**Integrating trait and phylogenetic distances to assess scale-dependent
community assembly processes**

Andros T. Gianuca¹, Steven A. J. Declerck², Marc W. Cadotte³, Caroline Souffreau¹,
Tom De Bie¹, Luc De Meester¹

¹Laboratory of Aquatic Ecology, Evolution and Conservation, KU Leuven, Charles
Deberiotstraat 32, B 3000, Leuven, Belgium

²Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW),
P.O. Box 50, 6700 AB Wageningen, The Netherlands

³Department of Biological Sciences, University of Toronto – Scarborough, 1265
Military trail, Toronto, ON, Canada M1C 1A4

Corresponding author: Andros T. Gianuca, Laboratory of Aquatic Ecology,
Evolution and Conservation, KU Leuven, Charles Deberiotstraat 32, B 3000, Leuven,
Belgium. E-mail: andros.taroucogianuca@bio.kuleuven.be

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Abstract

Biodiversity is structured by multiple mechanisms that are dependent, at least in part, on ecological similarities and differences among species. Integrating traits and phylogenies in diversity metrics may provide deeper insight into community assembly processes across spatial scales. However, different traits are influenced by processes at different spatial scales, and it is not clear how trait-spatial scale mismatches skew our ability to detect assembly patterns. An additional complexity is how phylogenetic distances, which might capture unmeasured traits, reflect spatially dependent processes. Here we analyze a freshwater zooplankton dataset from 91 ponds and show that different traits are associated with processes at different spatial scales. We first assessed the response of individual traits to processes at both α - and β -scales, and then quantified the power of different combinations of traits and phylogenetic distances to reveal environmental and spatial drivers of α - and β -diversity. We found that explanatory power was maximised when we accounted for environmental and spatial drivers with single, but different traits for α - and β -diversity. Using the most appropriate trait for each spatial scale outperformed phylogenetic information, but phylogenetic information outperformed the same traits when these were used at the wrong spatial scale, and all outperformed taxonomic analyses that ignore trait and phylogenetic information. We demonstrate that accounting for species' similarities and differences provides important information about dominant assembly mechanisms at different spatial scales, and that phylogeny is especially useful when measured traits are uninformative at a given spatial scale or when there is lack of trait data. Our study also indicates, however, that trait-scale mismatches among phylogenetically conserved traits may affect the performance of phylogenetic indices compared to indices that account only for the best single trait at each spatial scale.

Keywords. Functional ecology, spatial dynamics, metacommunity

Introduction

Traditionally community ecologists have quantified biodiversity using metrics based on species identities alone, which are blind to ecological similarities and differences among species. Increasingly, trait and phylogenetic-based metrics are being used to link diversity patterns to potential mechanisms operating within (α -diversity) and among (β -diversity) communities (Cavender-Bares et al. 2009, Leibold et al. 2010, Peres-Neto et al. 2012). The use of phylogenies in ecology assumes that closely related species resemble each other more in their traits than do distantly related species, and that phylogenetic indices would synthetically represent species multidimensional niches (Wiens et al. 2010). Phylogenetic measures are therefore expected to provide a richer understanding of both α - and β -diversity drivers than analyses based on a few traits or species identities alone (Graham and Fine 2008, Mouquet et al. 2012, Weinstein et al. 2014).

There are, however, two potential limitations associated with approaches that rely exclusively on phylogeny and disregard trait information. The first and more obvious one is that traits can be labile, so that phylogeny may not really capture species ecological similarities and differences (Gerhold et al. 2015). The second limitation, which is often neglected in community ecology studies, is that confounding or idiosyncratic effects among phylogenetically conserved traits can actually affect the performance of phylogenetic indices (Trisos et al. 2014). This can happen when traits have contrasting responses along environmental gradients, thus canceling each other's signal (Butterfield and Suding 2013, Spasojevic and Suding 2012). With this type of complexity, it seems logical that accounting separately for traits associated with different assembly processes can provide superior explanatory

power in analyses of community assembly than phylogeny [i.e., because phylogenies integrate variation in different traits simultaneously (Trisos et al. 2014)].

Paralleling diversity patterns along multiple environmental gradients, biodiversity patterns at different spatial scales (e.g. within and among communities) can also be controlled by different axes of niche space (Ackerly and Cornwell 2007, Messier et al. 2010, Silvertown et al. 2006). Species coexistence within communities is mainly determined by traits involved in resource exploitation, microhabitat use, or diet (Ackerly et al. 2006). Following Pickett and Bazzaz (1978), Silvertown et al. (2006) used the term α -niche to refer to this local scale dimension of niche differentiation. Conversely, at larger spatial scales species are sorted based on their habitat preferences or environmental tolerances (Cavender-Bares et al. 2006, Cavender-Bares et al. 2009). The term β -niche (Silvertown et al. 2006) has been used to refer to this larger-scale component of niche differentiation as it defines the positioning of species along environmental gradients. Accounting separately for α - and β -traits in analyses of community assembly may therefore enrich our understanding of biodiversity drivers across spatial scales and it is a fundamental problem of approaches that account solely for phylogeny that one cannot separate traits associated with different environmental axes or spatial scales.

