MACROECOLOGICAL METHODS

How many dimensions are needed to accurately assess functional diversity? A pragmatic approach for assessing the quality of functional spaces

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ABSTRACT

Aim Functional diversity is a key facet of biodiversity that is increasingly being measured to quantify its changes following disturbance and to understand its effects on ecosystem functioning. Assessing the functional diversity of assemblages based on species traits requires the building of a functional space (dendrogram or multidimensional space) where indices will be computed. However, there is still no consensus on the best method for measuring the quality of functional spaces.

Innovation Here we propose a framework for evaluating the quality of a functional space (i.e. the extent to which it is a faithful representation of the initial functional trait values). Using simulated datasets, we analysed the influence of the number and type of functional traits used and of the number of species studied on the identity and quality of the best functional space. We also tested whether the quality of the functional space affects functional diversity patterns in local assemblages, using simulated datasets and a real study case.

Main conclusions The quality of functional space strongly varied between situations. Spaces having at least four dimensions had the highest quality, while functional dendrograms and two-dimensional functional spaces always had a low quality. Importantly, we showed that using a poor-quality functional space could led to a biased assessment of functional diversity and false ecological conclusions. Therefore, we advise a pragmatic approach consisting of computing all the possible functional spaces and selecting the most parsimonious one.

Keywords

Diversity indices, functional dendrogram, functional dissimilarity, functional ecology, functional traits, multidimensional space.

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INTRODUCTION

In the current context of the sixth biodiversity crisis, assessing the level of biodiversity within species assemblages is a crucial step to better understand and predict the impacts of global change on ecosystem functioning (Cardinale *et al.*, 2012). Among the different facets of biodiversity, functional diversity, i.e. the breadth of functions performed by species in an assemblage (Petchey & Gaston, 2006), is closely related to the rates of ecosystems processes (Mouillot *et al.*, 2011; Naeem *et al.*, 2012) and is shaped by environmental conditions and anthropogenic disturbances (Flynn *et al.*, 2009; Villéger *et al.*, 2010). Functional diversity is thus increasingly measured to disentangle the pro-

cesses that structure assemblages and to assess how the biodiversity of assemblages affects ecosystem functioning, including large-scale studies through functional biogeography (Violle *et al.*, 2014).

The first step in measuring functional diversity is to describe the functional strategies of species using a set of functional traits, i.e. any biological feature measurable at the individual level that is directly related to an ecological function (Violle *et al.*, 2007). The number of traits and their type (quantitative versus qualitative) vary depending on the size and taxonomic diversity of the species present in the set of local assemblages being studied (Petchey & Gaston, 2006). Functional diversity within each species assemblage is then assessed based on the

functional strategies of the species present, i.e. their respective combination of functional traits values (Petchey & Gaston, 2006; Villéger *et al.*, 2008). Some functional diversity indices could be computed directly based on traits values (e.g. functional distance between species; Walker *et al.*, 1999; Ricotta & Szeidl, 2009) but most of indices require the placement of species within a functional space to determine their distribution (Mouchet *et al.*, 2008; Villéger *et al.*, 2008). In the last decade two frameworks were proposed for building functional spaces. The oldest is based on functional dendrograms, i.e. trees where species are the tips and node position and branch lengths reflect hierarchy in the functional dissimilarities between species (Petchey & Gaston, 2002; Mouchet *et al.*, 2008.) This representation allows us to compute indices similar to those developed for phylogenetic diversity (e.g. the total length of branches that link species present in an assemblage; Petchey & Gaston, 2002). However, an analysis of functional diversity patterns is generally required to assess which functional trait(s) are structuring the dissimilarity between species in an assemblage. Therefore, multidimensional functional spaces where species are plotted along trait axes, or synthetic axes capturing combined traits, have been proposed more recently (Cornwell *et al.*, 2006; Villéger *et al.*, 2008). These Euclidean multidimensional spaces allow the computation and visualization of several complementary facets of functional diversity (Mouillot *et al.*, 2013).

