synthesis for the multiple comparisons performed between the different levels. All statistical analyses and graphics were produced via the R platform (R Core Team 2012).

Results

A total of 35 taxa of the EPT group was collected from the streams sampled in our study region (Table 3), distributed in 4 families of Ephemeroptera, 1 family of Plecoptera and 9 families of Trichoptera. Overall, local stream macroinvertebrates assemblages were dominated by *Baetodes huaico*, *Camelobaetidius penai*, *Leptohyphes eximius*, *Thraulodes* sp., Hydroptilidae, *Metricchia* sp., *Smicridea* sp. Taxonomical richness ranged from 9 taxa in the upland site SM to 21 in the lowland site LJ3. Taxonomical richness was generally higher during the period of low waters.

The complete linkage dendrogram (Fig. 2) obtained from the functional dissimilarity between taxa can be partitioned into six groups (cutoff level = 0.4). Members of G1 (i.e. *Baetodes*, *Americabaetis*, *Varipes*, *Ca-*

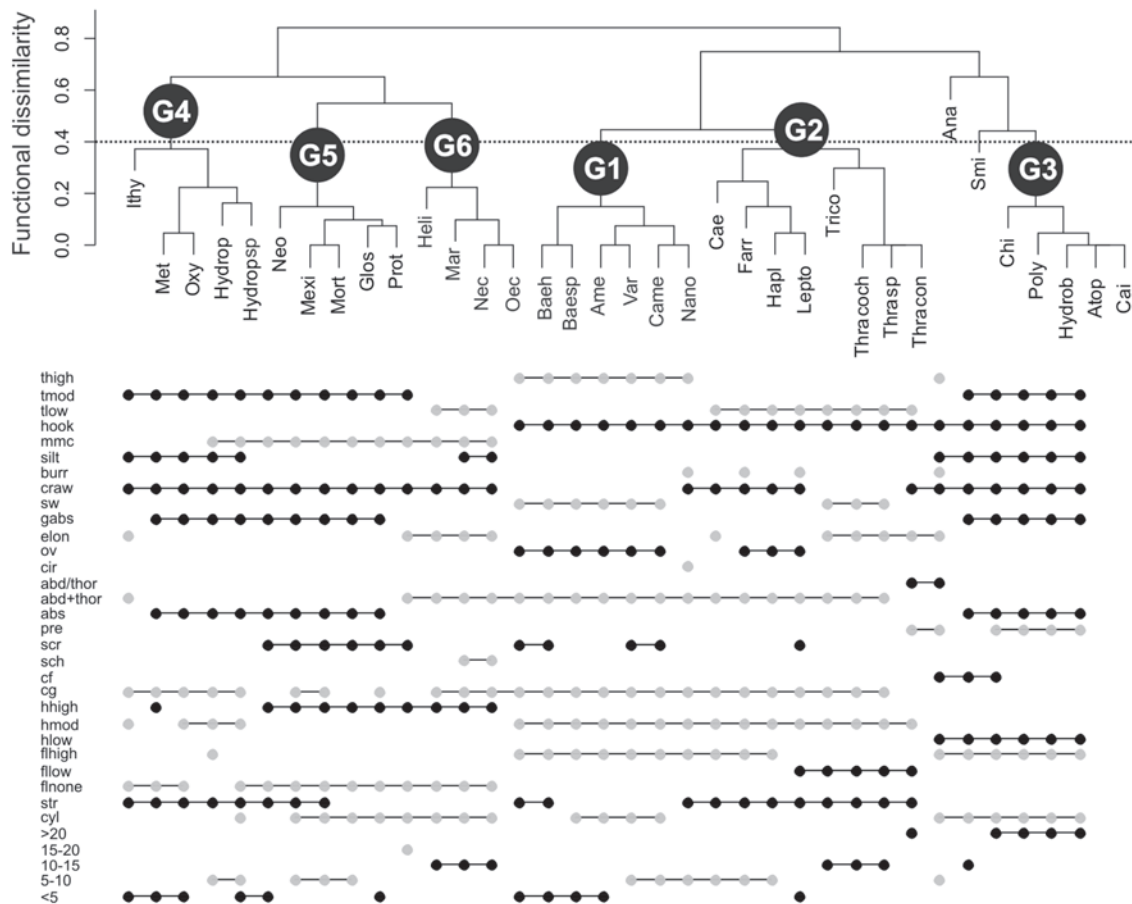


Fig. 2. Complete linkage clustering of taxa based on their functional dissimilarity. The profile of traits is indicated below the leaves of the dendrogram where points indicate presence of the respective trait modality. Sets of successive taxa that share the same trait are indicated through line segments joining the points. The dotted line cuts the tree at a height level of 0.4 and helps to define six functional groups. Codes used for referring the traits modalities are shown in Table 2. Abbreviations for the names of taxa are explained in Table 1.