Previous trait and phylogeny-based community ecology studies have either been limited to a single spatial scale or have not distinguished α - and β -niche traits in multi-scale analyses [e.g., (Cavender-Bares et al. 2004, Vogt et al. 2013, Weinstein et al. 2014)], thus ignoring potential confounding effects among traits. Because α - and β -niche traits present different responses to processes at different spatial scales (Silvertown et al. 2006), combining those traits may reduce explanatory power due to a trait-spatial scale mismatch. The latter highlights an important challenge in trait and

phylogenetic-based community approaches. Using too few traits can result in a lack of power because the selected traits do not represent the multitude of processes affecting community assembly across spatial scales (Lefcheck et al. 2014, Kraft et al. 2015).

Conversely, using several traits or phylogenetic information can introduce confounding effects in the analysis if these traits are associated with different structuring mechanisms (Spasojevic and Suding 2012, Trisos et al. 2014).

As a step forward, we here propose a conceptual framework for integrating trait and phylogenetic data into community analyses aiming at maximizing analytical performance and enhancing the interpretation of environmental and spatial drivers of community assembly and turnover. We propose that measured traits should be evaluated separately and then classified *a posteriori* based on their responses to α - and β -scale assembly processes. This avoids *a priori*, artificial trait categorization and can be easily done because α -niche trait(s) are by definition more informative locally whereas β -niche trait(s) are more informative at a regional scale (Fig. 1) (Silvertown et al. 2006). We then propose to integrate α - and β -traits separately with phylogenetic distances through the functional-phylogenetic approach developed by Cadotte et al. (2013). One important aspect of the functional-phylogenetic approach is that it allows differential weighting of traits and phylogeny so that investigators can assess the relative contributions of the two measures to explaining community patterns (Cadotte et al. 2013). While originally used to study α -diversity, we here extend its application to study β -diversity as well, thus achieving straightforward comparisons across spatial scales. This extension allows us to better understand if trait and phylogenetic approaches are equally informative across spatial scales or rather complement each other. The power of our approach is that one can account for α - and β -niche traits separately and use phylogenetic information as a benchmark to assess the

performance gain obtained by this more encompassing representation of species multidimensional niches.

To illustrate our conceptual framework we integrated traits and phylogenetic information to assess scale dependent assembly patterns in a regional zooplankton metacommunity involving 91 ponds and shallow lakes, using phylogenetic information as a benchmark to assess the informative power of different traits at different spatial scales. Specifically, we applied our framework to test three key ideas: (i) depending on the spatial scale considered (α -scale, β -scale), functional diversity in metacommunities is structured by different environmental or spatial processes that relate to different traits; (ii) different trait responses to α - and β -scale assembly processes affect the performance of combined trait indices in community analyses; and (iii) phylogenetic distances are informative at both spatial scales and can be used as a substitute for uninformative or missing traits, but might be outperformed by trait-based analyses that maximize the match between trait and spatial scale. We also compared trait and phylogenetic patterns with more traditional taxonomic-based approaches to assess the performance gain obtained by integrating traits and phylogeny into community analyses. We note that there are likely to be a multitude of traits involved in α - and β -niche processes. However, as a means of testing our proposed conceptual framework, we focus here on two key zooplankton traits that are expected to behave differently across spatial scales.

Methods

Functional-phylogenetic framework for α - and β -diversity analyses

Our framework is based on the idea that different traits can be integrated with phylogeny in α - and β -diversity analyses to maximize the match between relevant

niche dimensions and spatial scales. Functional trait (FDist) and phylogenetic distance (PDist) matrices can be combined into functional-phylogenetic distance matrices (FPDist) following Cadotte et al. (2013). This can be done by weighting the independent contributions of PDist (a) and FDist ($1-a$) to FPDist as follows: $FPDist = [aPDist^p + (1-a)FDist^p]^{1/p}$, where p represents a p-norm distance that is analogous to Euclidean distance when $p = 2$ (Cadotte et al. 2013). Functional-phylogenetic distance can thus be viewed as a compromise between functional and phylogenetic information, which can be tuned with the parameter, a . When $a = 1$, FPDist only represents the pure phylogenetic distances (i.e. is equal to PDist) and when $a = 0$, FPDist represents the pure functional trait distances (i.e. is equal to FDist). Intermediate values of parameter a imply that both sets of information are used and their relative contribution is weighted by a .

Integrating α - and β -traits with phylogeny in α - and β -diversity analyses provides a strong diagnostic tool to scale the explanatory power of specific traits to processes at different spatial scales as well as to account for unmeasured, phylogenetically conserved traits associated with each spatial scale (Fig. 2). Theoretically, there are several possible outcomes in trait and phylogeny-based community analyses across spatial scales, which ultimately depend on which traits are measured and which traits are labile or conserved (Fig. 2) [see also (Cadotte et al. 2013)]. First, confounding effects among α - and β -traits may affect the performance of phylogenetic-based approaches if both α - and β -niches are conserved along the phylogeny. Therefore, it may be more powerful to account directly for α - and β -traits separately in analyses of α - and β -diversity, respectively, than accounting solely for phylogeny (Fig. 2a-b). This pattern can be detected by an increase in explanatory power of the analysis towards pure trait information (i.e., low values of the parameter

α), with different traits responding to processes at different spatial scales. However, α - and β -niche traits may evolve under different rates (Ackerly et al. 2006, Silvertown et al. 2006), so that phylogeny can be uninformative at a given spatial scale (i.e., due to labile traits) and, at the same time, highly informative at another spatial scale (i.e., due to phylogenetic signal in unmeasured traits) (Fig. 2c-d). Additionally, phylogeny may be more informative than the measured traits at a given spatial scale if it better represents variation in unmeasured traits associated with that spatial scale (Fig. 2 e). Such pattern will lead to an increase in informative power towards pure phylogenetic information (i.e., high values of the parameter α). Finally, traits and phylogeny may complement each other when measured traits are highly informative but labile and phylogeny represents variation in additional, unmeasured traits that are highly informative at the given spatial scale [see also (Cadotte et al. 2013)]. This will lead to an increase in informative content for intermediate α -values.

