

**Untangling the assembly of macrophyte metacommunities by means of  
taxonomic, functional and phylogenetic beta diversity patterns**

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## Abstract

Metacommunity ecology has broadened considerably with the recognition that measuring beta diversity beyond the purely taxonomic viewpoint may improve our understanding of the dispersal- and niche-based mechanisms across biological communities. In that perspective, we applied a novel multidimensional approach including taxonomic, functional and phylogenetic data to enhance our basic understanding of macrophyte metacommunity dynamics. For each beta diversity metric, we calculated the mean overall value and tested whether the mean value was different from that expected by chance using null models. We also employed evolutionary and spatially constrained models to first identify the degree to which the studied functional traits showed a phylogenetic signal, and then to estimate the relative importance of spatial and environmental effects on metacommunity structure. We first found that most individual ponds were inhabited by species that were merely random draws from the taxonomic and phylogenetic species pool available in the study region. Contrary to our expectations, not all measured traits were conserved along the phylogeny. We also showed that trait and phylogenetic dimensions strongly increased the amount of variation in beta diversity that can be explained by degree of environmental filtering and dispersal limitation. This suggests that accounting for functional traits and phylogeny in metacommunity ecology helps to explain idiosyncratic patterns of variation in macrophyte species distribution. Importantly, phylogenetic and functional analyses identified the influence of underlying mechanisms that would otherwise be missed in an analysis of taxonomic turnover. Together, these results let us conclude that macrophyte species have labile functional traits adapted to dispersal-based processes and some evolutionary trade-offs that drive community assembly via species sorting. Overall, our exploration of different facets of beta diversity showed how functional and phylogenetic information may be used with species-level data to test community assembly hypotheses that are more ecologically meaningful than assessments of environmental patterns based on the purely taxonomic viewpoint.

47    **Keywords:** aquatic plant, dispersal limitation, diversity facets, null models, species sorting,  
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## 1. Introduction

Traditionally, species-based metrics (i.e. taxonomic-centric approaches) have primarily been used to quantify the relative importance of species sorting and dispersal limitation on variation in species composition among habitat patches (i.e. beta diversity; see Logue et al., 2011; Swenson et al., 2012). An important source of criticism to this traditional approach is that it is silent on functional and phylogenetic differences among species (Devictor et al. 2010), compromising our ability to untangle the mechanistic basis linked to the spatial and temporal dynamics of biodiversity (McGill et al. 2006). Measuring phylogenetic diversity (i.e. mean length of evolutionary pathways that connect a given set of taxa) in species assemblages was then proposed as a promising way to explain the role of species interactions, historical imprints and evolutionary legacy in community structure (Webb et al. 2002). Meanwhile, functional diversity, reflecting the diversity of morphological, ecological and physiological traits in the multidimensional niche space (Petchey & Gaston 2006), was shown to better explain ecosystem functioning than other classical measures of diversity (*sensu* Devictor et al. 2010).

In that perspective, adopting a multifaceted approach on beta diversity may provide a clearer picture of the selection pressures underlying different historical, ecological and evolutionary processes (Webb et al., 2002). For example, while the species composition of local communities (i.e. taxonomic facet) may sometimes fall short in providing information on how ecological communities are assembled, trait-based and phylogenetic approaches tell us more about the evolutionary constraints on community membership and how ecological processes may interact to shape patterns of niche divergence (Webb et al., 2002; Swenson et al., 2012).

The extent to which taxonomic, functional and phylogenetic beta diversities are related to different drivers may help us to identify the mechanisms structuring metacommunities along environmental gradients (Cisneros et al., 2014). Also, provided that phylogenetic relatedness aligns with functional similarity (i.e. phylogenetic inertia; Faith, 2015), the former can be

considered a proxy for the latter, and thus a more phylogenetically diverse community will have a greater functional complementarity (Poff et al., 2006; Fig. 1a). However, when the measured traits are labile, functional and phylogenetic patterns can be decoupled and provide complementary insights into community assembly (Fig. 1b). How strongly their patterns overlap depends on the strength of phylogenetic signal in the functional characters, which in turn depends on the underlying processes of species diversification and niche evolution (Burns and Strauss, 2011). Such patterns would be highly complementary to traditional taxonomic-based analyses by providing evidence of the impacts of past evolutionary history, specific phenotypic traits and current ecological processes on community assembly (Heino, 2011). However, the strength of phylogenetic signal in species traits is rarely examined in the metacommunity context (but see Pillar and Duarte, 2010).

An increasing number of studies (e.g. Gianuca et al., 2018; Roa-Fuentes et al., 2019) have attempted to characterise patterns of functional and phylogenetic beta diversity, and with the increase in species-level trait and phylogenetic data this trend will continue. However, to our knowledge, no study to date has examined to what extent dispersal limitation and species sorting drive the structure of macrophyte metacommunities, while simultaneously taking into account multiple facets of beta diversity. To overcome this shortage, we analysed variation in taxonomic, functional and phylogenetic beta diversity of pond macrophyte communities in a Mediterranean drainage basin. Previous studies in this system based purely on taxonomic diversity measures have shown that macrophyte assemblages are mainly structured by the interaction between spatial and environmental gradients (e.g. García-Girón et al., 2019a, 2019b).