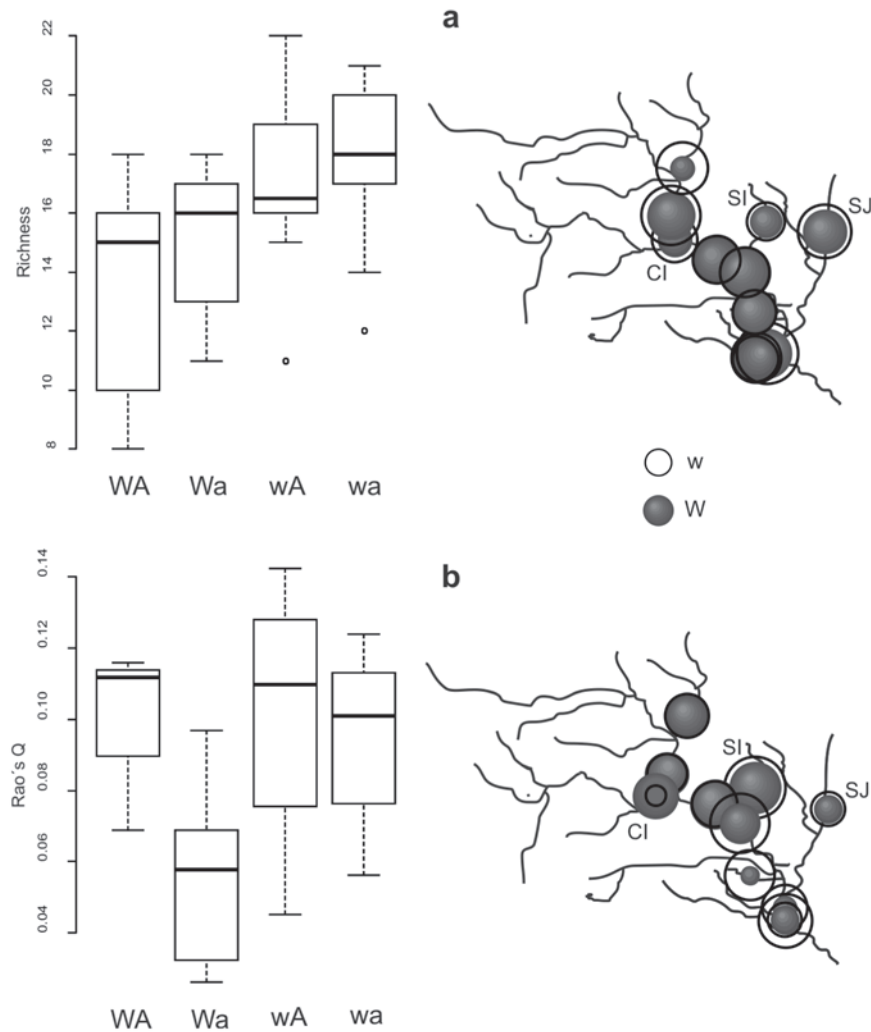


Fig. 3. Boxplots and whiskers of the scores of species richness **(a)** and FD **(b)** across four different groups associated to the spatio-temporal configuration of the sampling points. Each group results from combining one temporal level (flow regime: W = high waters, w = low waters) with another spatial level (altitude: A = upland, a = lowland). Bubble sizes in the spatial representation of sites are proportional to the scores of diversity measures averaged by sampling season.

Table 5. Results of one-way ANOVAs with species richness and FD as dependant variables; spatio-temporal categories as the predictive factor. Significance at the 0.05 (*) and 0.01 (**) levels are indicated

Response variable and source of variation	Df	SS	Mean S	F value	<i>p</i> (> F)
Species richness					
Spatio-temporal factor	3	113.5	37.83	4.052	0.014*
Residuals	36	336.1	9.34		
Functional diversity					
Spatio-temporal factor	3	0.01222	0.004073	6.105	0.002**
Residuals	36	0.02402	0.000667		

melobaetidius and *Nanomis*) exhibit a combination of traits characterized by small size (<5 mm), high flexible body, moderate hardness exoskeleton, collector-gatherers and scrapers feeding habit, with oval gills, tarsal hooks, surface swimmers and high tolerance

to oxygen deficiency. Members of G2 (i.e. *Farrodes*, *Thraulodes*, *Caenis*, *Tricorythodes*, *Haplohyphes* and *Leptohyphes*) are mainly collector-gatherers that show flattened and low flexible bodies, with gills, tarsal hooks and low tolerance to oxygen deficiency. The