Either dendrograms or functional spaces have been used to estimate functional diversity across space and time (Petchey & Gaston, 2006; Mouillot *et al.*, 2013; Violle *et al.*, 2014). However, the quality of the functional space, i.e. the extent to which it faithfully reflects the initial functional dissimilarity between species, is still rarely investigated. The use of a biased functional space can have profound implications for the subsequent assessment of functional diversity (Podani & Schmera, 2006; Villéger *et al.*, 2011). For instance, the dissimilarity metric used to compute the functional distance between species and the clustering algorithm used to build the functional dendrogram can significantly change the ranking of functional diversity among assemblages (Podani & Schmera, 2006; Mouchet *et al.*, 2008). In the same vein, the dimensionality of the functional space can affect conclusions about trends in functional diversity across time (Villéger *et al.*, 2011). There are already statistical methods for selecting functional traits based on their known correlation with response to ecological gradients or ecological roles (e.g. Pillar & Sosinski, 2003) and methods to select the best subset of traits to discriminate communities between user-defined types (e.g. fire regime; Ricotta & Moretti, 2010). Recently, criteria have been proposed to select, a posteriori, the functional dendrogram offering the least distortion from the initial species functional dissimilarity (Mouchet *et al.*, 2008; Mérigot *et al.*, 2010). However, surprisingly, there is still no objective way to compare the quality of dendrograms and Euclidean spaces or to determine how many dimensions are necessary to build a reliable functional space, and these two points are crucial for producing an unbiased assessment of functional diversity. Here we present a general

framework for quantifying the quality of species representation within a functional space. We then used simulated datasets to analyse the influence of species richness, the number of traits and their type on the identity and quality of the best functional space, and to test how the quality of functional space affect functional diversity patterns in local assemblages. Finally, using a real study case, we illustrated how the difference in quality of functional spaces can affect biogeographical conclusions.

MATERIAL AND METHODS

Building a functional space to measure functional diversity

Assessing functional diversity within a set of local assemblages first requires the building of a functional space using a three-step process (Petchey & Gaston, 2002; Villéger *et al.*, 2008) (Fig. 1).

Describing species using functional traits

The first step towards computing functional diversity is to functionally describe the species of interest using a set of relevant functional traits (Violle *et al.*, 2007). The number of traits used to describe the species depends on the taxa and on the focus of the study, but generally exceeds five. Similarly, the nature of the traits selected depends on the biological functions described and/or on the availability of relevant information (Violle *et al.*, 2007). Most of traits describing morpho-anatomy (e.g. size, shape) or biochemistry (e.g. nutrient content) of organisms are measured using continuous variables. Other facets of species niche are coded as categories (e.g. main diet, ability to fix nitrogen), including binary traits (e.g. migratory behaviour, clonal reproduction) and ordered categories (e.g. vertical position in an aquatic habitat – bottom, bottom and water column, water column). Ordinal traits could also be used to code continuous variables when it is not manageable to assess these traits with good confidence for all the species (e.g. size when both size range and species pool are large; Villéger *et al.*, 2011). Therefore, even if most studies are based only on morpho-anatomical continuous traits (e.g. Cornwell *et al.*, 2006; Villéger *et al.*, 2010), some studies account for a mix of categorical and continuous traits (e.g. Olden *et al.*, 2006; Buisson *et al.*, 2013) or even only for categorical traits (e.g. Villéger *et al.*, 2011; Belmaker *et al.*, 2013).