In this study, we used a novel combination of methods (including null models and phylogenetic signal evaluations) to assess whether the role of dispersal limitation, species sorting and combined effects was consistent for the three different beta diversity dimensions. We first

hypothesised that beta diversity patterns are non-random (Siefert et al., 2013), providing evidence that environmental and spatial processes induce ecological constraints on macrophyte community assembly patterns. Second, we expected that environmental change in this kind of highly fragmented ponds (see Fernández-Aláez et al., 2018) will lead to habitat filtering against specific functional characters, which may also lead to phylogenetic signal in species turnover. Third, based on previous findings (e.g. Gianuca et al., 2018), we hypothesised that functional and phylogenetic approaches should provide more explanatory power than the traditional taxonomic approach that treats all species as equally differentiated from each other. Fourth, we assumed that taxonomic and phylogenetic assemblages are controlled by both environmental filtering and dispersal limitation (see also García-Girón et al., 2019a, 2019b), whereas functional community component is primarily structured by environmental filtering. This is because species identity and evolutionary kinship should be more influenced by dispersal limitation and evolutionary divergence (Mouquet et al., 2012; Cai et al., 2019), whereas functional diversity should be more directly related to environmental filtering due to species trait-environment association (Hoeinghaus et al., 2007; Heino et al., 2013).

## **2. Material and methods**

### **2.1 Study area and macrophyte sampling**

We sampled 51 permanent ponds located within a heterogeneous and lowland area of approximately 94,000 km<sup>2</sup> in north-western Spain (Supplementary Fig. A.1). All of the ponds belong to the same biogeographical region, where aquatic plants have a shared evolutionary history (Molina, 2017). The study area presents a relatively flat slope and plains of quaternary fluvial sedimentary nature. The climate is Mediterranean with a wide seasonal variation in temperature and precipitation, since summers are typically hot and dry (average summer temperature of 18 °C and mean summer precipitation of 84.5 mm) and winters are primarily cold and wet (average winter temperature of 3.2 °C and mean winter precipitation of 173 mm;

1976-2015, data provided by the Spanish Met Agency – AEMET; <http://www.aemet.es>). This region is immersed in an agricultural intensive landscape consisting mainly on cereal crops (~50%), pastures (~30%), scattered scrubs (~10%) and pine plantations (~10%). However, the landscape has been fragmented in recent times for the development of dry farming and cropland irrigation (García-Girón et al., 2018a). The majority of the ponds studied are fed mostly by groundwater and rainfall and experience a strong reduction in water volume during the summer, ranging between 0.1 and 23 ha in aerial extent and 0.2 and 6.3 in depth. The study ponds display considerable variability in environmental conditions, including morphometry, nutrient content and mineralisation (Supplementary Table A.1).

Pond macrophytes (i.e. emergent, floating-leaved and submerged plants) were exhaustively surveyed using transects in June and July of either 2004 or 2005. Hence each pond was sampled once. Transects were distributed around each pond and quadrats (0.25 m<sup>2</sup>) were placed perpendicular to the shore-line at varying intervals of 0-5 m depending on the homogeneity of the aquatic flora. The number of transects and quadrats for each site was determined according to the pond area and shoreline complexity (Jensén, 1977), giving an accurate representation of local community composition and ranging between 1 and 3 and 5 and 83, respectively. Percent coverage of each macrophyte species was estimated in each quadrat and the mean coverage of each taxon in a pond was determined as the sum of percent coverages of that species in all quadrats divided by the number of quadrats used in the pond.

Nomenclature followed Flora Europaea (Tutin et al., 1980).

Further details on study site selection and macrophyte data collection can be found in García-Girón et al. (2019a, 2019b).

## **2.2 Local-scale variables**

The explanatory data comprised of well-known environmental variables structuring Mediterranean pond macrophyte assemblages (e.g. Fernández-Aláez et al., 2018; García-Girón

et al., 2018b, 2019b): pond area (ha), mean depth (m), Secchi depth (m), turbidity (NTU), oxygen ( $\text{mg l}^{-1}$ ), pH, conductivity ( $\mu\text{S cm}^{-1}$ ), total suspended solids (TSS;  $\text{mg l}^{-1}$ ), volatile suspended solids (VSS;  $\text{mg l}^{-1}$ ) total nitrogen (TN;  $\text{mg l}^{-1}$ ), nitrate ( $\text{NO}_3^- \text{-N}$ ;  $\text{mg l}^{-1}$ ), ammonium ( $\text{NH}_4^+ \text{-N}$ ;  $\text{mg l}^{-1}$ ), total phosphorous (TP;  $\mu\text{g l}^{-1}$ ), soluble reactive phosphorous ( $\text{PO}_4^{3-} \text{-P}$ ;  $\mu\text{g l}^{-1}$ ), dissolved organic carbon (DOC;  $\text{mg l}^{-1}$ ) and chlorophyll “a” (Chla;  $\text{mg l}^{-1}$ ).