Empirical example with freshwater zooplankton

In the summer of 2003, zooplankton samples were collected in 99 permanent farmland ponds across Belgium (c. 30,500 km²) (De Bie et al. 2012). To maximize variation in environmental conditions and disturbance levels, a stratified sampling was imposed in which 33 ponds were selected in nature reserves, 33 in extensive agricultural landscapes, and 33 in areas of intensive agriculture. Cladoceran community composition was characterized by counts and individuals were identified to species level (De Bie et al. 2012). We excluded ponds (n=8) where only one species was recorded. Physical, chemical and morphometric pond variables were also collected and are described elsewhere (De Bie et al. 2012) (see also Appendix S1 in supporting information). Based on previous work on cladoceran metacommunities,

we selected 12 environmental variables: concentration of total phosphorus and nitrates, pH, water transparency, conductivity, water temperature, submerged vegetation cover, floating vegetation cover, pond area, shaded area of the pond surface, abundance of fish, and presence of the invertebrate predator *Chaoborus*. We also included regional land use as an ordinal variable (i.e. 1 = nature reserve; 2 = extensive agricultural land use; and 3 = intensive agricultural land use). For a detailed description of the sampling methods see Appendix S1.

Trait data

We accounted for two key traits that have been repeatedly suggested to affect zooplankton community structure and turnover: body size and degree of association with (submerged) vegetation. Body size is linked to competitive ability and resource partitioning in cladoceran zooplankton (Brooks and Dodson 1965, DeMott and Kerfoot 1982, Gliwicz and Lampert 1990). Thus, we expected body size to behave primarily as an α -niche trait, even though it can also be associated with fish predation pressure (Brooks and Dodson 1965) and thus with species turnover across habitats. This illustrates challenges related to trait classification *a priori* in terms of α - and β -niches components. Species association with (submerged) vegetation is expected to represent an important β -trait for cladoceran zooplankton (Declerck et al. 2007, Declerck et al. 2011). Specifically, depending on their degree of association with submerged vegetation, zooplankton species tend to be sorted out along the typical turbid – clear water gradient in shallow waters (Scheffer 2004). In practice, however, it is likely that the association of traits with α - or β -niche axes are not mutually exclusive but rather vary in strength.

Information on body size values and on the degree to which the species were associated with (underwater) macrophytes were extracted from the literature. Body size is a continuous variable, whereas the strength of plant association was quantified as an ordinal variable (1 = pelagic; 2 = intermediate (opportunistic, generalist); 3 = littoral, strongly associated with macrophytes). All trait values and associated references can be found in Appendix S1.

Molecular-phylogenetic tree reconstruction

We built a molecular-phylogenetic tree for 35 of the 40 cladoceran species occurring in the sampled metacommunity following a recent protocol (Roquet et al. 2013). Information on four molecular markers (COI, and 16S, 18S and 28S rDNA) was extracted from Genbank for the 35 species using the browser “Geneious”. Maximum Likelihood (ML) tree reconstruction and bootstrapping was performed using RAxML (<http://phylobench.vital-it.ch/raxml-bb/>) (Stamatakis 2006). Finally, an ultrametric tree was constructed using the Penalized Likelihood method (Kim and Sanderson 2008). A complete description of the method used is given in Appendix S1.

Calculation and integration of phylogenetic and trait distances

A cophenetic phylogenetic distance matrix (PDist) was calculated from the branch lengths of the phylogenetic tree. A Euclidean trait distance matrix (FDist_{body size}) was calculated using body size values for all zooplankton species in the metacommunity. Finally, we also calculated a Euclidean trait distance matrix based on plant association (FDist_{plant assoc}).

Using these distance matrices, we constructed functional-phylogenetic distance matrices (FPDist) following Cadotte et al. (2013). We built 101 FPDist

matrices corresponding to a values ranging from 0 to 1 at intervals of 0.01. For $a = 0$, FPDist represents the pure functional trait distances, for $a = 1$ FPDist only represents the pure phylogenetic distances. Intermediate a -values indicate that both sets of information are integrated. These FPDist matrices were used *a posteriori* to estimate under which relative contribution of functional and phylogenetic information (i.e. at which a -value) our understanding of environmental and spatial drivers on biodiversity patterns is maximized using a variation partitioning approach (see further). We first combined information in our FPDist matrix based on the two traits (body size and strength of plant association), separately. We then also combined information on body size and plant association with phylogenetic information. Because one trait is categorical and the other is continuous, we used Gower's distance instead of Euclidean distance to calculate the combined trait distance matrix (Borcard et al. 2011).

