

# Correspondence between stream benthic macroinvertebrate assemblages and ecoregions in northwestern Argentina

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**Abstract:** The use of ecoregions to classify stream and river environments has been extensively tested in North America and Europe, but few such studies have been conducted in South America. In this study we tested whether taxonomic richness, composition, and organism abundance within benthic macroinvertebrate assemblages were associated with ecoregions in northwestern Argentina at the genus and family levels. We included 3 ecoregions and their respective subcoregions in this study: the Yungas subtropical cloud forest, the Western Chaco subtropical dry forest, and the Monte xeric shrublands. We used nonmetric multidimensional scaling, analysis of similarity, and rank–abundance curves to assess how assemblages varied among ecoregions and subcoregions. We used principal components analysis to describe how environmental factors varied among sites and regions. Most aspects of invertebrate assemblages were associated with both ecoregions and subcoregions. The structure of the macroinvertebrate assemblages was generally concordant with ecoregional classification at the genus level, although concordance was not evident at the subcoregion level of resolution, especially for family-level data. The segregation of assemblages was most strongly related to environmental variables associated with topography and less strongly related to physiochemical variables. Our results confirm that ecoregions may effectively predict the invertebrate biota inhabiting streams in northwestern Argentina, but it was difficult to delineate discrete assemblages. Future work should assess the effectiveness of modeling approaches that would better account for the gradual changes in assemblage composition that occur along environmental gradients and test how well both classification and modeling approaches partition biotic variation in other parts of South America.

**Key words:** ecoregion, benthic macroinvertebrates, stream, classification scheme, South America, Argentina

In recent decades, there has been an increasing interest in classifying freshwater ecosystems into water body types. Ideally, these classification schemes would allow researchers and managers to infer the environmental conditions and biota expected at specific individual water bodies based on their type. This type of classification is a prerequisite for assessing whether human activity has altered ecosystems, because assemblages can exhibit marked natural variability (Gibson et al. 1996). Some bioassessment methods do not depend on a classification approach but instead model how biota naturally vary across continuous environmental gradients (e.g., Hawkins et al. 2010, Bailey et al. 2014, Chessman 2014, Reynoldson et al. 2014). However, landscape and river type classifications are still being tested in different regions. For example, in Europe there has been a renewed interest

in the regionalization of aquatic ecosystems after the publication of the Water Framework Directive (WFD) (WFD2000/60/EC; European Commission 2000). Accordingly, many classifications have been used to test the concordance between landscape attributes and the structural and functional aspects of biological communities in Europe (Verdonschot and Nijboer 2004, Ferréol et al. 2005, Verdonschot 2006). The approach proposed by the WFD recognizes 2 systems for river classification for water bodies in Europe, based on the ecoregions proposed by Illies and Botosaneanu (1963) and Illies (1978). Furthermore, a recent conceptual framework posits that biomes (ecoregions) provide a meaningful way of understanding how lotic ecosystem structure and function vary across macroscale spatial scales (Dodds et al. 2015). Ecoregions are contiguous landforms that have similar ge-

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ology, soil, vegetation cover, and climate within their boundaries, and they are therefore expected to contain similar communities (Omernik 1987, Omernik and Griffith 1991).

It is especially important that we develop effective classification systems for macroinvertebrate assemblages, given their ecological importance. Aquatic macroinvertebrates are widely used to understand distributional patterns across spatial scales (Johnson et al. 2007) and are also used extensively as indicators of the biological quality of freshwater ecosystems (Resh et al. 1995). Further, macroinvertebrates play an important role in freshwater ecosystem functioning by cycling nutrients, processing organic matter, and providing food to higher trophic levels.

Many stream classification schemes based on ecoregions have been tested, but these tests have primarily been conducted in North America and Europe (e.g., Gerritsen et al. 2000, Johnson 2000, Oswood et al. 2000, Pan et al. 2000, Sandin and Johnson 2000, Van Sickle and Hughes 2000). Some studies have detected concordance between benthic macroinvertebrate assemblages and ecoregions in different states of the United States (Feminella 2000, Rabeni and Doisy 2000, Waite et al. 2000). Marchant et al. (2000) obtained similar results in Victoria, Australia. In a synthesis paper, Hawkins et al. (2000) concluded that significant biotic variation among stream sites can be associated with ecoregions, espe-

cially where they differ markedly in topography (e.g., mountains vs lowlands or plains). However, some studies have found no significant concordance between ecoregions and benthic invertebrates (e.g., the United States: Hawkins and Vinson 2000, Sweden: Sandin and Johnson 2000). It is important to understand why the associations among ecoregions and macroinvertebrates are strong in some places and weak in others. In some situations, measures of local habitat features may be necessary to allow accurate predictions of freshwater fauna composition (Hawkins et al. 2000). More generally, recent evidence has shown that modeling can outperform discrete classifications in terms of the accuracy and precision of predicting taxonomic composition (Hawkins et al. 2010, Johnson and Hallstan 2018). Importantly, associations between freshwater communities and landscape units are still poorly studied in South America, and these landscapes may provide additional insights into the conditions under which regional classification schemes are useful.

To date, a variety of stream classification systems have been evaluated in South America. Most of these studies have been done in Brazil. One study found that ecoregions had higher classification strengths for fish assemblages in the Paraíba do Sul River than did other classification systems (Pinto et al. 2009). In contrast, another study observed higher

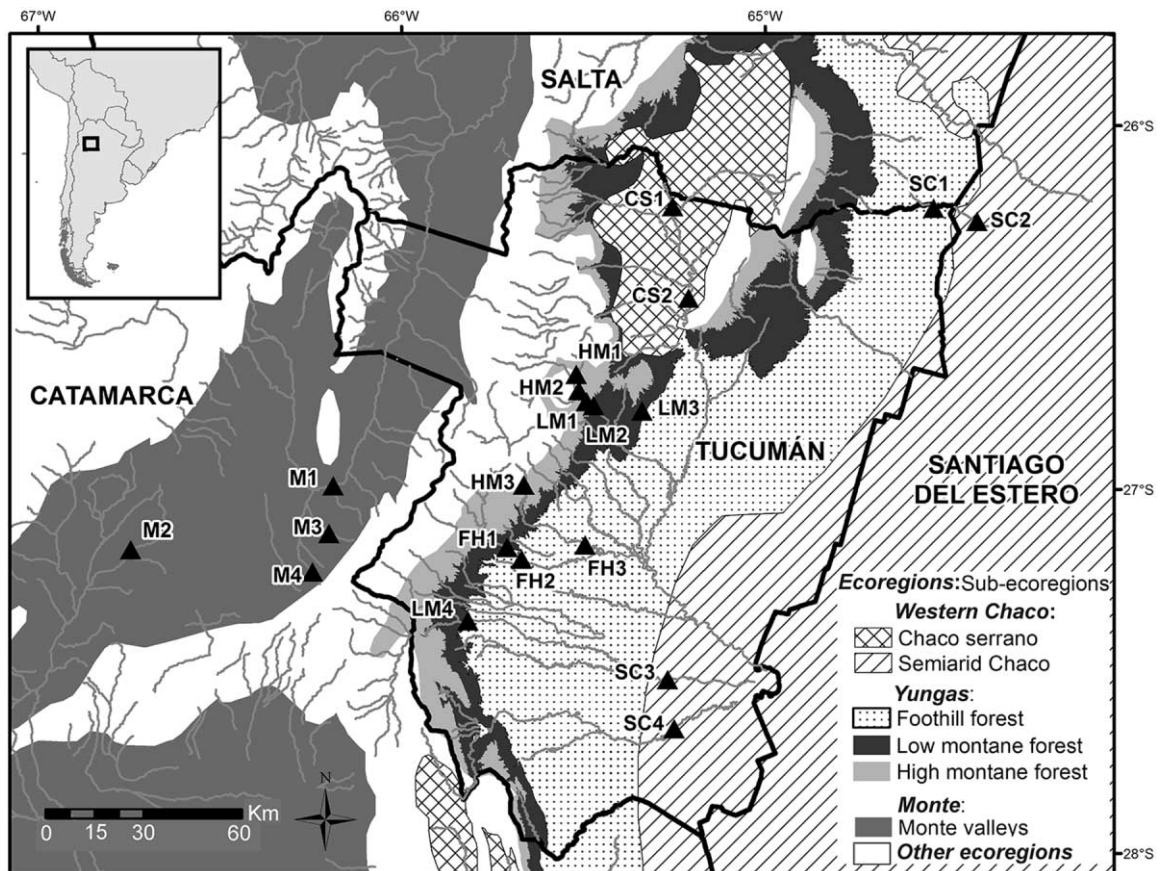


Figure 1. Study area and sampling site locations.

classification strength values for macroinvertebrate assemblages and for a classification derived from a select set of local environmental variables than for fish assemblages and other classifications, such as ecoregions and hydro-ecoregions in Rio Grande do Sul (Vasconcelos et al. 2013). In a recent study, macroinvertebrate assemblages were shown to vary among 3 landscape units defined by geomorphologic features within the Brazilian Cerrado ecoregion (Martins et al. 2017). In northwestern Argentina, the only studies of associations between biota and ecoregions have been conducted on terrestrial plant assemblages (Cabrera 1976, Burkart et al. 1999, Brown and Pacheco 2006). In this study, we tested how strongly benthic macroinvertebrate assemblages were associated with ecoregions and stream environmental features in northwestern Argentina. We addressed 4 questions: How do the taxonomic richness, composition, and organism abundance of benthic macroinvertebrate assemblages vary among streams that flow through different ecoregions and subecoregions? Do macroinvertebrate assemblages segregate into discrete groups by ecoregions? How do local environmental features vary among ecoregions and subecoregions? Which local abiotic features are most strongly associated with biotic variation?