Measuring functional dissimilarity between species

The second step in measuring functional diversity is to compute the matrix of functional dissimilarity between all pairs of species (Fig. 1). The choice of the distance metric to use depends on the nature of the functional traits (Mouchet *et al.*, 2008; Villéger *et al.*, 2008). Euclidean distance could be used when all the traits are continuous and requires a preliminary scaling of each trait to a null mean and a standard deviation of one to give the same weight to each trait. Gower's distance (Gower, 1966) and the
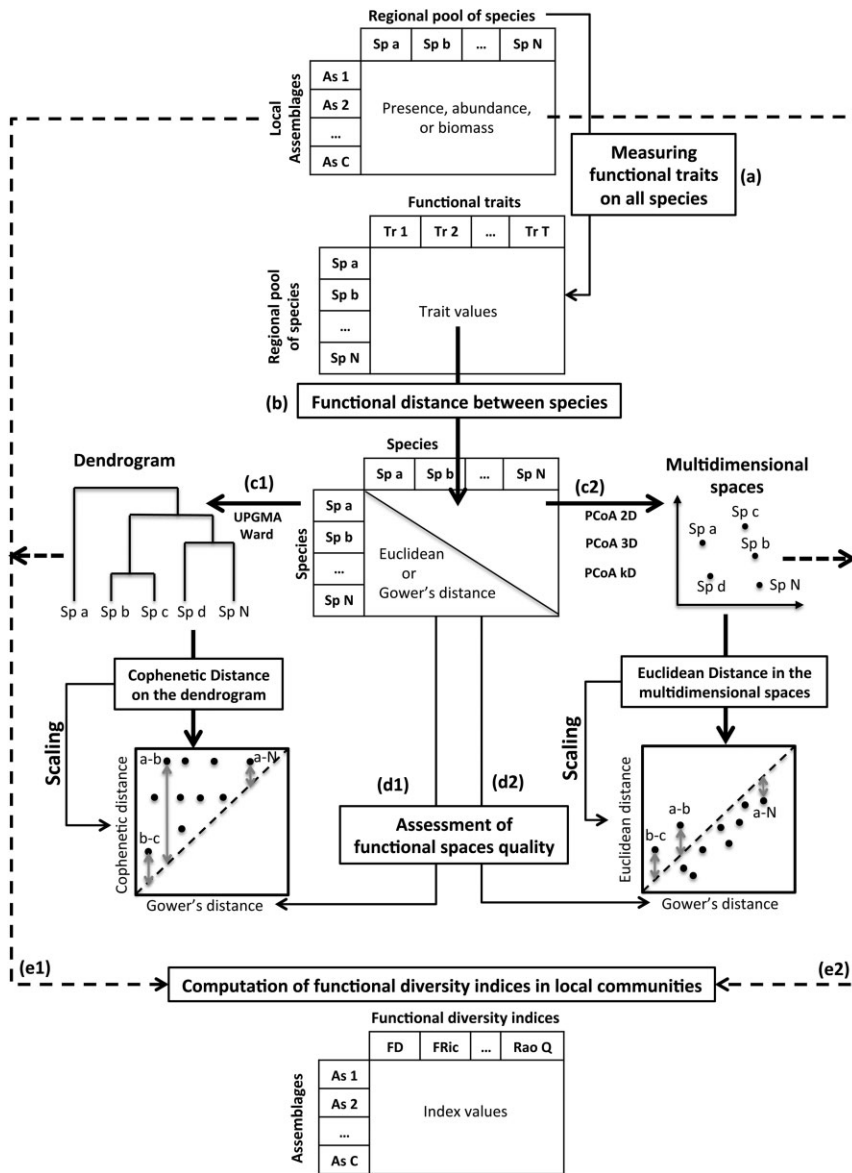
Figure 1 Framework for building and assessing the quality of functional spaces. The first step in building functional spaces is to assess a set of functional traits on all the species present in the regional pool, i.e. species present in at least one of the local communities studied (a). The functional distance between all pairs of species present in the regional pool is then computed (b), using the Euclidean (if all traits are continuous) or the Gower's metric (if there are only categorical traits or several types of traits). Two types of functional space can be computed using this functional distance matrix: (c1) a functional dendrogram selected as the best tree among all combinations of clustering methods; (c2) several multidimensional functional spaces, with two to *k* dimensions (*k* selected by the user, usually less than six), provided by a principal coordinates analysis (PCoA). The quality of these functional spaces can be assessed by computing the mean squared deviation (illustrated for three species pairs by grey double-arrows) between the initial functional distances (i.e. based on traits) and the scaled functional distances in the functional space: co-phenetic distance along tree branches (d1) or Euclidean distance in the multidimensional functional space (d2). The lower the mean squared deviation, the higher the quality of the functional space. Functional diversity indices in each of the local communities studied are computed based on species abundance/biomass and position of species in a functional space, i.e. topology and branch length of a dendrogram (e1) or coordinates of species in a multidimensional space (e2).

subsequent generalization proposed by Pavoine *et al.* (2009) can cope with all types of traits (i.e. traits coded as continuous, categorical, ordinal, circular or fuzzy variables), while giving them the same weight. It also tolerates missing trait values, which can be present in study cases with a rich species pool. Gower's distance can thus be applied to all study cases, including those with only continuous traits, and is therefore widely used in functional ecology studies (Podani & Schmera, 2006; Villéger *et al.*, 2011; Buisson *et al.*, 2013).