Pond area was measured using images available on SIGPAC (the Spanish Geographical Information System for Agricultural Parcels - <http://www.sigpac.jcyl.es/visor/>), whereas mean and Secchi depths were determined from point measurements at different sites along the same transect of which macrophytes were surveyed. Water chemistry measurements were based on a single water composite sample randomly collected at different depths along a shore-centre transect using a cylinder (diameter = 6 cm and length = 100 cm). The number of water samples ranged between 3 and 15, depending on pond area. Standard water characteristics (i.e. turbidity, oxygen, pH and conductivity) were measured *in situ* from the composite sample using WTW field probes (Model LF 323) and a portable turbidimeter (Model HACH 2100P). Then, the integrated water sample was immediately transported to the laboratory and preserved at 4 °C to determine TSS, VSS, TN,  $\text{NO}_3^- \text{-N}$ ,  $\text{NH}_4^+ \text{-N}$ , TP,  $\text{PO}_4^{3-} \text{-P}$ , DOC and Chla following standard methods (APHA, 1989).

We tested for an effect of sampling time (i.e. 2004 vs 2005) on pond environmental conditions and macrophyte species distribution, and the effect proved to be minimal and non-significant (see Fernández-Aláez et al., 2018).

### **2.3 Spatial features: a proxy for dispersal processes**

We derived a set of spatial variables from the geographical coordinates (UTM) of the sites using Moran eigenvector maps (MEMs; Dray et al., 2006). This technique maps neighbourhood relationships onto orthogonal and linearly independent spatial variables over a wide range of spatial scales, and thus is more efficient in capturing complex community patterns of spatial



variation than geographic distances alone (Borcard et al., 2011). We obtained results for distance truncation thresholds of 50 values ranging from the minimum to the maximum Euclidean distance between sites, selecting the threshold with the highest number of positive and significant eigenvectors. The first MEMs generated in the analyses represent broader spatial structures, while the last ones cover finer spatial scales (see Borcard and Legendre, 2002; Borcard et al. 2004 for details).

## **2.4 Trait and phylogenetic distances**

We selected a number of key traits to provide information on functional characters that could potentially come under selection by environmental filters. We collected information from literature (Castroviejo, 1986-2012; Willby et al., 2000; Cirujano et al., 2014; Fu et al., 2014) and existing databases (*TRY* Plant Trait Database; Kattge et al., 2011) on twelve different traits for all 51 species and 19 families occurring in the metacommunity: dispersal vector, growth form, leaf dry mass (LDM), offspring size, plant longevity, plant phenology, reproduction mode, reproduction time, seed length, seed longevity, seed number and specific leaf area (SLA) (Supplementary Table A.2). Physiological traits (e.g. extent of bicarbonate use, leaf carbon/nitrogen content and photosynthetic mechanism) were excluded because of inadequate coverage or poorly differentiated data. However, the relatively high number of traits considered should be sufficient to give a broad characterization of the realized niche of a species (*sensu* Willby et al., 2000) without an *a priori* assumption that some traits were more important than others. Since the relative adaptive significance of each character was unknown, we applied the orthogonal rotation in PCAmix (de Leeuw and Van Rijkevorsel, 1980) to clarify the covariance structure of trait variables and assess the general pattern of relationships within the functional data. PCAmix is a method implemented in the R package PCAmixdata (Chavent et al., 2017) that provides insight into the underlying structure and interdependence of a set of continuous and categorical samples by using a combination of principal component

analysis and multiple correspondence analysis. Then, we used the mixed-variables coefficient of distance (i.e. a generalization of Gower's distance; Borcard et al., 2011) to extract a functional distance matrix, which described the functional dissimilarity between all species pairs based on a suite of characters with the largest squared loadings on the first two PCAmix axes. The functional dissimilarity matrix was obtained using the vegan package (Oksanen et al., 2016) in R 3.4.4 (R Core Team, 2018).

Owing to lack of true phylogeny comprising all macrophyte species, we calculated (cophenetic) phylogenetic distances among all families present in our metacommunity using the package picante (Kembel et al., 2010). To do this, we used a recently released compilation of angiosperm phylogeny (*sensu* R20160415.new) based on APG IV (2016) [for details on family tree reconstruction, see Gastauer and Meira-Neto (2017) and supplementary information therein]. We acknowledge that this supertree is not ideal for answering strict evolutionary questions, but we believe that the phylogenetic information used here is valid for large-scale metacommunity studies requiring such information (but see Mueller et al., 2013).