## METHODS

### Study area

Northwestern Argentina (lat 22°–30°S, long 68°–62°W) covers a wide area of heterogeneous landscape that contains diverse ecosystems including deserts, xeric shrublands, mountain rain forests, dry forests, and grasslands. This region is made up of 5 ecoregions: Puna, High Andes grassland and tundra, Monte xeric shrublands, Yungas subtropical cloud forest, and Western Chaco subtropical dry forest (Brown and Pacheco 2006). In this study, we sampled streams in 3 of those ecoregions: Monte, Yungas forest, and Western Chaco (Fig. 1). Our study areas occur in 3 provinces (Catamarca, Santiago del Estero, and Tucumán) and span 2° of latitude and longitude (lat 26°–28°S, long 66°–64°W) (Fig. 1).

The Monte ecoregion is a desert that includes xeric shrublands and extends north to south across sub-Andean dry valleys of central and northwestern Argentina. The mean annual temperature is 17.5°C, and annual rainfall varies between 100 and 400 mm, with only summer thundershowers and scarce winter precipitation (Burkart et al. 1999). The dominant plants are bushes and small trees. The Monte ecoregion contains 2 subecoregions: Monte valleys and Monte plains (Brown and Pacheco 2006). Only the Monte valleys subecoregion is located in northwestern Argentina, and it occurs between 500 and 3500 m asl.

The Yungas subtropical cloud forest (Yungas forest) is a narrow belt of mountain rainforest that ranges from 400 to >3000 m asl (Brown 2000). The Yungas forest is part of a long chain of mountain cloud forests that extends along the east side of the Andes Mountains of South America

from Venezuela to northwestern Argentina. The climate is warm and humid, with mean annual temperatures ranging from 14 to 26°C and rainfall from 1000 to 2500 mm (Hueck 1978). The Yungas forest is stratified into 3 vegetation floors, or bands. In general, Yungas altitudinal floors are not considered subecoregion units, but in this study we evaluated them as differentiated units within the Yungas forest. The high montane forest (1500–3000 m asl) contains monospecific tree stands that are usually either *Alnus acuminata* or *Podocarpus parlatorei*. Rainfall reaches 1000 mm. The low montane forest (700–1500 m asl) has the most diverse vegetation, with many evergreen species, and is dominated by *Cinamomum porphyrium* and *Blepharocalyx salicifolius*. The low montane forest also has the highest precipitation (2000 mm annually) and the least seasonal hydrological regime. The foothill forest (400–700 m asl) contains deciduous trees and is dominated by *Tipuana tipu* and *Enterolobium contortisiliquum*. The annual rainfall varies between 1000 and 1500 mm during the wet season, and the 6-month dry season ( $\leq 50$  mm of rainfall) extends from June to November (Brown et al. 2001).

The Western Chaco ecoregion is a vast sedimentary fluvial plain formed by the streams or rivers that run northwest to southeast, and it includes parts of northwestern Argentina, southeastern Bolivia, northwestern Paraguay, and southwestern Brazil. The headwaters are located in the mountains, outside of the region, and transport great quantities of sediments into the region. Mean annual temperatures range between 19 and 24°C. Mean annual rainfall varies between 400 and 900 mm, with most precipitation falling in the summer and little falling in the winter (Minetti 1999). The vegetation is composed of dry forests and segregated grasslands. This ecoregion is made up of 3 subecoregions: Arid Chaco, Semiarid Chaco, and Chaco Serrano (Brown and Pacheco 2006). Only the latter 2 are represented in the study area. The Chaco Serrano is part of the western limit of the ecoregion and is characterized by low mountain topography. It is bordered in some places by the Yungas forest or Monte ecoregions. The Semiarid Chaco occupies the greater portion of the ecoregion and is a continuous xerophytic and semideciduous forest. A wide transition zone occurs between the Western Chaco and the Yungas forest, which includes species common in both ecoregions.

### Survey design and methods

We studied 20 sites (Fig. 1, Table S1). Sites were distributed across ecoregions and subecoregions as follows: 4 in the Monte (M), 10 in the Yungas subtropical cloud forest (3 in high montane [HM], 4 in low montane [LM], and 3 in foothill forests [FH]), and 6 in the Western Chaco (2 in Chaco Serrano [CS] and 4 in Semiarid Chaco [SC]). Each site consisted of a stream reach ~100 m long. We chose sites that were minimally disturbed, without indus-



trial impact, and with native riparian vegetation at least 100 m wide.

Data from 10 of the 20 sites (HM3, LM3, FH1, FH2, CS1, CS2, SC1, SC2, SC3, and SC4) were collected between 2014 and 2016 by the authors. Data for the 10 other sites (HM1, HM2, LM1, LM2, LM4, FH3, M1, M2, M3, and M4) were obtained from the IBN (Neotropical Biodiversity Institute, National Council of Technological and Scientific Research, National University of Tucumán) database. The IBN sites were sampled between 2005 and 2007 following the same collection procedures. Climate conditions were similar during these 2 periods according to local climate databases, and both periods corresponded to the Southern Oscillation phase of El Niño according to the Oceanic Niño Index ([http://www.cpc.noaa.gov/products/analysis\\_monitoring/ensostuff/ensoyears.shtml](http://www.cpc.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.shtml)). In addition, previous studies in the region observed that the macroinvertebrate assemblage composition and structure change seasonally rather than annually (Mesa et al. 2009, Mesa 2012). All sites were sampled once at the end of the low-water period (October–December) and once at the end of the high-water period (March–June), with the exception of 4 sites that were sampled only during the low-water period (LM4, FH3, SC3, and SC4).

*Benthic macroinvertebrates.* At each site we collected quantitative and qualitative samples. Three quantitative samples were collected with a Surber net (0.09-m<sup>2</sup> area with a 300- $\mu$ m mesh) and were subsequently pooled into a single composite sample. We took these samples in fast-water habitat units (riffles or runs, sensu Hawkins et al. 1993) that were separated by 50 m along a longitudinal transect. The qualitative samples consisted of samples collected with a D-frame net (300- $\mu$ m mesh) or with a kick-net (500- $\mu$ m mesh) or by manual sampling. Manual sampling included directly picking specimens from boulders, cobbles, leaves, and algae. The qualitative sampling took ~30 min to cover all habitats. Riffles, pools, and marginal vegetation habitats were most common. We did not collect quantitative data in sites SC3 and SC4 and did not use these sites in abundance analyses. However, we used these sites in the presence–absence analyses because they included qualitative data collected from all habitats. Quantitative data were used to analyze abundance patterns, and the combined quantitative and qualitative data were used to analyze presence–absence data. We brought all samples to the lab after collection, where we processed and identified each entire sample. Macroinvertebrates from all samples were identified by the same group of taxonomists.

*Environmental variables.* We characterized the environmental setting at each site to determine whether sites differed in habitat features within and among ecoregions and subcoregions. We recorded altitude (m asl) with a

Garmin eTrex 20™ global positioning system (Olathe, Kansas). Channel width (m), discharge (m<sup>3</sup>/s), sediment size (mean diameter, cm), stream power (W/m), water temperature (°C), pH, conductivity ( $\mu$ S/cm), turbidity (NTU), and dissolved oxygen (mg/L) were recorded at every visit. We estimated discharge by measuring cross-sectional area, taking depth measurements every 25 cm (for streams  $\leq$ 11 m wide) or 1 m (for rivers  $\geq$ 11 m wide) along 1 cross-sectional transect across the channel, and measuring velocity with a velocity meter at 2/3 the depth at each point (Global Water Flow Probe FP111, College Station, Texas). To estimate average sediment grain size at each site, we measured 20–130 clasts that were  $>$ 2 mm in diameter in a cross section of the fluvial bar close to the channel where we took the invertebrate samples. The sediment grain size deposited at a mid-central fluvial bar is related to the slope and discharge and hence stream power (Bridge and Demicco 2008). Stream power was estimated from the formula given by Gordon et al. (2004):  $W = \rho g Q S$ , where  $W$  is power in Watts,  $Q$  is discharge (m<sup>3</sup>/s),  $S$  is the stream slope (m/m) obtained from a digital elevation map (ASTER DEM 30  $\times$  30 m resolution),  $\rho$  is the density of water (kg/m<sup>3</sup>), and  $g$  is the acceleration due to gravity (m/s<sup>2</sup>). Physiochemical variables were measured with a Horiba™ multiprobe water quality checker U-50 series (Kyoto, Japan).