Building a functional space

The last step towards computing functional diversity indices is the computation of the functional space based on the functional dissimilarity matrix (Fig. 1). Two methods have been proposed during the last decade or so: functional dendrograms (Petchey & Gaston, 2002) and multidimensional functional spaces (Cornwell *et al.*, 2006; Villéger *et al.*, 2008). Different functional dendrograms could be obtained from the same dissimilarity matrix depending on the clustering method used (UPGMA, WPGMA, UPGMC, WPGMC or Ward's method). We recommend using the framework developed by Mouchet *et al.* (2008) which consists of selecting the best dendrogram among all the trees possible, including consensus trees among clustering algorithms, based on the two-norm quality criterion (Mérigot *et al.*, 2010).

Multidimensional functional spaces are computed based on the dissimilarity matrix using a principal coordinates analysis (PCoA)

(PCoA; a generalization of the principal component analysis, PCA), which produces a Euclidean space with two to the number of species minus one axes. Users have then to select a posteriori the number of dimensions to keep for computing functional diversity indices using species' position in this multidimensional space (Fig. 1; Villéger *et al.*, 2008). It should be noticed that in study cases involving only continuous functional traits, a multidimensional functional space could be obtained directly from traits (i.e. each axis is a trait) after scaling each trait (mean = 0 and standard deviation = 1). However, if there are too many traits (e.g. more than 10), computing functional diversity indices will be time-consuming while graphical representation of the functional space will not be manageable. Therefore, in such a situation it is often preferable to look for a functional space with fewer dimensions, which could be done by computing a PCA on scaled functional traits and keeping species coordinates on principal axes to build a multidimensional functional space (Villéger *et al.*, 2008).

Assessing the quality of a functional space

A functional space of high quality is a functional space where distance between each pair of species is congruent with the initial functional distance (i.e. Gower's or Euclidean distance computed on trait values) (Mérigot *et al.*, 2010). Distances between species in functional spaces are computed using co-phenetic distance for dendrograms (the total length of the branches linking two species) and Euclidean distance for multidimensional spaces (Fig. 1). Co-phenetic or Euclidean distances do not have the same unit and/or range as the initial functional distance computed on traits values. To compare initial and final functional distances, we propose to standardize the distance in the functional space according to the maximum value of the initial functional distance matrix:

$$y_{ij}^{st} = \frac{y_{ij}}{\max_{i,j}(y_{ij})} \cdot \max_{i,j}(x_{ij}),$$

where x_{ij} represents the initial distance between species i and j and y_{ij} represents the co-phenetic distance on the dendrogram or the Euclidean distance in a multidimensional space for the same pair of species. After this scaling, the initial and final functional distances have the same maximum and could thus be compared (Fig. 1).

To assess the congruence between the initial distance and the standardized distance in the functional space we propose to use a metric commonly used in statistics, the mean squared deviation (mSD):

$$mSD = \frac{\sum_{i=1}^{S-1}\sum_{j=i+1}^{S}(x_{ij} - y_{ij}^{st})^2}{\frac{S \times (S-1)}{2}},$$

where S is the number of species, x_{ij} represents the initial functional distance between species i and j and y_{ij}^{st} represents the standardized distance in the functional space for the same pair

of species. The mSD is minimal and equals 0 when the functional space perfectly represents the initial distance ($x_{ij} = y_{ij}^{st}$ for all pairs of species) and it increases when some pairs of species are poorly represented in the functional space, i.e. when species with similar trait values are far in the functional space or alternatively when species with different trait values are close in the functional space.