## **2.5 Data analysis**

### **2.5.1 Phylogenetic signal in functional characters**

The degree to which the studied traits showed a phylogenetic signal was assessed by means of a two-step approach consisting of a standard Mantel test followed by a test based on the Brownian evolutionary model (i.e. EM-Mantel; Debastini and Duarte, 2017). This new analytical procedure, which has appropriate Type I error and acceptable power, is a good alternative for measuring phylogenetic signal in continuous and categorical traits (Debastini and Duarte, 2017). While the conventional Mantel test examines whether more closely related families have more similar character states, the EM-Mantel test further contrasts the empirical phylogenetic signal to that simulated from a neutral (i.e. null) evolutionary model (for more details, please see Debastini and Duarte, 2017). Here we used the Brownian motion

evolutionary model, which assumes that differentiation in functional characters is proportional to evolutionary time among families. We ran the evolutionary model 999 times with the original R code provided by Debastini and Duarte (2017) to obtain the null distributions of the Mantel coefficient for the conventional Mantel and EM-Mantel tests, respectively. Similarly, we calculated the *K*-statistic of Blomberg et al. (2003) to estimate the strength of phylogenetic niche conservatism for different traits using the `multiPhylosignal` function in R package `picante`. The statistical significance of phylogenetic signal for each trait is determined by comparing observed patterns of the variance of independent contrasts of the functional character to a Brownian motion model of shuffling family labels across the tips of the phylogeny (Blomberg et al., 2003).

## **2.5.2 Quantifying taxonomic, functional and phylogenetic beta diversity**

We estimated pairwise taxonomic beta diversity for abundance data (i.e. Bray-Curtis dissimilarities) following Baselga (2013) with the function `beta.pair.abund` from the package `betapart` (Baselga et al., 2018). Phylogenetic beta diversity was calculated based on the phylogenetic distance matrix using mean pairwise phylogenetic dissimilarity (Swenson, 2014) with the function `COMDIST` in R library `picante`. We also used `COMDIST` to estimate functional beta diversity based on the Gower's multi-trait distance matrix (see Gianuca et al., 2018). Abundance values of taxa were used when calculating both phylogenetic and functional beta diversity metrics. Then, the pairwise output values obtained from Bray-Curtis and `COMDIST` were synthesized into principal coordinate analysis (PCO) using the Lingoes correction (Borcard et al., 2011) with the function `dudi.pco` from R package `ade4` (Dray et al., 2018). The final product is a matrix of orthogonal PCO eigenvectors, each of them describing taxonomic, trait and phylogenetic dissimilarity patterns, which may be used as response variables in direct gradient analyses (Duarte et al., 2012). In principle, all PCO eigenvectors may be used as descriptors of beta diversity patterns in constrained ordinations. However, using all

of them might introduce confounding effects in posterior analyses (see Anderson and Willis, 2003). We therefore made a selection of a subset of orthogonal eigenvectors that maximised the association between beta diversity patterns (taxonomic or functional-phylogenetic) and the set of explanatory variables. To select how many orthogonal eigenvectors should be used in successive analyses, we applied a procedure that is suitable for direct multiple regression analyses (*sensu* Anderson and Willis, 2003). We retained as many eigenvectors as required to maximise total  $adjR^2_{(Y|X)}$ , which is the exact number that optimises the fit between explanatory and response matrices (see also Duarte et al. 2012). For more details on this selection procedure check Anderson and Willis (2003) and Duarte et al. (2012).

For each beta diversity facet, we calculated their mean overall value using the extension of the alpha diversity metric, i.e. mean pairwise distance (MPD), to beta diversity (i.e.  $\beta MPD$ ; Webb et al., 2008) and tested whether the mean value was different from that expected by chance. Since we were using abundance data, we performed randomisations that fixed the entire community data matrix while randomising the functional and phylogenetic information for performing null models (Swenson, 2014). We randomised the identities of families and species 1,000 times in the phylogenetic tree and the Gower's multi-trait distance matrix, respectively. With this randomisation procedure, the species alpha and beta diversities and the abundance of species within and across communities are all fixed, while the observed dispersal limitation of species is conserved (see Swenson et al., 2012; Swenson, 2014). After each randomisation, the phylogenetic and functional beta diversities were calculated and these values were used to compute the null distribution. Using these metrics, we obtained the standardised effect size (SES) as:

$$SES = \frac{mpd.obs - mpd.rnd.mean}{mpd.rnd.sd}$$

where *mpd.obs* is the observed beta diversity, *mpd.rnd.mean* the mean of the null distribution and *mpd.rnd.sd* the standard deviation of the null model.

Negative SES values indicate phylogenetic and functional beta diversity lower than expected by null models, meaning that the assemblages comprised functionally similar species or closely related families. Conversely, positive SES values suggest that phylogenetic and functional beta diversities are higher than expected by chance, meaning that different communities generally contain distantly related plant families and/or functionally dissimilar species (Swenson et al., 2012; Swenson, 2014). To test whether the mean overall value of each  $\beta$ MPPD metric was different from the expected value of zero for random data, we performed a series of two-tailed  $t$  tests. Null models were prepared according to Swenson (2014) and both procedures were conducted using R statistical software.