## Data analysis

*Taxonomic richness (TR) and resolution.* We conducted the analyses at 2 target taxonomic levels of resolution: genus and family (see Table S2 for a list of all taxa). We identified individuals based on the regional keys of Domínguez and Fernández (2009). When possible, individuals of Ephemeroptera, Plecoptera, Trichoptera, Megaloptera, Lepidoptera, Coleoptera (Elmidae), and Mollusca were identified to genus level. Individuals of Crustacea, Odonata, Diptera, and the rest of Coleoptera were identified at family level. The latter groups were used in family-level analyses but not genus-level analyses. Representative individuals of Hydracarina, Nematoda, Platyhelminthes, and Annelida were not included in the analyses because they could not be identified to family.

We used the quantitative data to estimate the mean and 95% confidence intervals (CIs) for genus and family richness at each sampled site, subcoregion, and ecoregion. We accounted for multiple comparisons with Bonferroni corrections (Scheiner and Gurevitch 1993). Nonoverlapping CIs were considered to represent statistically significant differences among treatments (Cumming et al. 2007, MacGregor-Fors and Payton 2013). Prior to these tests, however, we ensured that our samples had the same coverage (Chao and Jost 2012), because this is necessary to make ecologically appropriate comparisons of TR. Same coverage is a measure of sample completeness that indicates sampling coverage relative to the estimated TR at the site (Chao and Jost 2012). We used the

R package *iNEXT* (version 2.0.12; Hsieh et al. 2016) (R version 3.3.0; R Foundation for Statistical Computing, Vienna, Austria) to evaluate the completeness of same coverage for each site within ecoregions and subcoregions, following the protocol for comparison of multiple samples proposed by Chao and Jost (2012). The rarefaction curve used by this method is  $1 - f_1/N$ , where  $f_1$  is the number of singletons and  $N$  is the total number of individuals in the sample. Thus, the completeness is given by the number of individuals that occur in the sample as singletons, and sample coverage can be reduced so the coverage between samples is equivalent.

**Dissimilarity.** We used the Sørensen index and the positive matching index (PMI, Dos Santos and Deutsch 2010) to analyze the presence–absence data. We used the Bray–Curtis and Dissim indices (Nieto et al. 2017) to estimate compositional dissimilarity between macroinvertebrate assemblages based on our abundance data. The PMI can vary between 0 and 1 and represents the mean proportion of positive matches relative to the complete list of taxa that could occur at a site. The PMI covers the range of richness encompassed by the 2 lists, i.e., the smaller and longer ones (Dos Santos and Deutsch 2010). Hence, if 2 lists of different lengths are compared, e.g., of 10 and 100 specimens, and the PMI is 0.3, that result indicates that the 2 lists share 30% of taxa, on average, given that the list sizes range from the smaller one to the longer one (Dos Santos and Deutsch 2010). In contrast, Euclidean and Bray–Curtis distances are 2 dissimilarity indices that are frequently used in ecological analyses (Nollet and De Gelder 2014). However, both of these indices are strongly influenced by dominant species and are only weakly affected by rare species (Valentin 2012), and they are therefore not as useful when there are gradual changes in composition along a gradient. The Dissim index can be used when the observed taxa are assumed to have been sampled from a common regional pool of species. The Dissim index assesses whether assemblages are similar based on both the taxa present and their abundance. Thus, 2 sites would be considered more similar if they grouped consistently near each other after successive orderings of sites by increasing values of consecutive taxa abundances (Nieto et al. 2017).

We used analysis of similarities (ANOSIM) (Legendre and Legendre 1998) to determine whether site taxonomic composition differed statistically among ecoregional and subcoregional classifications based on genus-level resolution, family-level resolution, presence–absence data, and abundance data. We also used multivariate analyses to determine whether differences in assemblage composition among sites were associated with regional classifications. We used non-metric multidimensional scaling (NMDS) based on dissimilarity values obtained from presence–absence and abundance data to visualize whether the positions of sites in taxa space were concordant with ecoregional and subcoregional classifications. We interpreted how discrete the ecoregions and

subcoregions were by drawing a convex polygon around each group of subcoregion on the NMDS plot. These polygons were based on whichever classification method had the highest ANOSIM value. We considered NMDS and ANOSIM to be complementary analyses.

It is well known that benthic macroinvertebrate assemblages can vary markedly with season (Minshall 1988, Poff and Ward 1989). We, therefore, separated the data by low- and high-water periods to verify that the differences among ecoregions and subcoregions were greater than the seasonal differences within each site. In addition, to test whether ecoregional and subcoregional differences in the fauna apply to both large and small streams, we assigned sites to 1 of 2 groups, small streams (<11-m mean width) and large streams (>11-m mean width), and we analyzed each group separately.

**Composition and structure of benthic macroinvertebrate assemblages.** We used rank–abundance (RA) curves (also known as dominance–diversity curves) to compare how assemblage structure varied across the different ecoregions and subcoregions. RA curves, in combination with species identity, can provide insight into specific patterns of species diversity, dominance, rarity, and composition (e.g., Feinsinger 2001, Andresen 2005, Vidaurre et al. 2006, Cultid-Medina and Escobar 2016). We used these analyses to complement the multivariate analyses and allow more detailed observations of compositional and structural differences among assemblages. Groups of dominant taxa and taxa that occurred exclusively in each ecoregion and subcoregion were identified. The 3 most abundant taxa at each site were considered the dominant taxa for each ecoregion and subcoregion (note that some regions could have more than 3 dominant taxa).

**Environmental variables.** We used principal components analysis (PCA) to describe how physiochemical factors varied within and across ecoregions and subcoregions. We used the function *dudi.pca* in the *ade4* R package (version 1.7–8, Dray et al. 2017) to perform these analyses. The PCA was based on the average values of each variable across all sampling points at each site. In addition, we determined whether site positions along the NMDS axes were correlated (Pearson correlation coefficients) with environmental PCA axes, and we accounted for multiple comparisons with a Bonferroni correction (Scheiner and Gurevitch 1993).

## RESULTS

### Biological classification of ecoregions

**Taxonomic richness.** After the Bonferroni adjustments for multiple comparisons, our adjusted significance values were  $p < 0.01$  for genus richness (GR) and  $p < 0.003$  for family richness (FR) comparisons. GR and FR in Yungas

were the highest (GR =  $51 \pm 2.1$ , FR =  $46 \pm 4.8$ ). In addition, TR was high in Western Chaco (GR =  $39 \pm 2.0$ , FR =  $38 \pm 4.0$ ) compared with Monte (GR =  $19 \pm 0.4$ , FR =  $24 \pm 1.4$ ). At the subcoregional level, TR was highest in the foothill forest (GR =  $42 \pm 2.5$ , FR =  $36 \pm 4.4$ ), followed by Chaco Serrano (GR =  $39 \pm 2.9$ , FR =  $38 \pm 2.7$ ), low montane forest (GR =  $37 \pm 4.1$ , FR =  $34 \pm 4.2$ ), and high montane forest (GR =  $34 \pm 1.8$ , FR =  $30 \pm 3.6$ ). In contrast, TR in the Semiarid Chaco (GR =  $25 \pm 2.3$ , FR =  $30 \pm 3.9$ ) and Monte valleys (GR =  $19 \pm 0.4$ , FR =  $24 \pm 1.4$ ) were substantially lower than in the above-mentioned subcoregions.

**Dissimilarity.** ANOSIM results ( $p = 0.001$ ) showed that assemblages were significantly associated with regions at both genus and family levels. However, the family-level data set calculated with the Bray–Curtis index had an  $R$  value close to 0 (Table 1), indicating that there was little distinction among ecoregion assemblages at the family level. The genus-level analyses tended to have higher positive  $R$  values. The overall structure of the macroinvertebrate assemblages was concordant with ecoregion classification at the genus level based on either presence–absence data sets (PMI index) or abundance data sets (Dissim index) (Fig. 2). At the genus level, NMDS axis 1 segregated 2 groups: one composed of the Yungas and Chaco Serrano sites and the other composed of the Semiarid Chaco and Monte sites. NMDS axis 2 separated the Western Chaco sites from the Yungas and Monte sites. However, macroinvertebrate assemblages were less strongly associated with the subcoregion level. The seasonal differences among sites were much lower than the ecoregional and subcoregional dissimilarities. Nevertheless, the assemblages from streams in the Chaco Serrano at high-water periods and from foothill forest at low-water periods were more similar to each other than to assemblages of their respective subcoregion collected at different water levels (Fig. 2). This result was especially strong at the genus level. Results based on separate analyses of small and large streams were similar to the results from the combined data (results not shown).