Disentangling the determinants of the quality of functional spaces

Simulation of regional pools of species and of their functional traits

We aimed to characterize the influence of three variables on the quality of a functional space: the number of species studied (i.e. the species present in at least one of the local assemblages for which functional diversity should be assessed) and the type and the number of traits used to describe them. We selected a set of modalities for these variables that correspond to the situations most frequently encountered in functional ecology studies (Fig. S1 in Supporting Information). We considered four levels of species richness: 50, 100, 200 and 400 species. These levels cover the range of species richness currently observed in functional ecology studies, from local to regional scales (e.g. Belmaker *et al.*, 2013; Villéger *et al.*, 2013). We considered two levels of trait number, 5 and 10, as the number of traits used is often between 3 and 20 (Cornwell *et al.*, 2006; Olden *et al.*, 2006; Villéger *et al.*, 2010; Buisson *et al.*, 2013). To analyse how trait coding can affect the quality of the functional space we considered three types of traits used to describe organisms: categorical, ordinal and continuous traits. We also considered a situation with mixed traits with the following proportions: 1/5 of continuous traits, 2/5 of categorical traits and 2/5 of ordinal traits. The combinations of modalities between the three variables gave a total of 32 types of regional pool of species. To simulate these 32 types of regional pool, we randomly sampled species in a global functional matrix containing 2000 species and 30 functional traits (10 per trait type) (Fig. S1). Trait values for these species were generated according to realistic evolution processes (a Brownian motion process for continuous traits and a Markovian process for categorical and ordinal traits) along a simulated pure-birth phylogenetic tree linking the 2000 species. For each categorical and ordinal trait, five potential states were considered. A thousand replicates per type of regional pool were sampled (Fig. S1).

Statistical analyses

For each of the 32,000 simulated functional traits datasets, we computed a distance matrix using Gower's distance and we compared the mean squared deviation of seven functional spaces: the best functional dendrogram obtained with the Mouchet *et al.* (2008) procedure and the six multidimensional functional spaces, from two to seven dimensions, corresponding to the axes provided by a PCoA. We did not consider spaces with

more than seven dimensions because in real study cases users want to keep a manageable number of dimensions to reduce computing time and allow graphical representation.

We assessed the relative importance of the number of species, the number of functional traits and their type, and the type of representation (dendrogram and two- to seven-dimensional spaces) on the quality of functional space. We used a linear model testing the influence of these four variables and of their six pairwise interactions on the mSD of the simulated functional spaces.

Impact of the quality of the functional space on functional diversity measures

Simulations of local assemblages

To assess how the quality of the functional space built on a regional pool of species affects the assessment of functional diversity in local assemblages, we simulated species assemblages with three levels of species richness (10, 20, 40 species) under a random sampling of species within the regional pool and under two types of non-random assembly processes (niche filtering, limiting similarity) following the procedure of Mouchet *et al.* (2010) (Fig. S1). The niche filtering process was simulated by randomly choosing an 'optimal' species, and then sorting species with the lowest raw functional distance (i.e. Gower's distance) to this optimal species until targeted local species richness was reached. The limiting similarity process was simulated by randomly choosing a species, then sequentially selecting the species with the highest average functional distance from the set of species already selected, until targeted species richness was reached. Species relative abundances were sampled using a log-normal probability law and attributed to species according to the type of assembly process: randomly for a random scenario, in decreasing order for niche filtering and limiting similarity scenarios, i.e. highest relative abundance for the first species sampled and lowest abundance for the last species selected.

Computing functional diversity of simulated local assemblages

Two types of functional diversity indices were computed for each simulated local assemblages using species position in each of the seven types of functional spaces built for their respective regional pool: (1) functional richness, i.e. the range of species position in the functional space, with the Functional Diversity (FD) index on a dendrogram (Petchey & Gaston, 2002) and the Functional Richness (FRic) index in multidimensional spaces (Villéger *et al.*, 2008), and (2) functional entropy, i.e. the distribution of species relative abundance in the functional space, with Rao's Q index (expressed as an equivalent number of species; Ricotta & Szeidl, 2009) using co-phenetic distance on the dendrogram or Euclidean distance in multidimensional spaces.