### **2.5.3 Quantifying the effects of environmental and spatial factors on beta diversity patterns**

The methods of direct gradient analysis and variation partitioning (hereafter referred as VP; Borcard et al., 1992) are the most widely used quantitative frameworks to evaluate the contributions of species sorting (i.e. environmental fraction) and dispersal limitation (i.e. spatial component) to metacommunity structure. Such a traditional approach can in principle only estimate the role of species sorting by analysing its non-spatialized environmental contribution (Smith and Lundholm, 2010). However, spatial autocorrelation can occur independently in both the environment [ES] and species distribution. In these cases, the classic VP framework may present high Type I error and inflated estimates of the importance of environmental determinism (*sensu* Clappe et al., 2018). To deal with these limitations, Clappe et al. (2018) proposed a new VP procedure using Moran Spectral Randomisation (MSR; Wagner and Dray, 2015) as a spatially constrained null model. This novel MSR-based spatially constrained VP allows estimating the statistical distribution of  $_{adj}R^2$  values under pure spurious species-environment relationships [SP] for a given species data matrix. Hence, the MSR-based VP adjusts for this spurious contribution in the total environmental fraction by reducing fraction [SP] to the contribution of the spatially structured environment [ES]. The significance

of each fraction (non-spatialized environment [E], dispersal limitation [D] and both in combination [ED]) is then estimated by a randomisation test where the sites of the predictors are permuted and appropriate *F*-statistics related to each fraction are calculated.

To identify significant metacommunity processes structuring variation in species composition, macrophyte lineages and functional traits, we first employed the forward selection procedure of Blanchet et al. (2008). This method is based on two criteria: (i) the  $_{adj}R^2$  of the global model; and (ii) the significance alpha level of 0.05. We selected the most parsimonious subset of environmental and spatial variables for each biodiversity dimension separately (i.e. taxonomic, functional and phylogenetic). This allowed us to minimise Type I error while maximising the fit between response and explanatory data matrices (Blanchet et al., 2008). Based on the resulting subset of explanatory variables, we performed MSR-based VP using as response matrices: (i) the PCO eigenvectors describing Bray-Curtis dissimilarities in species composition; (ii) the Hellinger-transformed species abundance per site; (iii) the PCO eigenvectors describing patterns of functional beta diversity; and (iv) the PCO eigenvectors describing phylogenetic beta diversity. The first two response matrices are two alternative but complementary approaches for the taxonomic analysis of metacommunity dynamics. The Hellinger-transformed species abundance data is the standard strategy for investigating community assembly processes. We run the additional approach based on PCO eigenvectors describing dissimilarities in species composition to check whether differences in the amount of explained variation between taxonomic and functional/phylogenetic procedures were not due to the number of dependent variables included in the spatially constrained randomisation model (see Gianuca et al., 2018). Metacommunity patterns of the two taxonomic approaches were very similar, thus we report only the results obtained through the Hellinger-transformed procedure in the main text (see Supplementary Fig. B.1 for the results of the Bray-Curtis approach). All analyses were run with packages *ade4* and *adespatial* (Dray et al., 2018) in the R environment. See Fig. 2 for the main steps of our statistical approach.

### 3. Results

A total of 51 macrophyte species from 19 different families were identified. A complete list of species and families is provided in Supplementary Table A.3. *Eleocharis palustris* was the most frequent plant species, occurring in 35 ponds (~70%). The second and third most frequent species were *Schoenoplectus lacustris* and *Potamogeton trichoides*, occurring in 32 (~60 %) and 25 (~50 %) ponds, respectively. The trait-based analysis (Supplementary Tables A.4 and A.5) indicated that the majority of macrophyte species in the study sites were submerged leaved (87.9 %), anemochorous (70.1 %) and seed-producing (100%).

#### 3.1 Phylogenetic signal in functional characters

Factorial analysis of mixed data (PCAmix) clarified the covariance structure of trait variables (Supplementary Fig. A.2), suggesting that all functional characters were likely to influence how plant species might respond to environmental and spatial constraints. The standard Mantel test revealed a significantly positive correlation between trait and phylogenetic distances among macrophyte families ( $R = 0.15$ ;  $p < 0.05$ ). Surprisingly, the Brownian motion evolutionary model did not showed any significant signal of phylogenetic conservatism (Null  $K = 1$ ;  $p > 0.05$ ), suggesting labile trait evolution in aquatic plant families. When using the angiosperm supertree, with a complete coverage of species but only family-level resolution, significant phylogenetic signal was only detected for dispersal vector and reproduction mode (Table 1).

#### 3.2 Metacommunity analyses

The mean values for taxonomic and phylogenetic beta diversities were not different from the expected value of zero, suggesting that taxonomic-based community assembly dynamics ( $p = 0.27$ ) and lineage composition ( $p = 0.33$ ) were likely to be random. By contrast, functional beta diversity was different from that expected by chance, suggesting that community assembly

processes underlying selection for particular traits in functional niche space were dominated by deterministic processes ( $p < 0.05$ ; Fig. 3).