**Composition and structure of benthic macroinvertebrate assemblages.** The most abundant taxa were relatively consistent across the cloud forest. At the genus level, *Baetodes* was among the most abundant taxa in all Yungas regions (Fig. 3). *Austrelmis*, *Simulium*, *Camelobaetidius*, *Leptohyphes*, *Thraulodes*, and *Andesiops* were also abundant in the high montane forest (Yungas subcoregion), whereas *Leptohyphes*, *Nanomis*, *Smicridea*, *Austrelmis*, *Simulium*, and *Farrodes* were among the dominant taxa in the low montane forest (Yungas subcoregion). In the foothill forest (Yungas subcoregion), *Austrelmis*, *Smicridea*, *Leptohyphes*, and *Simulium* were also abundant (Fig. 3). Twelve genera, mainly Ephemeroptera, were observed exclusively in the Yungas ecoregion (Table S4).

The dominant taxa varied across the 3 arid regions (Chaco Serrano, Semiarid Chaco, and Monte) (Fig. 4). In the Western Chaco subcoregions, *Americabaetis* was dominant at 3 of 4 sites. Within the Chaco Serrano, *Leptohyphes*, *Smicridea*, and *Simulium* were also dominant, whereas in the Semiarid Chaco, immature *Austrelmis*, *Smicridea*, *Heleobia*, and *Ostracoda* sp. were the most abundant taxa. We observed 8 genera, 4 of which were mollusks, exclusively in the Western Chaco (Table S4). Within the Monte, *Austrelmis*, *Andesiops*, and *Hydroptila* were among the most abundant genera. *Simulium* and *Bezzia* were also abundant in site M3. One genus, *Massartellopsis*, occurred exclusively in the Monte ecoregion (Table S4).

Some genera were widely distributed and occurred across all or most ecoregions and subcoregions (Figs 3, 4). *Americabaetis*, *Baetodes*, *Caenis*, *Camelobaetidius*, *Anacronetia*, *Austrelmis*, *Bezzia*, *Simulium*, and *Limnocoris* occurred in all regions. *Leptohyphes*, *Thraulodes*, and *Smicridea* occurred in both the Yungas and Western Chaco ecoregions and were absent from only the most arid region (Monte). An intermediate area that included the Yungas foothill forest and Chaco Serrano subcoregions occurred between the Yungas and Western Chaco ecoregions. This area appeared to be transitional, as it was inhabited by distinct mixed assemblages. These assemblages were composed of taxa found only in this transitional area, such as *Guajirolo* and *Oxyethira*, as

Table 1. Analysis of similarity (ANOSIM) statistics ( $R$  and  $p$ ) for the test of association between taxonomic composition and site groupings (ecoregion, subcoregion, and water period) for both genus and family levels. Dissimilarity values are based on presence–absence (Sørensen, positive matching index [PMI]) or abundance (Bray–Curtis, Dissim) data.

Index	Ecoregion				Subcoregion				Water period			
	Genus		Family		Genus		Family		Genus		Family	
	$R$	$p$	$R$	$p$	$R$	$p$	$R$	$p$	$R$	$p$	$R$	$p$
Sørensen	0.61	0.001	0.56	0.001	0.34	0.001	0.28	0.001	0.00	0.403	0.01	0.259
PMI	0.70	0.001	0.58	0.001	0.55	0.001	0.50	0.001	−0.01	0.441	0.01	0.284
Bray–Curtis	0.50	0.001	0.23	0.004	0.18	0.001	0.03	0.235	0.08	0.016	0.09	0.010
Dissim	0.75	0.001	0.58	0.001	0.56	0.001	0.45	0.001	0.02	0.263	0.08	0.033



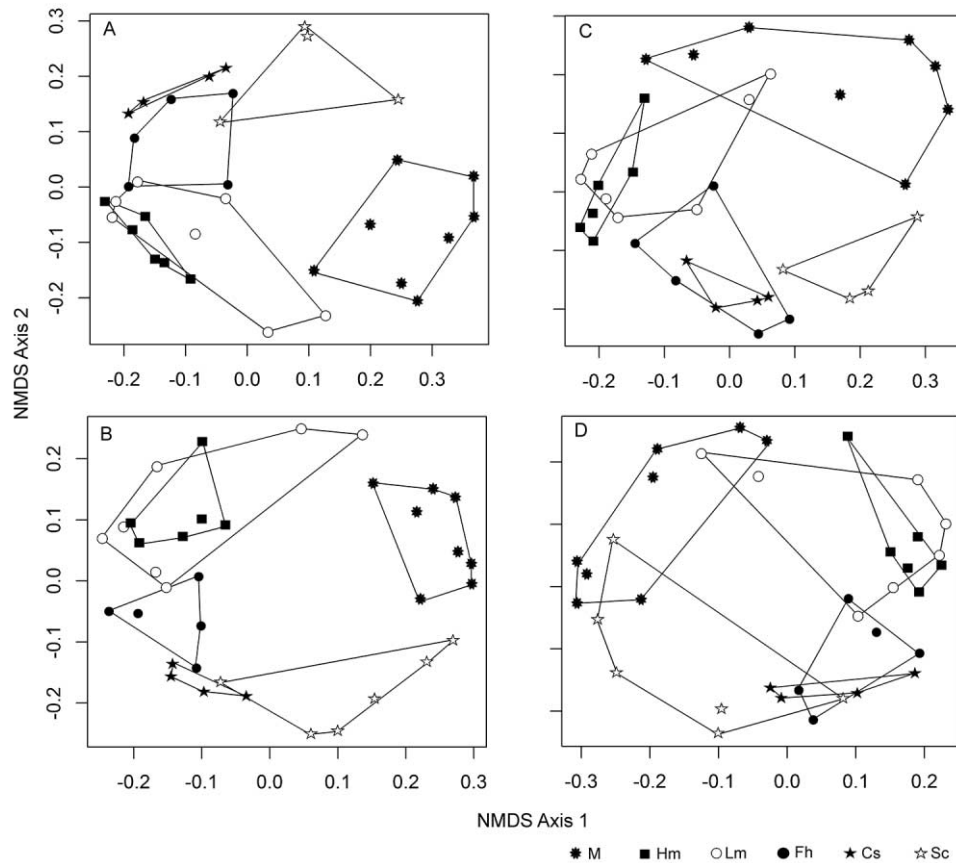


Figure 2. Nonmetric multidimensional scaling (NMDS) analyses of dissimilarity of sampling sites at the genus and family levels. A.—Genus abundance data (Dissim index), stress = 16.7. B.—Genus presence–absence data (positive matching index [PMI]), stress = 16.4. C.—Family abundance data (Dissim index), stress = 19.5. D.—Family presence–absence data (PMI), stress = 18.9.

well as others typical in either bordering ecoregion (Figs 3, 4). Many species that occurred in the Yungas were also found in the Chaco Serrano but not in the Semiarid Chaco.

At the family level (Figs 3, 4, Tables S4–S6), Chironomidae, Elmidae, and Baetidae were among the most abundant families in all ecoregions and subcoregions. Leptohiphidae and Hydropsychidae were abundant in Western Chaco, and Hydroptilidae was abundant in some Monte sites. Eight families occurred exclusively in the Yungas, 1 exclusively in the Western Chaco, and 3 exclusively in the Monte (Table S4).

### Environmental characteristics of ecoregions

Three principal components accounted for most (69.7%) of the variation among sites in physiochemical features studied (Fig. 5A, Table S2). PC1 accounted for 31.5% of total variation and was most strongly correlated with factors associated with stream size (discharge, channel width, sediment size, and altitude; Fig. 5). PC2 accounted for 24.9% of total variation and was strongly correlated with water temperature, turbidity, pH, dissolved oxygen, and stream power. PC3 accounted for 13.3% of total variation and was correlated with conductivity.