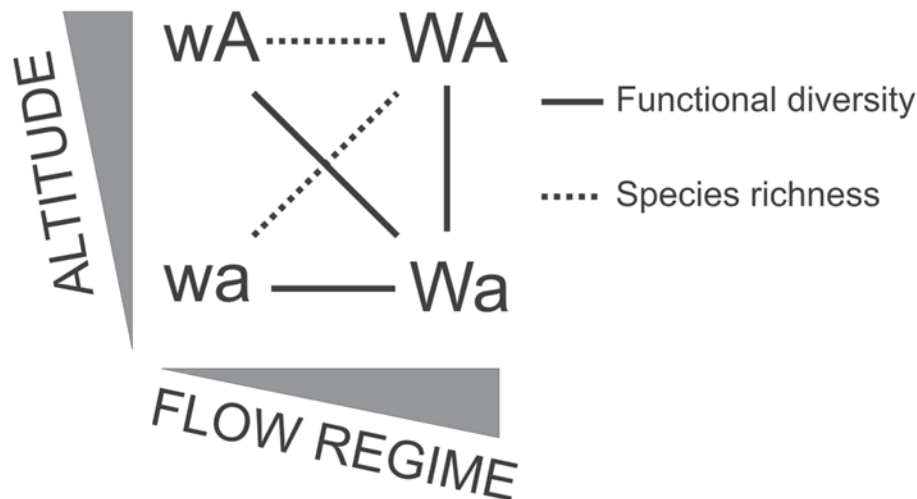


Fig. 4. Graphical synthesis for the multiple comparisons performed between the different levels of the spatio-temporal factor. These levels come from combining a temporal level (flow regime: W = high waters, w = low waters) with another spatial one (altitude: A = upland, a = lowland). Pairs of levels are linked if they show statistically significant differences in their scores either of species richness (dotted lines) or of FD (solid lines). Note the discrepancy between both variables for detecting differences. Pairwise comparisons are numerically summarized in Table 5.

Table 6. Multiple comparisons between the levels of the spatio-temporal factor for the values of species richness and FD. Levels are: WA (high waters at upland), Wa (high waters at lowland), wA (low waters at upland) and wa (low waters at lowland). Pairwise comparisons were performed through the technique of Tukey's Honest Significant Difference. End points of the 95 % CI in addition to the *p*-values adjusted for multiple comparisons are reported. Significance at the 0.05 (*) and 0.01 (**) levels are indicated.

Pairwise comparisons	Differences in mean	Lower	Upper	<i>p</i> -adj
Species richness				
Wa-WA	1.6667	-2.2126	5.5459	0.6572
wA-WA	3.6667	0.0379	7.2954	0.0469**
wa-WA	4.3667	0.5856	8.1477	0.0183**
wA-Wa	2.0000	-1.6287	5.6287	0.4572
wa-Wa	2.7000	-1.0810	6.4810	0.2365
wa-wA	0.7000	-2.8235	4.2235	0.9499
Functional diversity				
Wa-WA	-0.0434	-0.0762	-0.0106	0.0055*
wA-WA	-0.0008	-0.0315	0.0299	0.9999
wa-WA	-0.0045	-0.0365	0.0274	0.9807
wA-Wa	0.0426	0.0119	0.0733	0.0034*
wa-Wa	0.0389	0.0069	0.0709	0.0119**
wa-wA	-0.0037	-0.0335	0.0261	0.9866

cluster G3 (i.e. *Chimarra*, *Polycentropus*, *Cailloma*, *Hydrobiosidae*, *Atopsyche*) comprises large-sized organisms (size > 20 mm) characterized by cylindrical and soft bodies, which are crawlers with tegumentary respiration and provided of specializations that prevent individuals from being easily drifted such as silt gland and anal hooks and predators and collector-filterers with moderate tolerance to oxygen deficiency.