Only the first PCO eigenvector was selected as response variable for the taxonomic, functional and phylogenetic facets of beta diversity. Combining spatial and environmental drivers of metacommunity assembly revealed that total explained variation (i.e. total  $_{adj}R^2$ ; Fig. 4) obtained through MSR-based VP was highest for the phylogenetic approach ( $_{adj}R^2 = 0.49$ ), followed by the multi-trait ( $_{adj}R^2 = 0.36$ ) and the classic taxonomic approaches ( $_{adj}R^2 = 0.19$ ). Furthermore, functional and phylogenetic metrics significantly explained variation in the residuals of the best performing spatially constrained model on species composition (i.e. Hellinger-transformed species abundance data; Supplementary Fig. B.2). This finding suggests that accounting for plant lineages and species-specific functional characters improved predictions on the role of dispersal limitation and species sorting on community assembly.

MSR-based spatially constrained VP models revealed some differences in the relative contribution of species sorting and dispersal limitation to variation in each facet of beta diversity. Pure spatial effects (i.e. dispersal limitation) better explained taxonomic and multi-trait beta diversity dimensions than the pure effect of the non-spatialized environment. Spatial variables with small eigenvalues (e.g. MEM2; Table 2), indicating broad-scale variation in geographical structure, were the most influential for both species composition and community trait structure. By contrast, the pure effect of species sorting explained a significant proportion of phylogenetic variation (Fig. 4 and Table 2). Clearly, the greatest amount of explained variation ( $_{adj}R^2 = 0.11 - 0.18$ ) in all beta diversity facets was seen for the shared effects between environmental and spatial processes. The explained variation of shared effects was, however, not statistically significant for the multi-trait and phylogenetic beta diversity facets (Fig. 4 and Table 2).



Despite the differences in the amount of explanatory power, there was a relatively good agreement among the environmental and spatial variables selected as important predictors of each beta diversity dimension (Table 2). For example, the spatially constrained models revealed that both species and lineage composition varied mainly as a function of pH and nutrient concentration. Emergent macrophytes were likely to be positively associated to high productivity levels (here measured as nutrient concentration and pH), and the opposite pattern was observed for the floating- and submerged-leaved plant species, which lean towards the centre and the beginning of the productivity gradient, respectively (Fig. 5). Similarly, the MSR-based VP procedure associated variation in macrophyte trait structure and species composition at the extent of approximately 300 km (Supplementary Fig. A.3).

#### **4. Discussion**

In this study, we applied a multidimensional approach including taxonomic, functional and phylogenetic data to enhance our basic understanding of macrophyte metacommunity dynamics. Importantly, our study is unique in bridging together different facets of beta diversity that have traditionally been used separately to infer macrophyte community assembly scenarios. By using them systematically on the same data set together with null models, we were able to show their complementarity in explaining variation in spatial and environmental components of macrophytes. Our findings suggest that species inhabiting most ponds were merely random draws from the taxonomic and phylogenetic species pool available in the study region. In addition, we evidenced lack of phylogenetic conservatism across all the studied species traits based on Brownian motion evolutionary model, and only dispersal vector and reproduction mode of all species traits showed phylogenetic signal when individual species traits were considered separately using the angiosperm supertree. Our results also revealed that although the environmental and spatial variables together contributed to variation in taxonomic, functional and phylogenetic macrophyte communities, dispersal

limitation drove more than environmental filtering for taxonomic and functional dimensions, and pattern was opposite for phylogenetic community component.

#### **4.1 Null models: disentangling the role of stochastic and deterministic processes in community assembly**

We found that functional beta diversity was different from that expected by chance, suggesting the prevalence of deterministic processes in structuring variation of trait composition. This finding partially supported our first hypothesis that some facets of beta diversity are non-random. Nevertheless, the taxonomic and phylogenetic beta diversity dimensions showed signs of stochasticity, indicating that processes explaining taxonomic variation and lineage composition were mostly shaped by random draws from the regional species pool (Hawkins et al., 2015). This type of randomness may also stem from the combined effects of different habitat affinities and dispersal dynamics across phylogenetic lineages (Roa-Fuentes et al., 2019).

More strikingly, the observed multi-trait beta diversity was consistently low relative to random expectations, suggesting that the trait composition of the studied ponds was very similar (Chalmandrier et al., 2017). We interpreted this pattern as a consequence of low functional complementarity between ponds (Heino and Tolonen, 2017). For example, most emergent macrophytes are functionally very different from many groups of aquatic hydrophytes by having different growth forms, life history strategies, phenology, SLA and dispersal vectors (e.g. Willby et al., 2000). Accordingly, if there is at least one helophyte and one hydrophyte in each site, the multi-trait differences among ponds appear to be relatively small. Most of the study ponds harboured at least one species from different major macrophyte functional groups (i.e. emergent, floating-leaved and submerged plants). Hence, as these groups are functionally distantly related and at least one representative of them occurred in most sites,

among-pond variation in functional community composition was low in comparison to that expected from a null distribution (see Heino and Tolonen, 2017).