The PCA ordination revealed that sites from the same ecoregion and subcoregion were closely associated in principal component space and well segregated from one another (Fig. 5B, C). The Yungas sites had low values of water temperature, turbidity, pH, and conductivity but high values of sediment size, stream power, and dissolved oxygen. Western Chaco sites had low values for sediment size, stream power, and dissolved oxygen but high values for water temperature, turbidity, pH, and conductivity. Differences were also apparent at the subcoregional level. For example, in the Yungas high montane forest, streams had larger sediment size and higher stream power than streams in low montane and foothill forest. Conversely, foothill forest streams had higher turbidity and water temperature than high and low montane forest streams. Consequently, the Yungas altitudinal gradient was apparent in the PCA ordination. Chaco Serrano sites occupied an intermediate position between the Semiarid Chaco sites and the Yungas sites. Finally, the sites from the Monte ecoregion were clearly distinct from the other ecoregions. All the Monte sites are part of the same subcoregion (Monte valleys), but there was a great distance among them in PC space. Sites M1 and M2 were located at lower altitude and registered higher water temperature and turbidity, whereas

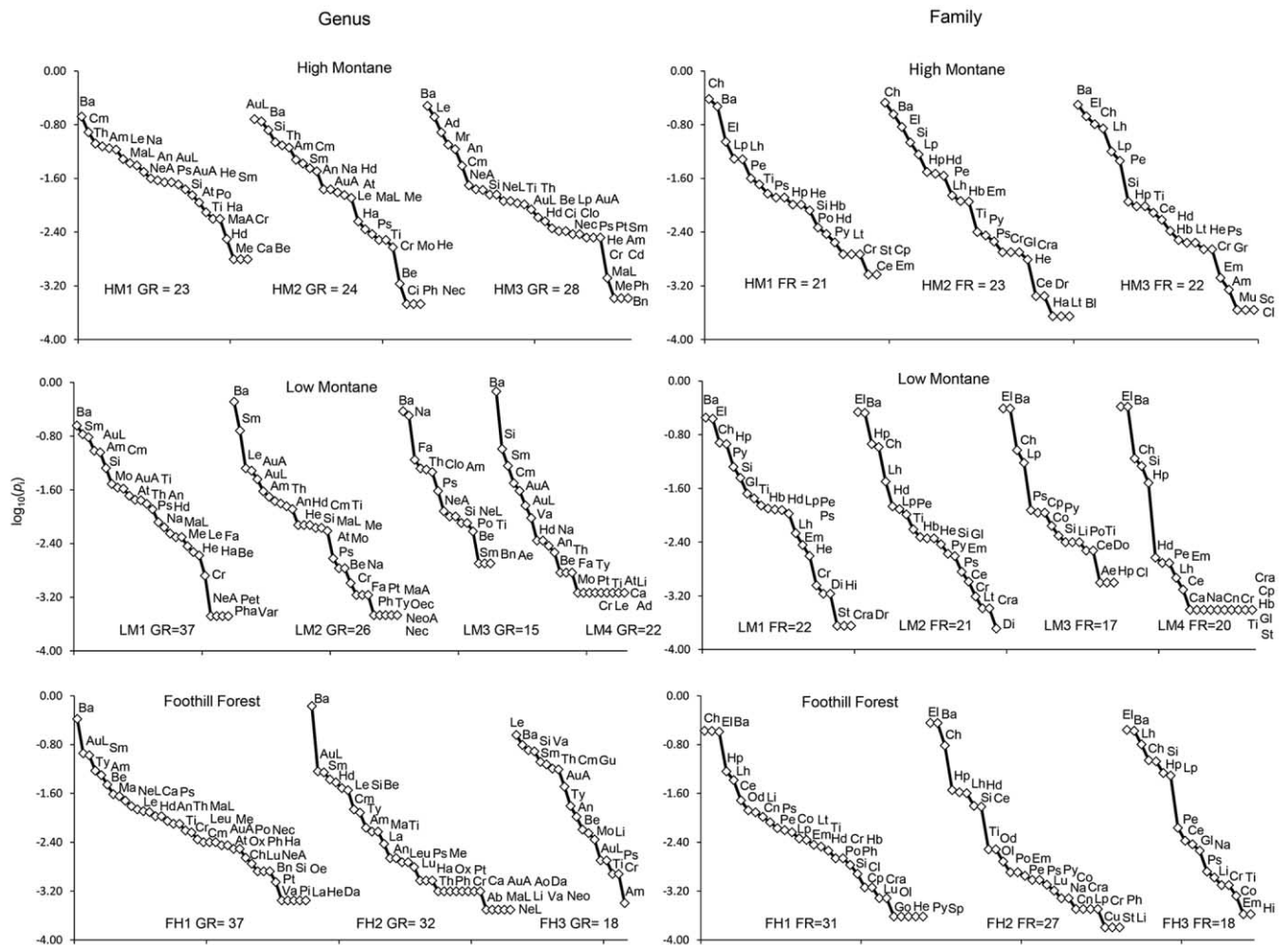


Figure 3. Rank–abundance curves for the Yungas assemblages (HM = high montane, LM = low montane, FH = foothill forest) at genus level (left panels) and family level (right panels). Abundance is expressed as  $\log_{10}(p_i)$  ( $p_i = n_i/N$ , where  $n_i$  = number of individuals of the taxa and  $N$  = total number of individuals found in the assemblage). See taxa abbreviation in Table S3.

sites M3 and M4 were situated at higher altitude and showed higher values of sediment size and stream power. Thus, there was an apparent altitudinal gradient within this arid ecoregion.

### Concordance of biological and environmental classifications

No significant correlations existed between genus TR and individual environmental variables, following adjustments for multiple comparisons (adjusted significance:  $p < 0.006$ ). Genus-based NMDS axes were significantly correlated with some PCA axes after they were adjusted for multiple comparisons (adjusted significance:  $p < 0.001$ ). For presence–absence data used with the Sørensen index, NMDS-2 was most strongly correlated with PC1 (–), but NMDS-1 based on the PMI index was strongly correlated with PC2 (–), and NMDS-2 was correlated with PC1 (+). For abundance data used with the Bray–Curtis index, NMDS-1 was most strongly correlated with PC2 (+), whereas with the Dissim index, NMDS-1 was strongly correlated with PC2 (–), and NMDS-2

was correlated with PC1 (–) (Table 2). Similar patterns occurred at the family level (Table 2). The correlations among axes showed that the segregation between the Yungas and Monte assemblages from Western Chaco was most strongly related to PC1 (discharge, channel width, sediment size, and altitude), whereas the separation of Yungas and Chaco Serrano from Semiarid Chaco and Monte was related to PC2 (water temperature, turbidity, pH, dissolved oxygen, and stream power).

### DISCUSSION

We found that taxonomic richness, composition, and organism abundance in stream benthic macroinvertebrate assemblages varied across the Argentinian ecoregions and subcoregions studied. The ecoregions were environmentally distinct, and our results show that differences in biotic distribution across these regions are related to their environmental dissimilarities. The overall structure of the macroinvertebrate assemblages was concordant with ecoregional