Phylogenetic signal in traits

We assessed the level of phylogenetic signal in body size and plant association through the K-statistic (Blomberg et al. 2003). The K-statistic can be compared to randomized K values and significant p-values indicate non-random correspondences between traits and phylogeny. Values of K higher than 1 indicate that traits are more conserved than expected by a Brownian Motion Evolutionary model (Blomberg et al. 2003). Values lower than 1 indicate that traits are less conserved along the phylogeny than expected by this model.

Functional-phylogenetic α -diversity across a -values

We calculated the abundance-weighted version of the mean functional-phylogenetic distance across α -values (MFPD, hereafter α -FPD) within communities. α -FPD considers information on the average sum of evolutionary and/or trait distances (depending on the α -values) among species in local communities (Cadotte et al. 2013), and quantifies the level of clustering (i.e., communities that contain species that are more similar/more related to each other than expected by chance) or overdispersion (i.e., communities comprised of species that are less similar/less related than expected by chance). In order to ensure that differences in trait-phylogenetic structure were not an artifact of the number of species, we calculated the standardized effect size. To do this, we shuffled species names in the distance matrix 999 times to create a functional-phylogenetic null distribution of taxa in local communities, keeping the entire community data matrix constant (i.e. abundance values and frequencies remained untouched) (Swenson 2014).

Functional-phylogenetic β -diversity across α -values

β -diversity was calculated based on each of the 101 FPDist matrices using mean pairwise functional-phylogenetic dissimilarity among pairs of local communities (Swenson 2014), using the function COMDIST in picante statistical package (Kembel et al. 2010). For simplicity, we hereafter refer to this index as β -FPD. Abundance values of species were considered when calculating β -FPD. We then applied Principal Coordinates Analysis (PCoA) over the 101 β -FPD dissimilarity matrices (Swenson 2014). The final product is a matrix of orthogonal PCoA eigenvectors, each of them describing functional-phylogenetic β -diversity patterns, which can be used as response variables in constrained ordinations (Anderson and Willis 2003, Swenson 2014).

Taxonomic α - and β -diversity

We used traditional taxon-based community analyses as a benchmark to assess the performance gain achieved by more ecologically detailed trait- and phylogenetic approaches. To describe taxonomic α - and β -diversity patterns we choose widely used indices that take into account abundance values of species, making it comparable to the above-described functional-phylogenetic indices. We used the exponent of the Shannon-Wiener index (i.e., Shannon entropy, Jost 2006) as our metric of taxonomic α -diversity patterns. We compared results obtained for Shannon entropy with those obtained for taxonomic species richness and evenness (see Appendix S1). Results obtained for these three taxonomic diversity metrics were very similar (Appendix S1), and therefore we report in the main text only results of Shannon entropy. As a metric of taxonomic β -diversity, we used the Bray-Curtis index of dissimilarity. We then applied PCoA to the Bray-Curtis dissimilarity matrix using Lingo's correction (Borcard et al. 2011) and kept the eigenvectors to be used as response variables in the subsequent analyses.

Generating spatial descriptors

We used the geographical coordinates (UTM) of the sites to generate spatial variables by means of Principal Coordinates of Neighboring Matrices (PCNM) (Borcard and Legendre 2002). This technique allows assessing multiple spatial structures over the entire range of scales covered by the geographical sampling area. The first PCNMs generated in the analyses represent broader spatial structures, while the last ones cover finer spatial scales (Borcard and Legendre 2002).

Selection of response variables: orthogonal eigenvectors describing β -diversity patterns

In principle, all eigenvectors generated by PCoA could be used as descriptors of β -diversity patterns in subsequent analyses. However, using all of them might introduce confounding effects in the analyses. Since each eigenvector is an orthogonal synthetic variable representing gradients in β -diversity patterns, it is likely that some of these gradients are unexplained by the measured factors. A solution is to select a subset of orthogonal eigenvectors that maximizes the association between patterns of β -diversity (taxonomic or functional-phylogenetic) and a set of explanatory variables. To select the appropriate number of PCoA eigenvectors to be used in subsequent analyses, we followed the procedure proposed by Anderson and Willis (2003) (for more details see Appendix S1).

Disentangling the environmental and spatial drivers of α - and β -diversity

Variation partitioning allows disentangling the variation in community data (here also weighed by trait-phylogenetic distances) into a pure environmental component, a pure spatial component, a spatially structured environmental component, and an unexplained component of variation (Borcard et al. 1992). Before performing variation partitioning, explanatory variables were selected using forward selection (Blanchet et al. 2008). We selected the most parsimonious subset of spatial and environmental variables for each *FPD*-metric (i.e., across *a*-values). This allowed us to maximize the fit between explanatory and response data matrices while minimizing type I error. We also compared these results with analyses keeping the same number and type of explanatory variables for the entire range of *a*-values. Results of both approaches were qualitatively very similar, thus we report the results of the former