## **4.2 Phylogenetic signal in traits**

Contrary to our expectations (hypothesis 2), not all measured traits were conserved along the phylogeny. This mismatch stemmed in the moderately low phylogenetic signal of the functional characters both taken individually and together (Table 1). As a consequence, functional trait and phylogenetic patterns appeared quite decoupled in the studied regional metacommunity. The relatively large number of macrophyte families that have repeatedly adapted to diverse freshwater habitats in a relatively short period of time (Barret et al., 1993) might explain the seemingly labile trait evolution pattern. Although weak genetic constraints can also lead to low levels of phylogenetic signal, this is less likely to be the reason since aquatic plants might have experienced frequent convergence and local adaptation (see Barret et al., 1993). These findings suggest that phylogenetic lineages of aquatic macrophytes may not be used as proxies for functional dissimilarity due to weak phylogenetic signal exhibited in species traits. However, these results are extremely dependent on the considered functional characters (Corbelli et al., 2015); thus, the inclusion of alternative or additional traits could even reverse our findings if there is a phylogenetic signal in them. This point certainly requires further attention and would need to be complemented with similar analyses in order to clarify the debate over phylogenetic conservatism in aquatic plant lineages.

## **4.3 Drivers of taxonomic, functional and phylogenetic beta diversity patterns**

As expected from our third hypothesis, we observed that the explanatory power of the analyses increased from taxonomic to multi-trait and phylogenetic metrics. This was also supported by our residual analysis (Supplementary Fig. B.2), although the explanatory powers were generally relatively low. High residual variation is typical for metacommunity studies based on survey data (Cottenie, 2005; Heino et al., 2015) due to site-specific context

dependency, priority effects, historical legacies and stochastic species distribution (e.g. Capers et al., 2010; Alahuhta and Heino, 2013; Heino et al., 2013). Presumably, this unexplained variability in our data again emphasises the importance of stochastic events to the assembly of pond macrophyte metacommunities. Such stochasticity is probably related to small-scale disturbances and chance colonisation, establishment and extinction, which are often related to connectivity between ponds (Capers et al., 2010). It may thus be that apparently idiosyncratic species responses to multivariate gradients are in fact largely trait and phylogenetically mediated in our study (but see Gianuca et al., 2018).

The MSR-based VP models pointed to a strong role of both environmental filtering and spatial processes on macrophyte community assembly patterns. This finding only partly supported our fourth hypothesis, because functional community assemblage should primarily have been driven by environmental filtering (Hoeinghaus et al. 2007, Heino et al. 2013; Cai et al., 2019). Our results suggest that there should be no strong dichotomy between dispersal limitation and species sorting – both community assembly scenarios are extremes along a continuum of processes underlying observed biogeographical patterns (Heino et al., 2015).

Taxonomic and functional beta diversity patterns appeared to be partially spatially controlled, suggesting effective dispersal limitation at the regional extent. Specifically, dispersal limitation interfered with species sorting in determining taxonomic and trait composition by hindering species' tracking of local environmental conditions. After correction for the spatially structured environment, functional data were relatively straightforward in reflecting distributional patterns that are generated by pure spatial effects and dispersal limitation. The lack of spatial signal on phylogenetic patterns suggests that dispersal limitation is probably mediated by the measured functional traits, such as dispersal vector, growth form and seed number (García-Girón et al., 2019a). These findings agree with Capers et al. (2010), O'Hare et al. (2012) and

García-Girón et al. (2019a), suggesting that dispersal limitation can hinder the ability of plant species to reach suitable habitat patches and thereby weaken the strength of species sorting.

Where we did find evidence for dispersal limitation, our results showed that species sorting overrode the effects of spatial factors in explaining phylogenetic beta diversity at the metacommunity scale. This indicates that some of the macrophyte families can occur in a certain limited part of the environment (e.g. Alahuhta and Heino, 2013; Fu et al., 2019). It is hence possible that, as individualistic as the species responses may be, there must be some evolutionary or physiological trade-offs associated with important environmental thresholds (Meynard et al., 2013), which will translate into similar macrophyte lineages occurring and disappearing at the same ponds along the environmental gradient.

Nutrient concentration and pH were the most important environmental drivers in our studied regional metacommunity (Table 2). The importance of pH and nutrient content is related to the use of different forms of carbon (Madsen & Sand-Jensen, 1991) and the chain of effects generally hypothesised in eutrophication processes (Penning et al., 2008), respectively. The influence of both environmental drivers has been well documented in northern Europe (e.g. Elo et al., 2018), North America (e.g. Capers et al., 2010) and elsewhere (e.g. Fernández-Aláez et al., 2018). Overall, despite confounding factors, our results support the idea that productivity is an important driver of both taxonomic and phylogenetic beta diversity patterns.