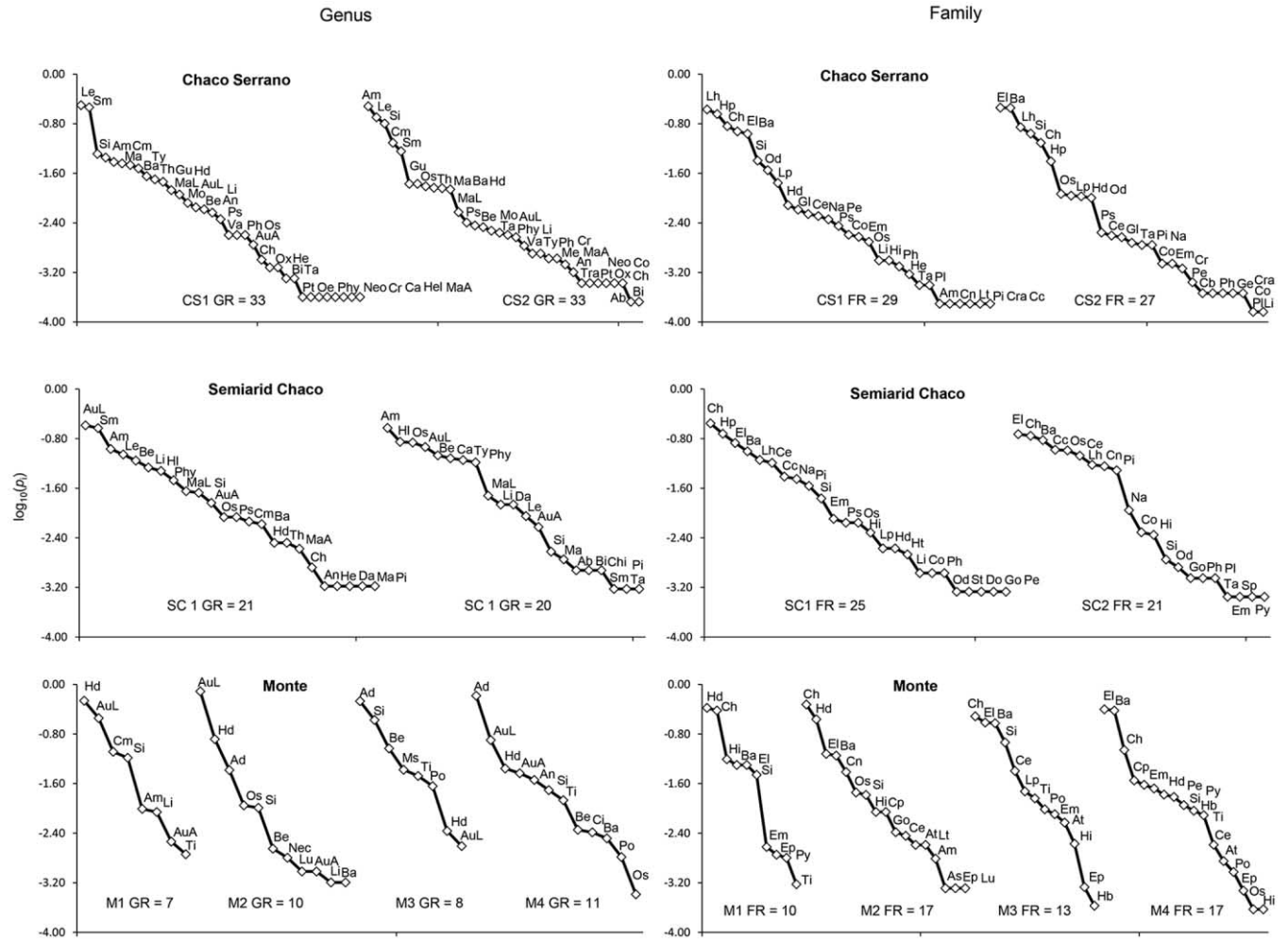


Figure 4. Rank–abundance curves of the Western Chaco (CS = Chaco Serrano, SC = Semiarid Chaco) and Monte assemblages at genus level (left panel) and family level (right panel). Abundance is expressed as  $\log_{10}(p_i)$  ( $p_i = n_i/N$ , where  $n_i$  = number of individuals of the taxa and  $N$  = total number of individuals found in the assemblage). See taxa abbreviations in Table S3.

classifications at the genus level, although discrete assemblages were not always apparent at the subcoregion level.

Our results show that segregation of assemblages among regions was most strongly related to topography and associated physiochemical variables. Many previous studies have also found this hierarchical pattern. For example, biotic variation among stream sites is higher when ecoregions have marked differences in topography (Hawkins et al. 2000). Another study analyzed macroinvertebrate data sets in relation to environmental and biogeographical variables from Europe and found 3 major stream types that corresponded with 3 major landscape types: mountains, lowlands, and Mediterranean (Sandin and Verdonschot 2006). Another study used a top-down approach to develop a stream typology based on abiotic variables in Luxembourg and determined that stream dimension, elevation, and geology were the main typological descriptors, whereas mineral and nutrient concentrations were the main variables that characterized sites within groups (Ferréol et al. 2005). Thus, in combination

with previous studies, our results provide support for landscape topography as a key component in classifying assemblage structure. In addition, our results suggest that this framework, which has primarily been applied in North America and Europe, may extend to macroinvertebrates in South America.

Some ecoregions may be more likely to harbor distinct macroinvertebrate assemblages than others. Our results indicate that distinct macroinvertebrate assemblages are most likely when ecoregions are delineated based on biome and when ecoregions differ in terms of a strong environmental gradient such as elevation. On one hand, differences in richness among ecoregions could be more pronounced when ecoregions correspond with different biomes, given we observed a higher richness in rain forest in comparison with more arid regions. Analysis of several biomes has consistently suggested that, in general, forests have the highest freshwater vertebrate and invertebrate richness and endemism, followed by grasslands and then deserts (Vinson

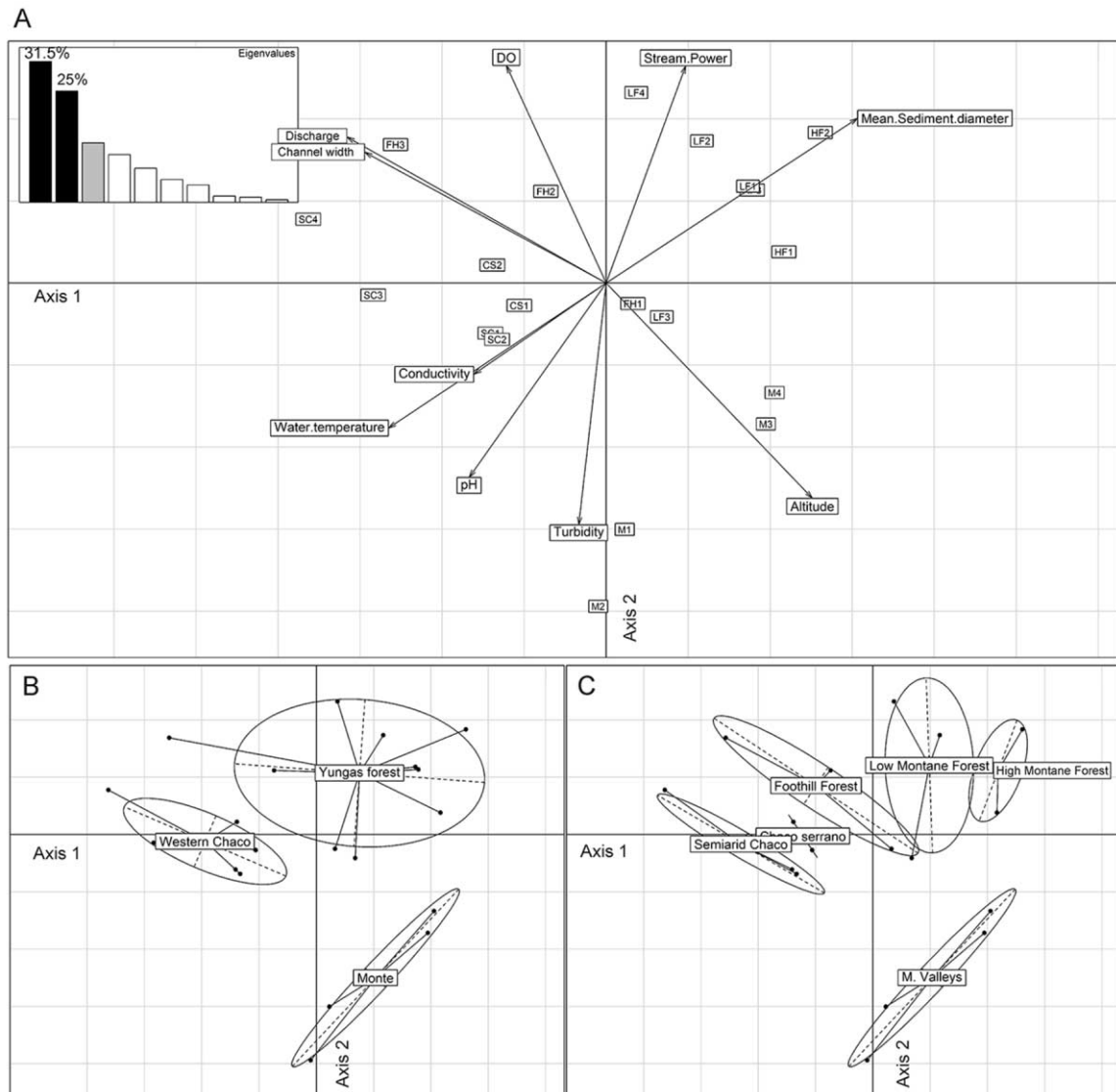


Figure 5. Principal components analysis (PCA) ordination of environmental variables measured at the 20 sampling sites. A.—PCA biplot of environmental variables and sampled sites, with the inset showing the bar plot of eigenvalues. The 95% confidence ellipses are shown for ecoregions (B) and subcoregions (C).

and Hawkins 2003, Dodds et al. 2015). On the other hand, ecoregions could have distinct assemblages if they contain distinct habitats, which would foster unique assemblages.

In our study area, topography varied throughout the South American biogeographical transition zone (Morrone 2014). Thus, both drivers of assemblage composition could act synergistically. Besides the environmental differences that occur among ecoregions, ecoregions also can have different biogeographical histories that could explain some biotic variation (Morrone 2014). Biogeographically, some taxa could be associated with the high Andean regions (including the Monte ecoregion), whereas some taxa are probably associated with the Amazon regions (including the Chaco and the Yungas). Ecologically, temperature often appears to structure freshwater taxa distributions (e.g., Ephemeroptera assemblages,

Dos Santos et al. 2018), although these distributional patterns are likely a consequence of multiple constraints. Based on our study results, the environmental variables influenced by topography, such as hydrology or sediment size, could be important related factors. The structure of macroinvertebrate assemblages will probably be strongly associated with ecoregion when the topographical and biogeographical contrasts occur together. Such joint effects may be the case for landscapes all along the Andean mountains and their contiguous lowlands in South America.

We note that ecoregions and subcoregions did not always clearly define discrete assemblages in our study. Instead, some taxa occurred in multiple subcoregions located in boundary zones (Tables S6–S8). Other studies have also identified boundary zones where taxonomic com-

Table 2. Pearson correlations between and principal components analysis (PCA) axis values and nonmetric multidimensional scaling (NMDS) axis values based on the different dissimilarity indices applied (Sørensen, positive matching index [PMI], Bray–Curtis, and Dissim) at 2 taxonomic levels of resolution (genus and family). \* $p < 0.001$ .