Functional diversity values (i.e. an index *I* measured in a functional space *f*) measured in a local assemblage simulated under a niche filtering process were compared with the 100 values of the same index computed in the same functional space for the 100 local assemblages simulated with random sampling in the same regional pool, using the standardized effect size (SES) metric: $SES(I_f) = [I_f - \text{mean}(I_{\text{Random}})]/SD(I_{\text{Random}})$. The SESs of functional diversity indices for limiting similarity process were computed in the same way. Comparison of the SESs of functional diversity indices between species assemblages is commonly used to detect non-random assembly processes and map functional diversity patterns independently of differences in local species richness (e.g. Swenson *et al.*, 2012). Niche filtering simulations promoted a functional clustering of species and of their abundances in the functional space (compared with the random scenario) and thus should lead to a negative SES. On the contrary, limiting similarity processes promoted a functional overdispersion and thus should lead to a positive SES.

To test whether the identity of the functional space affects the difference in functional diversity between random and the other two assembly processes, the effects of functional space type and of the characteristics of the regional species pool (number of species and number and type of functional traits) and the local assemblage species richness on the four SES (two functional diversity indices × two non-random assembly processes) were assessed using a linear model accounting for the pairwise interactions between these five factors.

A functional biogeography study case

To illustrate the potential consequences of differences in the quality of functional space on functional diversity patterns we assessed the functional biogeography of a vertebrate taxon at a continental scale. We used a database on native fish occurrences in 137 river basins of Europe (Fig. S3; Villéger *et al.*, 2014). The 246 fish present in this regional species pool were described using six functional traits, two continuous traits (size and body shape), one categorical trait (foraging position) and two ordinal traits (main diet, rheophily and vertical habitat). These functional traits were assessed for each species based on pictures and information available in the scientific literature (Kottelat & Freyhof, 2007; Froese & Pauly, 2013; see details in Table S1).

The quality of five functional spaces (the best functional dendrogram and multidimensional space from two to five dimensions) was computed using the R function 'quality_funct_space' provided in Appendix S1. The functional richness of each fish assemblage was computed in these five functional spaces. For each of the 137 fish assemblages we replicated 1000 times a random sampling of the observed number of species present in the assemblage among the pool of 246 species. Then, using these expected values we computed in each assemblage the SES of each functional richness index in each type of functional space. For each type of functional space, SES values were correlated with average latitude and average longitude of the river basins to test for geographical patterns in functional richness.

RESULTS

Identity and quality of the best functional space

The quality of the simulated functional spaces was highly variable among situations, with mSD ranging from less than 0.001 to 0.212 (Fig. 2). The quality of functional space depended mainly on interactions between the type of representation selected and the types of traits used to describe them (Table 1, Fig. 2). For instance, for regional pools described with five ordinal traits, the mSD of the dendrogram was higher than 0.1 for all species richnesses, while it was lower than 0.01 for the five multidimensional spaces with more than two dimensions (Fig. 2). The lowest difference in quality between functional spaces was observed when species were described with 10 continuous traits (Fig. 2). The quality of functional space decreased with increasing species richness, especially for the functional dendrogram built on the basis of categorical traits (Fig. 2). Overall, the number of traits only marginally influenced the quality of functional space (Table 1, Fig. 2).

The quality of the best functional space for a given type of regional pool was high for all cases with mSD lower than 0.01 in 25 of the 32 situations and a maximum value of 0.025 (when 400 species were described with five categorical traits) (Fig. 2). Functional spaces with at least four dimensions were the best, i.e. they had the highest percentage of minimum mSD among the 1000 simulations, for all types of regional species pools (Table 2). More precisely, seven-dimensional space performed the best for 23 of the 32 cases (Table 2). Dendrograms and two-dimensional functional space were never selected as the best functional space among the 32,000 simulated regional pools (Table 2). The quality of the seven-dimensional space was on average close to that of the four-dimensional space for cases involving non-categorical traits (mean ± SD of difference in mSD: −0.0024 ± 0.0024).