## **Conclusions**

Historically, taxonomic-based analyses of community assembly have been the most common approach used to characterise regional biodiversity and biogeographical processes (Heino et al., 2015). However, recent efforts to incorporate functional and phylogenetic dimensions into the metacommunity framework have provided further insights into niche- and dispersal-based processes (e.g. Mouquet et al., 2012; Gianuca et al., 2018; Roa-Fuentes et al., 2019). We continued in this path by exploring different beta diversity facets of macrophyte

metacommunities. We used a novel multidimensional approach (including null models and phylogenetic signal evaluations) to examine metacommunity structuring and showed that each dimension of beta diversity responded slightly differently to the underlying spatial and environmental gradients, suggesting distinct community assembly scenarios. Importantly, our analyses based on functional and phylogenetic data identified the influence of underlying mechanisms that would otherwise be missed in an analysis of taxonomic turnover. For instance, these results let us conclude that macrophyte species have labile functional traits adapted to dispersal-based processes and some evolutionary trade-offs that drive community assembly via species sorting. However, the interpretation of results is not straightforward, emphasising the need to go beyond the predominant thinking of considering dispersal limitation and species sorting as two alternative and mutually exclusive scenarios of community assembly (see also Heino et al., 2015; Brown et al., 2017). Our multidimensional study approach may also have repercussions for conservation biology and environmental management, raising the dilemma of which and how diversity facet should be favoured in conservation strategies. We suggest that implementing and assessing conservation strategies using a given diversity dimension as a cure-all should be avoided (Devictor et al. 2010). Instead, conservation biology and environmental management should benefit from pluralistic approaches connecting taxonomic, functional and phylogenetic data. We strongly believe that bridging biogeography, functional and evolutionary ecology holds the key for advancing our discipline and encourage similar analyses for other areas across the globe, which we hope will be found equally beneficial.

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## **Data statement**

The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## **CRedit authorship contribution statement**

**Jorge García-Girón:** Conceptualization; Data curation; Formal analysis; Methodology; Investigation; Writing - original draft; Writing - review & editing. **Camino Fernández-Aláez:** Funding acquisition; Project administration; Resources; Supervision; Validation. **Margarita Fernández-Aláez:** Funding acquisition; Project administration; Resources; Supervision; Validation. **Janne Alahuhta:** Writing - original draft; Writing - review & editing; Supervision; Validation; Visualization.

## **Competing financial interests**

The authors declare no competing financial interests.

## Figure captions

**Fig. 1** Hypothetical scheme depicting scenarios of taxonomic, phylogenetic and multi-trait (functional) beta diversity patterns along a multivariate (e.g. environmental or spatial) gradient. In both cases (a, b), rectangles represent local macrophyte communities (A, B, C). **(a)** Scenario where biogeography acts as a filter for particular functional characters (e.g. growth form, represented by geometric sizes and shapes), resulting in a strong pattern of trait and phylogenetic turnover (i.e. measured traits are conserved along the phylogeny). **(b)** Scenario where the measured functional characters are labile (e.g. growth form varies randomly along the phylogeny), whereas macrophyte lineages are more informative because they represent unmeasured traits that respond uniformly along the multivariate gradient (represented by matching branch colours along the gradient).

**Fig. 2** Flow chart of the statistical analyses used. Check the main text for details. s: species; p: ponds; t: traits; PCAmix: factorial analysis of mixed data; COMDIST: 'COMDIST' function in R package picante; PCO: Principal Coordinate Analysis; SES: Standardised Effect Size; mpd.obs: observed beta diversity; mpd.rnd.mean: mean of the null distribution; mpd.rnd.sd: standard deviation of the null model; e: environmental features; MEM: Moran Eigenvector Maps; MSR: Moran Spectral Randomisation; and VP: Variation Partitioning.

**Fig. 3** Standardised effect size values for each beta diversity dimension: species composition (mean overall value -  $\beta$ MPD; Taxo), functional structure (Trait) and phylogeny (Phylo). Differences from the expected value of zero for random data were assessed through a series of two-tailed *t* tests. Note that only multi-trait beta diversity was different from that expected by chance ( $p < 0.05$ ). Boxplot bold lines = median; box = interquartile range (IQR); whiskers = maximum and minimum up to  $1.5 \times$  IQR.

**Fig. 4** Results of MSR-based spatially constrained VP depicting the relative contribution ( $\%_{\text{adj}}R^2$ ) of local environmental features (green), MEM eigenvectors (yellow) and shared effects



(orange) to variation in different dimensions of beta diversity: species composition (Taxo), functional structure (Trait) and phylogeny (Phylo). Asterisks indicate significant results (\*p < 0.05; \*\*p < 0.01).

**Fig. 5** Evolutionary-traitgram showing patterns of phylogenetic and trait (i.e. growth form) clustering along a gradient of increasing degree of productivity (here measured as nutrient concentration and pH; see O'Hare et al. 2012). The evolutionary-traitgram posits the tips of the phylogeny according to a trait axis (here growth forms; emergent, floating-leaved and submerged), while keeping the internal nodes proportional to evolutionary time (i.e. genetic distance in this example). Each column of the heat-map represents one of the 51 sampled ponds and the colour of each of the circles indicates the relative abundance at the family level in each pond. For more details on the evolutionary-traitgram see Cadotte et al. (2013).

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