Taxa resolution	Index	NMDS			
		axes	PCaxis1	PCaxis2	PCaxis3
Genus	Sørensen	1	-0.39	-0.19	-0.13
		2	-0.52*	0.13	-0.20
	PMI	1	-0.15	-0.80*	0.15
		2	0.76*	0.01	0.38
	Bray–Curtis	1	-0.16	0.57*	-0.15
		2	0.13	-0.30	0.13
	Dissim	1	-0.07	-0.81*	0.12
		2	-0.76*	0.02	-0.47
Family	Sørensen	1	-0.17	-0.68*	0.21
		2	-0.10	-0.09	-0.05
	PMI	1	0.33	0.77*	-0.06
		2	0.63*	-0.12	0.35
	Bray–Curtis	1	-0.06	0.06	0.27
		2	-0.03	-0.58*	0.10
	Dissim	1	-0.48	-0.74*	-0.13
		2	0.56*	-0.46*	0.35

position is not distinctly different from adjacent ecoregions or stream types, primarily in mountainous areas (Rabeni and Doisy 2000, Verdonshot and Nijboer 2004). These fuzzy ecoregional boundaries make it difficult to assign the taxa that occur in these boundary areas to any ecoregion. However, differences in overall assemblage structure, such as in taxa dominance and the presence of some taxa exclusively in 1 ecoregion, can be sufficiently large that ecoregion partitions a measurable amount of biotic variation. The recently developed dissimilarity indices (PMI and Dissim) that we used take assemblage structural attributes, such as gradual abundance variations and shared presence of rare species, into account. These indices may better show the gradual and continuous changes that probably occur across regions.

Our results also showed that seasonal variation could influence similarities among assemblages in transitional areas. Seasonal variations in both environmental and biological features have generally not been considered when classifying water body types (Hawkins et al. 2000, 2010, Verdonshot and Nijboer 2004). However, we found that seasonal variation in low- and high-water periods was strongly associated with variation in assemblage structure in transitional areas, making it more difficult to detect ecoregion associations. We observed transitional and gradual patterns of changes in assemblage composition most clearly between the contiguous Yungas and Chaco regions. In our study, the Monte ecoregion was distant and separated by grassland from the

Yungas and Chaco. Future work should examine whether there are transitional or distinct assemblage changes across the Monte–grassland–Yungas/Chaco interface.

Weak associations between macroinvertebrate assemblages and ecoregions may be a result of assemblages changing gradually along environmental gradients (e.g., Hawkins and Vinson 2000, Sandin and Johnson 2000). Consequently, gradient modeling, where site assemblages are predicted based on site-specific differences in environmental conditions (e.g., River Invertebrate Prediction and Classification System–type models, Moss et al. 1987, Wright 1995), generally accounts for more variation than parsing assemblages by region (Davy–Bowker et al. 2006, Sandin and Verdonshot 2006, Hawkins et al. 2010, Johnson and Hallstan 2018). However, freshwater ecosystems from some regions are still poorly studied in South America, especially those in arid, semiarid, and highland areas. More extensive survey data are needed to develop such predictive models and compare the performance of different approaches (regionalizations, typologies, or modeling) to partitioning biotic variation in South American streams and rivers.

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## LITERATURE CITED

- Andresen, E. 2005. Effects of season and vegetation type on community organization of Dung Beetles in a tropical dry forest. *Biotropica* 37:291–300.
- Bailey, R. C., S. Linke, and A. G. Yates. 2014. Bioassessment of freshwater ecosystems using Reference Condition Approach: comparing established and new methods with common data sets. *Freshwater Science* 33:1204–1211.
- Bridge, J. S., and R. V. Demicco. 2008. Earth surface processes, landforms and sediment deposits. Cambridge University Press, Cambridge, UK.



- Brown, A. D. 2000. Development threats to biodiversity and opportunities for conservation in the mountain ranges of the upper Bermejo River Basin, NW Argentina and SW Bolivia. *Ambio* 29:445–449.
- Brown, A. D., H. R. Grau, L. R. Malizia, and A. Grau. 2001. Argentina. Pages 623–659 *in* M. Kapelle and A. D. Brown (editors). *Bosques Nublados del Neotrópico*. INBio, Heredia, Costa Rica.
- Brown, A. D., and S. Pacheco. 2006. Propuesta de actualización del mapa ecorregional de la Argentina. Pages 28–31 *in* A. Brown, U. Martínez Ortiz, M. Acerbi, and J. Corcuera (editors). *La situación ambiental Argentina 2005*. Fundación Vida Silvestre, Buenos Aires, Argentina.
- Burkart, R., N. O. Bárbaro, R. O. Sánchez, and D. A. Gómez. 1999. Ecorregiones de la Argentina. Administración de Parques Nacionales, Buenos Aires, Argentina.
- Cabrera, A. L. 1976. Regiones fitogeográficas Argentinas. Enciclopedia Argentina de Agricultura y Jardinería. 2<sup>nd</sup> edition. Editorial Acme S.A.C.I., Buenos Aires, Argentina.
- Chao, A., and L. Jost. 2012. Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. *Ecology* 93:2533–2547.
- Chessman, B. C. 2014. Predicting reference assemblages for freshwater bioassessment with limiting environmental difference analysis. *Freshwater Science* 33:1261–1271.
- Cultid-Medina, C. A., and F. Escobar. 2016. Assessing the ecological response of Dung Beetles in an agricultural landscape using number of individuals and biomass in diversity measures. *Environmental Entomology* 45:310–319.
- Cumming, G., F. Fidler, and D. L. Vaux. 2007. Error bars in experimental biology. *Journal of Cell Biology* 21:7–11.
- Davy-Bowker, J., R. T. Clarke, R. K. Johnson, J. Kokes, J. F. Murphy, and S. Zahrádková. 2006. A comparison of the European Water Framework Directive physical typology and RIVPACS-type models as alternative methods of establishing reference conditions for benthic macroinvertebrates. *Hydrobiologia* 566:91–105.
- Dodds, W. K., K. Gido, M. R. Whiles, M. D. Daniels, and B. P. Grudziński. 2015. The Stream Biome Gradient Concept: factors controlling lotic systems across broad biogeographic scales. *Freshwater Science* 34:1–19.
- Domínguez, E., and H. R. Fernández. 2009. *Macroinvertebrados bentónicos sudamericanos—Sistemática y biología*. 1<sup>st</sup> edition. Fundación Miguel Lillo, Tucumán, Argentina.
- Dos Santos, D. A., and R. Deutsch. 2010. The Positive Matching Index: a new similarity measure with optimal characteristics. *Pattern Recognition Letters* 31:1570–1576.
- Dos Santos, D. A., C. Molineri, C. Nieto, M. C. Zuñiga, D. Emmerich, P. Fierro, P. Pessacq, B. Ríos-Touma, J. Márquez, D. Gomez, F. F. Salles, A. C. Encalada, R. Príncipe, G. C. Gómez, C. V. Zarges, and E. Domínguez. 2018. Cold/warm stenothermic freshwater macroinvertebrates along altitudinal and latitudinal gradients in western South America: a modern approach to an old hypothesis with updated data. *Journal of Biogeography* 45:1571–1581.
- Dray, S., A. B. Dufour, and J. Thioulouse. 2017. ade4: analysis of ecological data: exploratory and Euclidean methods in environmental sciences. (Available from: <https://rdrr.io/rforge/ade4/>)
- European Commission. 2000. Directive 2000/60/EC of the European Parliament of the Council of 23<sup>rd</sup> October 2000 establishing a framework for community action in the field of water policy. Official Journal of the European Communities L327:1–72.
- Feinsinger, P. 2001. *Designing field studies for biodiversity conservation*. Nature Conservancy, Island Press, Washington, DC.
- Feminella, J. W. 2000. Correspondence between stream macroinvertebrate assemblages and 4 ecoregions of the southeastern USA. *Journal of the North American Benthological Society* 19:442–461.
- Ferréol, M., A. Dohet, H.-M. Cauchie, and L. Hoffmann. 2005. A top-down approach for the development of a stream typology based on abiotic variables. *Hydrobiologia* 551:193–208.
- Gerritsen, J., M. T. Barbour, and K. King. 2000. Apples, oranges and ecoregions: on determining pattern in aquatic assemblages. *Journal of the North American Benthological Society* 19:487–497.
- Gibson, G. R., M. T. Barbour, J. B. Stribling, J. Gerritsen, and J. R. Karr. 1996. Biological criteria: technical guidance for streams and small rivers. EPA 822-B-96-001. Environmental Protection Agency, Office of Water, Washington, DC.
- Gordon, N. D., T. A. McMahon, B. L. Finlayson, C. J. Gippel, and R. J. Nathan. 2004. *Stream hydrology: an introduction for ecologists*. 2<sup>nd</sup> edition. John Wiley and Sons, Chichester, UK.
- Hawkins, C. P., J. L. Kershner, P. A. Bisson, M. D. Bryant, L. M. Decker, S. V. Gregory, D. A. McCullough, C. K. Overton, G. H. Reeves, R. J. Steedman, and M. K. Young. 1993. A hierarchical approach to classifying stream habitat features. *Fisheries* 18(6):3–12.
- Hawkins, C. P., R. H. Norris, J. Gerritsen, R. M. Hughes, S. K. Jackson, R. K. Johnson, and R. J. Stevenson. 2000. Evaluation of the use of landscape classifications for the prediction of freshwater biota: synthesis and recommendations. *Journal of the North American Benthological Society* 19:541–556.
- Hawkins, C. P., J. R. Olson, and R. A. Hill. 2010. The reference condition: predicting benchmarks for ecological and water-quality assessments. *Journal of the North American Benthological Society* 29:312–343.
- Hawkins, C. P., and M. R. Vinson. 2000. Weak correspondence between landscape classifications and stream invertebrate assemblages: implications for bioassessment. *Journal of the North American Benthological Society* 19:501–517.
- Hsieh, T. C., K. H. Ma, and A. Chao. 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution* 7:1451–1456.
- Hueck, K. 1978. *Los Bosques de Sudamérica: ecología, composición e importancia económica*. Sociedad Alemania de Cooperación Técnica (GTZ), Berlin, Germany.
- Illies, J. 1978. *Limnofauna Europaea*. Gustav Fischer Verlag, Stuttgart, Germany.
- Illies, J., and L. Botosaneanu. 1963. Problèmes et méthodes de la classification et de la zonation écologique des eaux courantes, considérées surtout du point de vue faunistique. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* 12:1–57.
- Johnson, R. K. 2000. Spatial congruence between ecoregions and littoral macroinvertebrate assemblages. *Journal of the North American Benthological Society* 19:475–486.
- Johnson, R. K., M. T. Furse, D. Hering, and L. Sandin. 2007. Ecological relationships between stream communities and spatial scale: implications for designing catchment-level monitoring programmes. *Freshwater Biology* 52:939–958.
- Johnson, R. K., and S. Hallstan. 2018. Modelling outperforms typologies for establishing reference conditions of boreal lake and stream invertebrate assemblages. *Ecological Indicators* 93:864–873.