G4 (i.e. *Ithytrichia*, *Hydroptila*, *Hydroptilidae*, *Metri- chia*, *Oxyethira*) is composed of small sized organisms with moderate tolerance to oxygen deficiency, they are collector-gatherers and have bivalve cases of silk. G5 and G6 are characterized by medium-sized organisms, collector-gatherers and scrapers, provided of cylindrical body and mineral material-case. These latter groups differ in the degree of tolerance to oxygen de-

iciency taxa of G5 (i.e. Glossosomatidae, *Protophila*, *Neotrichia*, *Mexitrichia*, *Mortoniella*) have moderate tolerance whereas those of G6 (i.e. *Helicopsyche*, *Marilia*, *Nectopsyche*, *Oecetis*) have low tolerance to oxygen deficiency.

Table 4 displays all the values of FD and taxonomic richness calculated for the entire set of sampling units in each site. The lack of correlation between FD and taxonomic richness values for sites grouped by flow regimes (low water: $r = -0.1343$, $p > 0.1$; high water: $r = -0.3091$, $p > 0.1$) suggests that these measures entail different contents of information. The joint display of boxplots and bubble maps in Fig. 3 highlights this differential behavior. The taxonomic richness (Fig. 3a) and the FD (Fig. 3b) are not comparable in terms of their performance since they exhibit contrasting behavior throughout the basin. In general terms, both measures are modulated by the simultaneous action of time and space through different directions of influence: 1) taxonomic richness varies greatly according to seasonality in both upland (SM, GA) and lowland sites (SJ, LJ3); 2) FD is sensitive to the flow regime only at the lower altitude (DU, ME, PT, LJ2). As remarkable singularities we refer to the sites SJ, SI and CI. The behavior of the diversity measures in the SJ site is decoupled from that behavior recorded at the neighboring SI site. The CI site is the only one that showed a conspicuous reduction ($> 50\%$) in their FD values during the period of high water.

We found significant differences in the diversity measures among the various spatio-temporal levels of the influence factor. The respective ANOVAs for taxonomic richness and FD are summarized in Table 5. Interestingly, the pairwise comparisons via Tukey's HSD (Table 6) revealed a complementary rather than a redundant pattern of statistically significant differences ($p < 0.05$) between the effects of the influence factor (Fig. 4).

Discussion

It is commonly assumed that changes in species richness lead to changes in FD, but the relationship between them remains largely unknown for most ecological systems (Cadotte et al. 2011). The correlation between taxonomic richness and FD ranges theoretically from negligible to a one-to-one relationship. A debate exists about the redundancy or complementarity of these quantitative surrogates of diversity. Our results support the latter and allow us to emphasize the spatio-temporal mismatch between both indexes.

While the taxonomic richness measured at periods of low waters differ from that measured in upland sites during spates, the FD enables us to differentiate lowland sites sampled during spates against the remaining spatio-temporal pools of sites. One exception to this complementarity already stated is that both indexes showed no significant response to altitude during the period of low waters. Thus FD may fluctuate fairly independently of taxonomic richness. This pattern could be seen if functionally unique species colonize an area following loss of functionally redundant species, or *vice versa* (Mayfield et al. 2010). Ultimately, complementarity means here that the replacement in taxonomic composition is a process decoupled from the replacement in trait composition, a point also made by Bêche et al. (2006) following a different analytical strategy in Californian streams. Our finding has implications for conservation policies since the disparate behavior of both measures would result in different priorities being set. Using high-resolution bird abundance data in France, Devictor et al. (2010) showed that there were significant spatial mismatches in the distribution of FD and species richness. Further, they found that existing reserves seem to protect many of the most species rich sites, but high FD sites were actually under represented. Future reserves should attempt to maximize the protection of FD. There is evidence that FD is under even greater threat from human activities than is species richness. In a study of the effects of land-use intensification on species richness and FD, agricultural development caused significant reductions in both measures of diversity, but FD declined the most (Flynn et al. 2009). Similarly, Biswas & Mallik (2010) reported that FD of plant communities dropped when the system moved from moderately to greatly disturbance scenarios despite an increase in richness.

In general terms, the spatio-temporal pattern of species richness is characterized by homogeneity along the altitudinal gradient regardless of the flow regime, thus we cannot separate sites at higher altitude from those located at lower altitude when the component of flow regime is fixed. In contrast, taxonomic richness showed changes between successive periods of flow regime highlighting the effect of rainfall in the landscape. Richness is lower in periods of spates, probably due to the disruptive impact of floods that cause substratum instability (Gordon et al. 2004). The profile of FD values reflects the opposite influence of temporal and spatial components of variability. The higher the altitude of the basin the higher the recorded values of FD. Furthermore, FD at higher altitude does not re-

