SUPPLEMENTARY MATERIAL S2 | Investigating the effects of phylogenetic uncertainty.

In this supplementary material we report the results of the analysis of multivariate linear models and homogeneity of variances (adonis2 and betapart) by accounting for phylogenetic uncertainty due to polytomies in the synthesis phylogeny. For this we used 1000 randomly fully-resolved phylogenetic trees obtained following the method of Martins *et al.* (2013), for which we calculated phylogenetic beta diversity and its components (nestedness and turnover) using the method of Baselga *et al.* (2018). For each pairwise matrix of phylogenetic beta diversity (total, turnover and nestedness) we performed the adonis2 model using the same predictors presented in Tab. 1 of the main text and the betapart function for phylogenetic structure of the Paraná and Paraguai River basins. This approach allowed us to investigate the influence of phylogenetic uncertainty due to the presence of polytomies in the synthesis tree (Fig. 2 in the main text).

We stored F and p values for each of the 1000 models performed for total, turnover and nested components of phylogenetic beta diversity and environmental variables, and compared their distributions with the F and p values obtained from the analysis using the synthesis phylogenetic tree containing polytomies. If the F and p values obtained for the model that used the phylogenetic hypothesis containing the polytomies do not differ significantly from the F and p distribution obtained using the 1000 trees, it can be concluded that patterns of phylogenetic beta diversity not significantly influenced by the uncertainty presented in the terminal nodes with polytomies in the synthesis phylogeny.

Figure S1 shows the distributions of F and p values for each component of phylogenetic beta diversity obtained when relating environmental variables in the adonis2 model. We observed that all F and p values obtained from the phylogenetic tree with polytomies differed slightly from the mean values obtained from distribution of F and p values with the set of fully resolved phylogenies. In particular, variables that presented a significant effect for phylogenetic structure, like pH and water velocity, had F values that were highly concordant with the mean values of the F distribution; the same was true for p values as well.

We also performed an analysis of homogeneity of variance of groups in each basin for each component of phylogenetic beta diversity (betadisper) using the 1000 fully-resolved phylogenies. For each phylogeny we calculated the pairwise matrices of phylogenetic beta diversity (total, turnover and nestedness) and tested the homogeneity of variance of each matrix between basins. We stored the F and p-values that returned in each analysis resulting in a distribution of 1000 F and p-values for each component of phylogenetic beta diversity. We assessed whether the F and p values obtained with the phylogenetic hypothesis with polytomies fell within the distribution comprised by 1000 values (Fig. S1).

Figure S1 shows that the F and p values obtained from analysis of homogeneity of variances are very similar to the mean F and p values derived from the 1000 randomly fully solved phylogenies. This indicates that, for this analysis, the phylogenetic uncertainty presented due to the lack of resolution in some genera does not play an important role in the ability to interpret the patterns of clade distribution across the analyzed communities.

Therefore, we concluded that phylogenetic uncertainty due to the lack of resolution of some nodes does not have a significant effect on conclusions regarding the influence of environmental factors on the components of phylogenetic beta diversity.
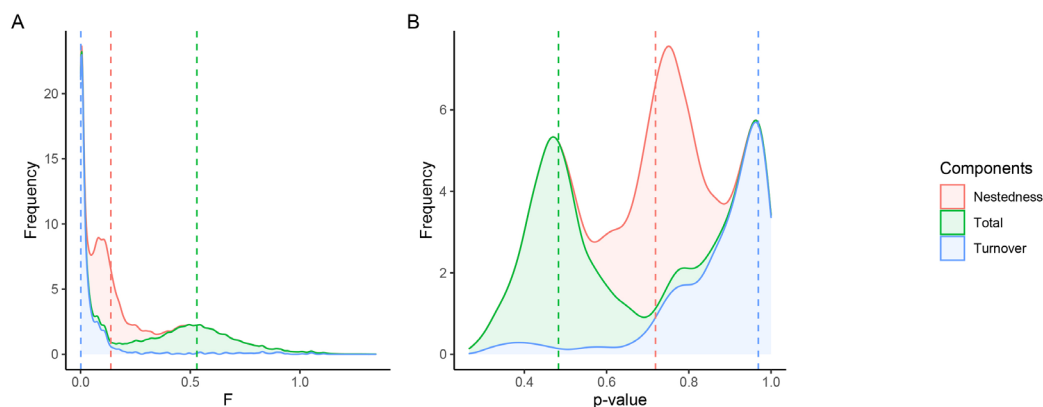
FIGURE S2 | Distributions for F (**A**) and p-values (**B**) obtained from the analysis of homogeneity of variances for total, turnover and nestedness components of phylogenetic beta diversity using 1000 fully randomly resolved phylogenies. Dashed lines indicate the F and p-values obtained from the analysis using a phylogenetic tree that contains polytomies.

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