- Legendre, P., and L. Legendre. 1998. Numerical ecology: developments in environmental modelling. 2<sup>nd</sup> English edition. Elsevier Science, Amsterdam.
- MacGregor-Fors, I., and M. E. Payton. 2013. Contrasting diversity values: statistical inferences based on overlapping confidence intervals. *PLoS ONE* 8:e56794.
- Marchant, R., F. Wells, and P. Newall. 2000. Assessment of an ecoregion approach for classifying macroinvertebrate assemblages from streams in Victoria, Australia. *Journal of the North American Benthological Society* 19:497–500.
- Martins, I., R. Ligeiro, R. M. Hughes, D. R. Macedo, and M. Callisto. 2017. Regionalisation is key to establishing reference conditions for neotropical savanna streams. *Marine and Freshwater Research* 69:82–94.
- Mesa, L. M. 2012. Interannual and seasonal variability of macroinvertebrates in monsoonal climate streams. *Brazilian Archives of Biology and Technology* 55:403–410.
- Mesa, L. M., H. R. Fernández, and M. V. Manzo. 2009. Seasonal patterns of benthic arthropods in a Subtropical Andean basin. *Limnologia* 39:152–162.
- Minetti, J. L., S. A. Albarracín, M. E. Bobba, C. M. Hernández, E. R. López, L. A. Acuña, M. C. Costa, I. J. Nieva, and E. Mendoza. 1999. Atlas climático del noroeste Argentino. Laboratorio Climatológico Sudamericano. Fundación Zon Caldenius, Tucumán, Argentina.
- Minshall, G. W. 1988. Stream ecosystem theory: a global perspective. *Journal of the North American Benthological Society* 7:263–288.
- Morrone, J. J. 2014. Biogeographical regionalization of the Neotropical region. *Zootaxa* 3782:1–110.
- Moss, D., J. F. Wright, M. T. Furse, and R. T. Clarke. 1987. The prediction of the macro-invertebrate fauna of unpolluted running-water sites in Great Britain. *Freshwater Biology* 17:41–52.
- Nieto, C., D. A. Dos Santos, A. Izquierdo, and H. R. Grau. 2017. Modelling beta diversity of aquatic macroinvertebrates in High Andean wetlands. *Journal of Limnology* 76:555–570.
- Nollet, L. M. L., and L. S. P. De Gelder. 2014. Handbook of water analysis. 3<sup>rd</sup> edition. CRC Press, Boca Raton, Florida.
- Omernik, J. M. 1987. Ecoregions of the conterminous United States. *Annals of the Association of American Geographers* 77:118–125.
- Omernik, J. M., and G. E. Griffith. 1991. Draft ecoregions and southeastern Plains subregions of Mississippi and Alabama. Environmental Research Laboratory, US Environmental Protection Agency, Corvallis, Oregon. (Available from: Alabama Department of Environmental Management, P.O. Box 301463, Montgomery, Alabama, USA 36130–1463.)
- Oswood, M. W., J. B. Reynolds, J. G. Irons III, and A. M. Milner. 2000. Distributions of freshwater fishes in ecoregions and hydroregions of Alaska. *Journal of the North American Benthological Society* 19:405–418.
- Pan, Y., R. J. Stevenson, B. H. Hill, and A. T. Herlihy. 2000. Ecoregions and benthic diatom assemblages in Mid-Atlantic Highlands streams, USA. *Journal of the North American Benthological Society* 19:518–540.
- Pinto, B. C. T., F. C. Araujo, V. D. Rodrigues, and R. M. Hughes. 2009. Local and ecoregion effect on fish assemblages structure in tributaries of the Rio Paraíba do Sul, Brazil. *Freshwater Biology* 54:2600–2615.
- Poff, N. L., and J. V. Ward. 1989. Implications of streamflow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. *Canadian Journal of Fisheries and Aquatic Science* 46:1805–1818.
- Rabeni, C. F., and K. E. Doisy. 2000. Correspondence of stream benthic invertebrate assemblages to regional classification schemes in Missouri. *Journal of the North American Benthological Society* 19:419–428.
- Resh, V. H., R. H. Norris, and M. T. Barbour. 1995. Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. *Australian Journal of Ecology* 20:108–121.
- Reynoldson, T. B., S. Strachan, and J. L. Bailey. 2014. A tiered method for discriminant function analysis models for the Reference Condition Approach: model performance and assessment. *Freshwater Science* 33:1238–1248.
- Sandin, L., and R. K. Johnson. 2000. Ecoregions and benthic macroinvertebrate assemblages of Swedish streams. *Journal of the North American Benthological Society* 19:462–474.
- Sandin, L., and P. F. M. Verdonschot. 2006. Stream and river typologies: major results and conclusions from the STAR project. *Hydrobiologia* 566:33–37.
- Scheiner, S. M., and J. Gurevitch. 1993. Design and analysis of ecological experiments. Chapman and Hall, New York.
- Valentin, J. L. 2012. Ecologia numérica: uma introdução à análise multivariada de dados ecológicos. Editora Interciência, Rio de Janeiro, Brazil.
- Van Sickle, J., and R. M. Hughes. 2000. Classification strengths of ecoregions, catchments, and geographic clusters for aquatic vertebrates in Oregon. *Journal of the North American Benthological Society* 19:370–384.
- Vasconcelos, M. C., A. S. Melo, and A. Schwarzbald. 2013. Comparing the performance of different stream classification systems using aquatic macroinvertebrates. *Acta Limnologia Brasiliensia* 25:406–417.
- Verdonschot, P. F. M. 2006. Evaluation of the use of Water Framework Directive typology descriptors, reference sites and spatial scale in macroinvertebrate stream typology. *Hydrobiologia* 566:39–58.
- Verdonschot, P. F. M., and R. C. Nijboer. 2004. Testing the European stream typology of the Water Framework Directive for macroinvertebrates. *Hydrobiologia* 516:35–54.
- Vidaurre, M., L. F. Pacheco, and A. I. Roldán. 2006. Composition and abundance of birds of Andean alder (*Alnus acuminata*) patches with past and present harvest in Bolivia. *Biological Conservation* 132:12–21.
- Vinson, M. R., and C. P. Hawkins. 2003. Broad-scale geographical patterns in local stream insect genera richness. *Ecography* 26: 751–767.
- Waite, I. R., A. T. Herlihy, D. P. Larsen, and D. J. Klemm. 2000. Comparing strengths of geographic and nongeographic classifications of stream benthic macroinvertebrates in the Mid-Atlantic Highlands, USA. *Journal of the North American Benthological Society* 19:429–441.
- Wright, J. F. 1995. Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. *Australian Journal of Ecology* 20:181–197.