approach only, which has a better compromise between explanatory power and type I error. Following Cadotte et al. (2013) we used the $_{\text{adj}}R^2$ (i.e., environment and space combined) as our measurement of information content for different combinations of trait and phylogenetic information (i.e., across α -values) for α - and β -diversity analyses. Based on the conceptual scheme in figure 2, we tested the idea that functional diversity is structured by different traits at different scales. This idea is supported when the most informative trait at one spatial scale (i.e., quantified by the $_{\text{adj}}R^2$; α -value = 0) is the least informative at the other spatial scale and vice-versa (Fig. 2). Similarly, we tested the idea that combining α - and β -traits reduces overall explanatory power (Fig. 1). This was done by quantifying the decrease in informative power ($_{\text{adj}}R^2$) obtained for single traits and that obtained when combining both traits in α - and β -diversity analyses. To test the idea that phylogenetic distances are informative at both spatial scales but can be outperformed by trait-based analyses that ensure a good match between trait and spatial scale, we quantified, for each spatial scale the increase or decrease in information content ($_{\text{adj}}R^2$) obtained by increasing phylogenetic weighing (i.e., increasing α -values; Fig. 2). All analyses were performed in R (R Core Team 2014) and a detailed description of the methods and packages used can be found in Appendix S1.

Results

Taxonomic patterns for α - and β -diversity

Taxonomic α -diversity was not significantly explained by any environmental or spatial variable (Table 1). For the taxonomic β -diversity, environmental and spatial factors combined explained approximately 18% of variation in species composition among ponds ($_{\text{adj}}R^2 = 0.182$, $p = 0.001$). From this total, about three quarters was

determined purely by the environmental model (i.e, submerged vegetation; *Chaoborus*; pH and nitrates), approximately one quarter was purely explained by spatial processes, and the shared effects of spatial and environmental variables accounted for 1.5% (Table 1).

Phylogenetic signal in traits

K-statistics revealed that body size was not significantly conserved along the phylogeny ($K = 0.04$, $p = 0.243$). In contrast, plant association was strongly conserved along the phylogeny ($K = 2.034$, $p = 0.001$).

Testing idea I: Is functional diversity structured by different traits at different scales?

When analyzing traits ($\alpha = 0$) separately in α -diversity analyses we found that body size responded stronger than plant association to both environmental and spatial processes operating at this local scale (Fig. 3a, Table 1). Body size was 22.8% more informative (as measured by differences in $_{adj}R^2$) than plant association for α -diversity analyses. Conversely, β -diversity analyses indicated that plant association responded much stronger than body size to (environmental) processes, even though body size was still informative for β -scale analyses (Fig. 3b, Table 1). Plant association was 30.8% more informative than body size for β -diversity analyses. Environmental (pH and NO^3) and spatial variables were similarly important in affecting body size diversity at local scales (Table 1). With respect to the environmental signal at the local scale, we observed a shift from size clustering to overdispersion along a gradient of pH (Appendix S2). Importantly, the pattern of size overdispersion in ponds with high pH was accompanied by an increase in abundance of larger species (Fig. 4).

Under low pH values, small species dominated, while at higher pH values both small- and large-bodied species coexisted (see Fig. S2 in Appendix S3).

Testing idea II: Combining traits reduces overall explanatory power

We found that combining both body size and plant association in trait-based analyses ($a = 0$) was suboptimal compared to accounting separately for body size at the α -scale and for plant association at the β -scale (Fig. 3, Table 1). In contrast, we found that integrated trait indices outperformed indices accounting solely for body size alone when this trait was used at the β -scale (Fig. 3b). Integrated trait indices similarly outperformed an index weighed by plant association alone for α -diversity analyses (Fig. 3a, Table 1).

Testing idea III: Phylogeny is informative at both spatial scales but is outperformed by trait analyses accounting only for the most informative trait at each spatial scale

Integrating trait and phylogenetic information via phylogenetic weighing revealed that the explanatory power of α -diversity patterns was maximized when focusing on body size information and decreased as more phylogenetic information was considered (i.e., towards large a -values) (Fig. 3a, Table 1). This indicates that a single trait (body size) was more informative than phylogeny in determining diversity patterns at this spatial scale. Conversely, integrating information on plant association and phylogeny resulted in increasing explanatory power with increasing phylogenetic weighting (i.e., towards large a -values) for the analysis of α -diversity (Fig. 3a, Table 1). For a complete overview of $_{\text{adj}}R^2$ values and the list of significant explanatory variables across a -values see Appendix S2.

When considering β -diversity patterns, we observed that the maximal explanatory power was obtained towards pure plant association information, and decreased with increasing phylogenetic weighing (towards large α -values) (Fig. 3b, Table 1). In contrast, while the correlation between explanatory variables and body size was significant (Table 1, Appendix S2), accounting for body size information was less informative than accounting for phylogenetic information at this spatial scale (Fig 3b).

Discussion

Increasingly, trait and phylogenetic data is being used to get new insights into biodiversity drivers across spatial scales. The underlying assumption is that using phylogenetic data or combining different traits into trait-based analyses can capture a significant portion of species fundamental niches, thus enhancing our understanding of the processes structuring biodiversity (Mouquet et al. 2012, Spasojevic et al. 2014). Here we examined community assembly in freshwater zooplankton and found that mixing traits associated with processes that operate at different spatial scales (here: α - and β -scale processes) in diversity metrics was suboptimal compared to using a single, most informative trait for each spatial scale. This supports that idea that the measured traits are associated with different axes of niche space and can provide complementary insights on community assembly across spatial scales. The most sensitive trait to α -scale processes, body size, contributed little to diversity patterns among communities, while plant association was very strongly related with species turnover among habitats but was not informative for α -diversity. At both scales, using the most informative trait resulted in a higher explanatory power of environmental and/or spatial processes than incorporating phylogenetic distances. However, phylogenetic distances were more informative than the same traits when these were

used at the wrong spatial scale, suggesting that phylogeny represents both α - and β -niches (Fig. 2a-b). Integrating phylogenetic and trait information is thus important in adding a benchmark to scale the explanatory value of specific traits, and allows optimization of explanatory power in terms of unmeasured, phylogenetically conserved traits. Turning this reasoning around, it is also possible, by measuring phylogenetic signal in traits associated with processes at different spatial scales (Blomberg et al. 2003), to understand how long-term evolutionary processes affect current species distributions across spatial scales.