spond to seasonality. This pattern does not follow for the sites sampled at lower altitude where instead the FD varies as a function of the flow regime. Lowland sites are very interesting since they yielded the lowest scores of FD when sampled during spates, a final outcome not reflected by the scores of taxonomic richness. The riparian area of these sites has been greatly impacted due to the over grazing of cattle and the invasion of exotic vegetation (Mesa 2010). We suspect that this anthropogenic impact translates into a magnified effect on the EPT group in the rainy season. This effect consists of an increased transport of particulate material from the surroundings leading to a uniformity of habitat and, consequently, to the dominance of a few traits such as collector-gatherer groups with a moderate to high tolerance to oxygen deficiency. Interestingly, Gordon et al. (2004) found this feeding strategy related to the change in land use and increased run-off and detritus transport. From a theoretical point of view, we suspect that FD decreases with increasing environmental constraints or stress (Statzner et al. 2004, Mouillot et al. 2006). When environmental constraints increase, coexisting species are more likely to be similar to each other because environmental conditions (i.e. abiotic properties of the habitat) act as a filter allowing only a narrow spectrum of species to survive. We hypothesize that the modification of the riparian forest is the main environmental stress causing functional uniformity in the EPT assemblages.

There is a limiting factor for the achievement of accurate estimation of FD, namely the right definition of traits for the organisms collected in the working area. Knowledge of the ecology and biology of Neotropical aquatic insects remains largely incomplete. For this region, the functional approach has been highly influenced by the work performed on temperate zones of Northern hemisphere. Consequently, traits defined for taxa from outside the Neotropics (e.g. Tachet et al. 2002) are used in functional analyses. However, this practice should be discouraged given the purported mismatch between traits exhibited by taxa occurring in different zones (Tomanova et al 2008). An important contribution of our work was the compilation of traits empirically defined for the EPT taxa inhabiting mountain streams of a Neotropical basin.

Conclusion

Neotropical freshwater systems harbor an astonishing faunal diversity. Political decisions oriented to protect them are urgent and necessary, but such decisions

should be based on the grounds of integral programs of assessment that go beyond the emphasis on taxonomic richness. The conservation of ecological processes is also of great concern and FD seems to be the best proxy to evaluate them. We suggest the implementation of an integrative approach of diversity prior to the delineation of priority areas. Certainly, the obstacle to carrying out this conservation strategy relies on the knowledge we have about the biological attributes of taxa. Although some progress has been made on the systematic knowledge of benthic taxa for the region, much effort is still necessary to compile information on life histories, physiological responses to environmental stress and use of resources in order to estimate more accurately the functional diversity of Neotropical running waters. Our work contributes towards advances in these final guidelines and will help to understand better the patterns of functional diversity in these relatively little known systems.

Acknowledgments

We are grateful to CONICET and ANPCyT for financial supporting via postdoctoral grants and projects PIP-CONICET 11220110100330, PICT-2012-1067 and PICT-2012-1910. We also express gratitude to all the taxonomists of our work lab (IBN, UNT) for their assistance in sampling and identification of taxa. We would also like to thank Anne Robertson (handling editor) and two anonymous reviewers for the comments and suggestions that greatly improve the structure of our manuscript.

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Submitted: 30 April 2013; accepted: 3 September 2013.

Appendix 1. R script that calculates the dissimilarity between two profiles of affinity associated to a fuzzy-coded ordinal variable.

The following function calculates the dissimilarity measure between two profiles of affinity associated to a fuzzy-coded ordinal variable. The inputs should be two vectors of identical length and could represent, for instance, the frequency of occurrence of each variable attribute for the variable under consideration within the respective item of analysis.

```
ordiss <- function(item1, item2){
  stopifnot(length(item1) == length(item2))
  n <- length(item1)
  item1 <- item1/sum(item1)
  item2 <- item2/sum(item2)
  prop <- matrix(0, n, n)
  aux1 <- item1
  for(j in 1:n){
    aux2 <- item2[j]
    for(i in 1:n){
      prop[i,j] <- pmin(aux1[i], aux2)
      aux2 <- aux2 - prop[i, j]
    }
    aux1 <- aux1 - prop[,j]
  }
  idx <- sum(prop*abs(row(matrix(0,n,n))- col(matrix(0,n,n))))/(n-1)
  return(idx)
}
```

Example. We will consider a variable consisting of five levels. There are two items called Pop1 and Pop2 with the following frequency distributions across the respective attributes:

```
Pop1 <- c(10, 0, 0, 5, 4)
Pop2 <- c(5, 1, 3, 10, 2)
ordiss(Pop1, Pop2)
```