Impact of the quality of functional space on functional diversity

Among the simulated local assemblages, the deviations of functional richness and of functional entropy between random and the two non-random assembly rules (limiting similarity and niche filtering) were all significantly affected by the type of functional space (Table 3, Fig. S2). Overall, dendrogram-based indices overestimated the intensity of niche filtering and underestimated limiting similarity (Fig. S2). For instance, absolute difference in SES of functional richness was higher than two between dendrograms and seven-dimensional space for all situations (Fig. S2). In addition, ranking of SES values among local assemblages was not congruent between functional spaces for a given assembly process. For instance, SES values of functional richness measured in seven-dimensional space were moderately correlated to SES of functional richness measured on a dendrogram (rho = 0.359 and rho = 0.314 for niche filtering and limiting similarity processes, respectively).

For the 246 freshwater fish species described with a mix of six categorical, ordinal and continuous traits, the best functional space was the multidimensional functional space with four dimensions (Fig. 3). The quality of this functional space was slightly higher than the quality of spaces with three or five dimensions, but was more than three times higher than the quality of the two-dimensional space and 57 times higher than the quality of the best functional dendrogram (built with the UPGMA clustering algorithm).

Functional richness was significantly correlated with species richness in the three functional multidimensional spaces from two to four dimensions and on the functional dendrogram (Fig. 4). However, the deviation of functional richness from the null hypothesis measured as SES was different between these four functional spaces (Kruskal–Wallis test, $P < 0.001$). More importantly, the strength and significance of the correlation between the SES of functional richness and latitude and longitude differed between the functional spaces (Figs 4 & S3). Indeed, the SES of functional richness measured in the four-dimensional (i.e. the best functional space) or in the three-dimensional space was not significantly correlated with latitude, while the SES of functional richness measured in the two-dimensional space or on the functional dendrogram was significantly correlated with latitude (Fig. 4). Similarly, the significant negative correlation between longitude and SES of functional richness measured in the three- and four-dimensional spaces was not observed with the SES measured in the two-dimensional space or on the functional dendrogram (Fig. 4).

DISCUSSION

Building an optimal functional space is a critical step towards a good assessment of functional diversity. Here we propose an index to quantify a posteriori the quality of a functional space (Fig. 1). This index, mSD, the mean squared deviation between the initial functional distance and the scaled distance in the functional space, differs from the traditional cophenetic correlation coefficient based on the linear relationship between initial and final distances, which is commonly used for comparing dendrogram quality (Mouchet et al., 2008). Indeed, mSD accounts explicitly for the deviation between the initial and final distance and penalizes the strong deviation because of the square power. This index is also different from the two-norm proposed by Mérigot et al. (2010), measuring the inertia in the deviation, which accounts for the deviation between distances but does not allow us to compare study cases with different distance metrics. Our index is widely used in statistics to assess errors and works in all situations. In addition, when using Gower's distance (as in our simulations and in most study cases) the mSD ranges from 0 and 1, which helps to interpret quality.