Body size was more informative for α -diversity patterns than phylogeny, plant association or taxonomic information. Surprisingly, fish abundance – often a key variable determining zooplankton size structure (Brooks and Dodson 1965) - was not significantly related with body size α -diversity in our study. A potential explanation for this is that most of the ponds in our regional metacommunity were either fishless or presented low fish abundances. Conversely, we observed that local communities shifted from clustering to overdispersion along a gradient of pH. In our study system, pH ranged from approximately 6.5 to 9.5 (Appendix S3), so that the pattern of trait clustering in ponds with low pH unlikely results from physiological stress associated with acidic conditions, as reported for Canadian lakes (Helmus et al. 2010). While it is not possible to identify the mechanism mediating the positive relationship between size diversity and pH from our dataset with any certainty, it may reflect a response to a productivity gradient. High pH values in aquatic systems are often associated with high productivity [alkaline lakes being often more productive, and the consumption of CO₂ by photosynthesis increasing pH directly; (Moss 2010)], which in turn tends to favor dominance by the larger *Daphnia* species (Dodson et al. 2000). To further explore this possibility, we quantified to what extent pH in our dataset was correlated

to environmental variables linked to productivity. We indeed observed an overall positive correlation between values of pH, turbidity, total phosphorus, and chlorophyll *a* concentrations, which are all productivity related variables (total phosphorus being the key limiting nutrient in most freshwater systems and chlorophyll *a* quantifying algal biomass, see Appendix S3 for the relationships). For cladoceran zooplankton, observational and experimental studies have demonstrated that low productivity may result in the elimination of larger species from communities likely due to food limitation (Brett et al. 2000, Dodson et al. 2000, Tillmann and Lampert 2011). Low pH or low associated productivity may thus have acted as a filter on body sizes in our study systems, preventing the establishment success of large species (Appendix 3). Conversely, the establishment of larger species in more productive ponds with higher pH may have resulted in the observed pattern of overdispersion in these ponds. We also note that the presence of the invertebrate predator *Chaoborus*, along with pH, significantly explained the shift from small to large zooplankton species in our system of ponds (Appendix S2, Table 5). This predator tends to select smaller zooplankton species (Riessen et al. 1988), so that predator release may have allowed small species to dominate in some ponds, resulting in size clustering.

While body size was very informative and plant association was uninformative for α -diversity, the information content of phylogenetic information was intermediate. The decrease in explanatory power from pure body size towards phylogenetic information within communities probably reflects the lack of phylogenetic signal in body size and suggests that size variation is more structured by the studied environmental and spatial drivers than other, more conserved traits. Lack of phylogenetic signal in key traits is known to erode the phylogenetic signal on community assembly (Mouquet et al. 2012, Wiens et al. 2010). Similar results were

found for butterflies (Pavoine et al. 2014) and marine herbivores (Best et al. 2013), in which lack of conservatism in key traits associated with species coexistences resulted in low phylogenetic signal on α -diversity.

Plant association had high explanatory power for β -diversity in the zooplankton metacommunity we studied, reflecting strong habitat filtering. Specifically, we observed that as environmental conditions changed along the typical turbid – clearwater habitat gradient in shallow waters (Scheffer 2004), zooplankton communities shifted from a dominance of pelagic species to a dominance of littoral species. This resulted in a pattern of clustering within communities and a high turnover among communities. The patterns for α - and β -diversity observed for plant association are therefore strongly linked, as they both reflect habitat filtering [see also Messier et al. (2010) for a similar example]. Similarly, for body size the patterns of α - and β -diversity are also linked, as the strong signal for α -diversity is the result of a shift from clustering to overdispersion, which is fueled by species turnover involving large species being excluded from low pH systems. Although body size is less informative for β -diversity than plant association or phylogenetic information, it still explains 17% of the observed variation in communities among habitats.

Despite the strong phylogenetic signal in plant association, explanatory power for β -diversity analyses decreased from pure plant association towards pure phylogenetic information. While this seems counterintuitive at first sight, it likely reflects the fact that incorporating information on multiple trait axes that are associated with different processes may reduce the explanatory power of phylogenetic analyses (Butterfield and Suding 2013). Indeed, a recent simulation study demonstrated that such contrasting signals among phylogenetically conserved traits affect the performance of phylogenetic metrics (Trisos et al. 2014). Recent empirical

studies have similarly shown that different traits can respond in contradictory ways to environmental gradients, so that focusing on single traits in separation may increase the explanatory power and interpretability of diversity patterns compared to multi-trait and phylogenetic analyses (Butterfield and Suding 2013, Spasojevic and Suding 2012). This is also illustrated by our own trait-based analysis, where we observed that including body size and plant association was suboptimal compared to using the best single trait for each scale of species distributions.

While our results show the usefulness of distinguishing traits for their association with within- or among-habitat processes, they also highlight that patterns in nature are more complex and that traits can behave both as α - and β -niche traits. For example, we observed that cladoceran body size was very important for α -diversity, but at the same time was still important for β -diversity patterns, as the pH gradient and the presence of the predator *Chaoborus* created a shift in body size among ponds. The degree to which a trait may be more α - or β -niche related can vary among metacommunities and environmental conditions. It is therefore not an easy task to identify *a priori* which trait should be classified as α - and β -niche traits. Instead, the method we apply here can actually be used as a diagnostic tool to determine the degree to which a trait is associated with α - and β -niches, using phylogenetic information as a benchmark (Fig. 2). This is important because, due to limitations in time and resources, trait-based approaches often depend on *a priori* decisions on which traits should be measured. In our data set, phylogenetic distances did provide additional explanatory power at both spatial scales over a non-informed taxonomic analysis based on species identity alone and also when there was a trait-scale mismatch, such as when using the β -niche trait to study α -diversity patterns. Our approach differs from that proposed by Ackerly and Cornwell (2007) in that it does

not rely exclusively on intraspecific trait information. Our approach integrates trait and phylogeny to account for potentially informative, unmeasured α - and β -traits, and it can be used in a multivariate environmental and spatial context.

Besides increasing the overall explanatory power of the analyses, the trait-phylogenetic approach also differed in the relative importance of environmental and spatial drivers of metacommunity structure compared to the taxonomic approach. The variation partitioning analysis indicated that taxonomic β -diversity was both structured by environmental determinism as well as pure spatial processes. The influence of spatial processes was, however, not significant when we analyzed trait and phylogenetic β -diversity patterns. We see at least two plausible explanations for this. First, as we include more trait-related information, we better approximate species niches, so that we better capture the impact of ecological determinism in structuring communities. Alternatively, the pure spatial signal for taxonomic β -diversity may reflect true dispersal limitation, which is not occurring at the trait or phylogenetic level. This would occur when, for example, communities are assembled through trait sorting, while there is dispersal limitation with respect to which species represents a given trait. The lack of spatial constraints on the phylogenetic species pool is in line with previous findings for freshwater cladoceran communities in North America (Leibold et al. 2010). While we did not observe a pure spatial signal in the trait-phylogenetic analysis of β -diversity, body size α -diversity was spatially structured. To explain this, we conducted additional analyses, which reveal that this pattern is likely mediated by dispersal limitation of large-bodied species, which are strong competitors (see Appendix 4). Hence, traits related to α - and β -scale processes also differed in their environmental determinants and in the importance of spatial signals. This difference was not detectable when considering overall functional diversity. Thus,

accounting separately for traits related to different environmental gradients and spatial scales allows more straightforward interpretation of the drivers of diversity within and among communities.

In summary, our results support the hypothesis that different functional traits can be associated with distinct aspects of species' niche use, and that confounding effects among such traits compromise the performance of metacommunity analysis compared to single trait metrics (Butterfield and Suding 2013, Spasojevic and Suding 2012). Such confounding effects can also affect the performance of phylogenetic metrics (Trisos et al. 2014). To overcome these limitations, we propose an approach in which α - and β -traits are analyzed separately and evaluated for their explanatory power using phylogenetic information as a benchmark in an integrated analysis of functional and phylogenetic distances (Fig. 2). We demonstrated that phylogenetically informed analysis provides a strong benchmark for assessing the explanatory power of trait-based approaches at different spatial scales. Therefore, if measured traits do not match the spatial scales considered or if there is lack of trait data, phylogeny provides a valuable substitute for taxonomy-based community approaches. While we here only considered local and regional spatial scales, we envision that a similar approach can be used to analyze patterns ranging from local to continental and global scales.

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Table Legend

Table 1. Proportion ($_{\text{adj}}R^2$) of the total variation in α -FPD and β -FPD explained by the total environmental and spatial model (EUS), by a pure environmental signal (E/S), by a pure spatial signal (S/E), and by the shared effect of space and environment ($E \cap S$), as estimated by variation partitioning, and the corresponding p-values. For trait-phylogenetic metrics we only report the best combination between trait and phylogenetic information and list the α -value for which this maximum is obtained. Body size + Phylogeny refers to diversity metrics integrating information on body size and phylogeny. Plant association + Phylogeny refers to diversity metrics integrating information on plant association and phylogeny. Body size and plant association + Phylogeny refers to diversity metrics integrating information on both traits with phylogeny.

Index	$_{\text{adj}}R^2$			
	EUS	E/S	S/E	$E \cap S$
<i>α-diversity</i>				
Taxonomic (Shannon entropy)	0	0	0	0
Body size + Phylogeny				
α -FPD ($\alpha = 0$) (i.e., pure body size)	0.228***	0.091**	0.074**	0.061
α -FPD ($\alpha = 0.01$) (maximal R^2 obtained)	0.229***	0.092**	0.071**	0.064
Plant association + Phylogeny				
α -FPD ($\alpha = 0$) (i.e., pure plant association)	0	0	0	0
α -FPD ($\alpha = 0.73$) (maximal R^2 obtained)	0.131***	0.131***	0	0

Index	$\text{adj}R^2$			
	EUS	E/S	S/E	$E \cap S$
α -FPD ($a = 1$) (i.e., pure phylogeny)	0.060**	0.060**	0	0
Body size and plant association + Phylogeny				
α -FPD ($a = 0$) (maximal R^2 obtained)	0.141***	0.141***	0	0
β -diversity				
Taxonomic (Bray-Curtis)	0.182***	0.121***	0.045**	0.015
Body size + Phylogeny				
β -FPD ($a = 0$) (i.e., pure body size)	0.175***	0.175***	0	0
β -FPD ($a = 0.71$) (maximal R^2 obtained)	0.296***	0.296***	0	0
Plant association + Phylogeny				
β -FPD ($a = 0$) (i.e., pure plant assoc.)	0.483***	0.483***	0	0
β -FPD ($a = 0.04$) (maximal R^2 obtained)	0.495***	0.495***	0	0
β -FPD ($a = 1$) (i.e., pure phylogeny)	0.291***	0.291***	0	0
Body size and plant association + Phylogeny				
β -FPD ($a = 0.13$) (maximal R^2 obtained)	0.410***	0.410***	0	0 ¹

¹ Significant results are indicated with asterisks as follows: $p < 0.001$ “***”; $p < 0.005$ “**”.

Figure Legends

Figure 1. Information on α -niche traits provides increased explanatory power on drivers of α -diversity but will be of no or low information content for patterns of β -diversity. Conversely, information on β -niche traits provides increased explanatory power on drivers of β -diversity but will be of no or low information content for patterns of α -diversity. Combining both types of traits results in reduced information content compared to only including the trait matching the spatial scale of interest, but provides increased information compared to only including traits that are associated with processes at the other spatial scale. The added value of including phylogenetic information over purely taxonomic metrics or trait-based approaches will depend on whether α - or β -niche traits are phylogenetically conserved. If phylogeny captures information on both α - and β -niches then phylogenetic-based metrics will behave similarly to trait-based metrics that combine α - and β -niche traits.

Figure 2. Conceptual scheme on how to optimally integrate trait and phylogenetic distances in α - and β -diversity analyses. In the vertical axis, information content describes the strength of the response of different traits and phylogeny to α - and β -scale assembly processes. In the horizontal axis, a -values refer to the phylogenetic weighing parameter, which allows integration of trait and phylogenetic distances [see methods for details and also Cadotte, M. et al. (2013)]. For a -value = 0 only trait information is taken into account; for a -value = 1 only phylogenetic information is considered. Intermediate a -values indicate that both trait and phylogenetic distances are combined. In (a) and (b) both α - and β -niches are phylogenetically conserved so that phylogeny is informative at both spatial scales. In such cases it may be more powerful to account only for α -traits in α -diversity analyses (a) and only for β -traits in β -diversity analyses (b). In (c-d) phylogeny captures information on the β -niche, but not the α -niche, so that phylogeny is uninformative for α -diversity but highly informative for β -diversity analyses (the opposite is true when phylogeny captures information on the α -niche, but not the β -niche; not shown). In (e) phylogeny better represents variation in the α -niche than the measured α -trait(s). The same can occur for β -diversity (not shown). In (f) phylogeny complements the information provided by the measured traits, so that higher information content is obtained for intermediate a -values. This can also happen for α -diversity [not shown; but see also Cadotte, M. et al. (2013)]. The schemes depicted here are a simplification of reality as we assume one single α - and β -niche trait, whereas in reality there are a multitude of traits that are integrated when using phylogenies, and there are a multitude of traits involved in α - and β -niche processes.

α - and β -niches phylogenetically conserved

α -niche labile and β -niche conserved

Phylogeny more informative than measured α -traits

Phylogeny complements measured β -traits

α -niche trait(s) integrated with phylogeny ———

β -niche trait(s) integrated with phylogeny ———

Figure 3. Proportion of total explained variation (i.e., environmental and spatial processes combined; as given by $_{\text{adj}}R^2$) for functional-phylogenetic α -diversity (a) and β -diversity (b) across a -values (a ranges from 0 where only trait information is taken into account to 1 where only phylogenetic information is considered, in steps of 0.01). At intermediary a values, both trait distance and phylogenetic distance are combined. The red line refers to analyses where body size information is combined with phylogenetic information; the blue line refers to analyses where plant association information is combined with phylogenetic information. The purple line refers to a situation in which both traits are combined with phylogenetic information. LOESS smoothing was used to aid visualizing and interpreting the general patterns.

Figure 4. Evolutionary-trait gram depicting patterns of trait and phylogenetic (i.e., α -FPD body size + phylogeny) clustering versus overdispersion for each of the communities of all the 91 sampled ponds. The evolutionary-traitgram combines phylogenetic and trait information and is useful to assess patterns of trait evolution (i.e., divergences, convergences) because it posits the tips of the phylogeny according to a trait axis (here body size, ranging from 0.26 to 4 mm), while keeping the internal nodes proportional to evolutionary time (i.e., genetic distance in this example). Each column of circles represent one of the 91 sampled ponds and the color of each of the circles represents the relative abundance of each species represented at the tip of the phylogeny in each of the ponds. Communities are ordered according to α -FPD, ranging from clustered to overdispersion.