Using simulated datasets, we demonstrated that the type of representation (dendrogram or multidimensional Euclidean space) and the types of traits selected to describe species are the main determinants of the quality of functional spaces (Table 1).
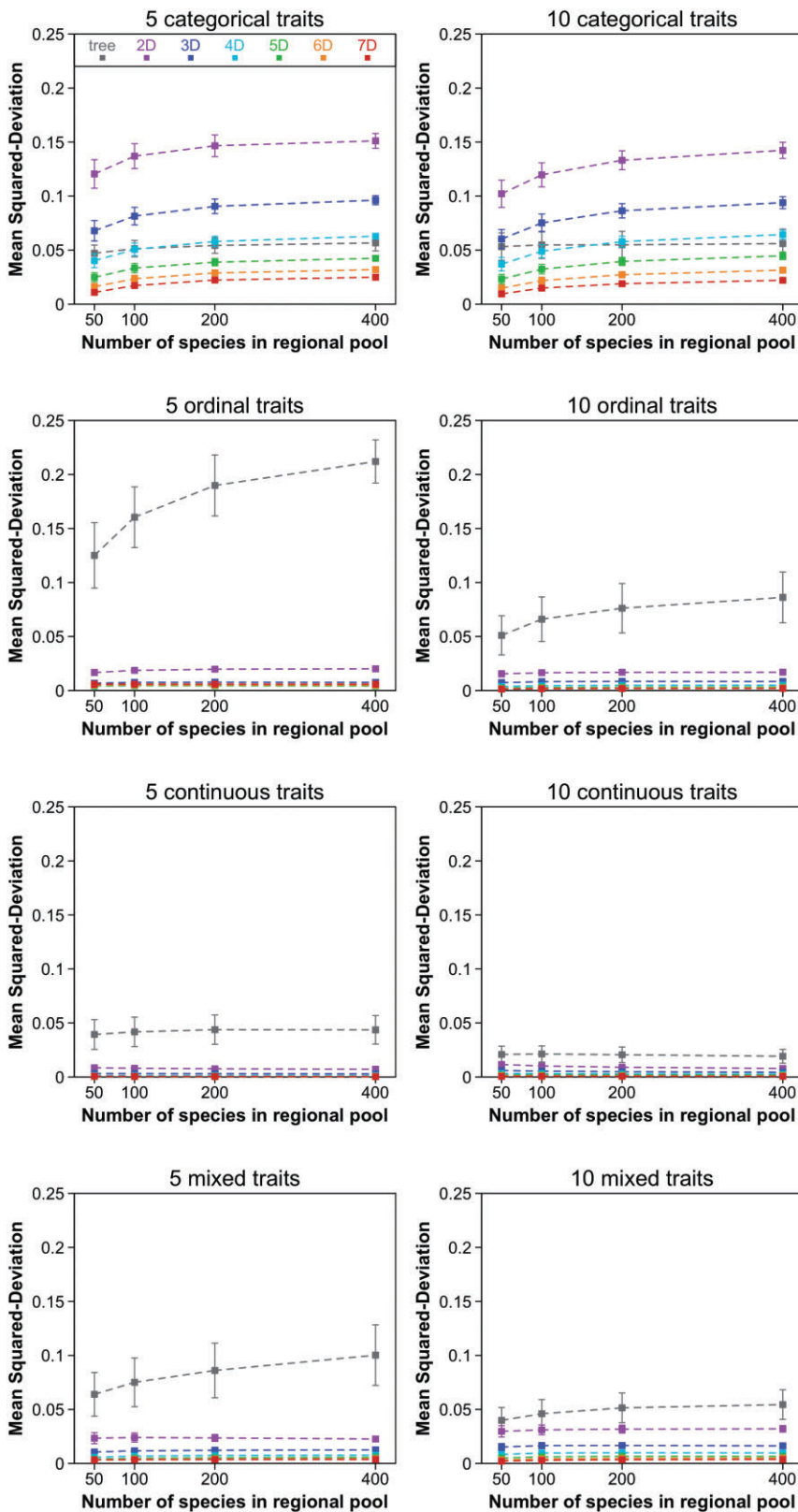
Figure 2 Effect of the number and type of functional traits and number of species on functional space quality. Points represent the average values (± standard deviation) of the mean squared deviation between the initial functional distance between species pairs based on trait values and the final distance based on the position of species in the functional space among the 1000 simulated datasets for each level of species richness. The quality of functional space increases when the mean squared deviation tends to zero. Coloured points represent the quality of multidimensional functional spaces (from two to seven dimensions) obtained from a principal coordinates analysis and grey points represent the quality of the best functional dendrogram (i.e. tree). tree (■); 2D (■); 3D (■); 4D (■); 5D (■); 6D (■); 7D (■).

Table 1 Determinants of the quality of functional space.

Variable	Degree of freedom	Relative sum of squares	*F*-value
Type of functional space	6	0.318	135,396
Type of trait	3	0.241	205,666
Number of traits	1	0.006	15,903
Number of species	1	0.006	15,849
Type of functional space × type of trait	18	0.295	41,867
Type of functional space × number of traits	6	0.032	13,494
Type of functional space × number of species	6	0.003	1,452
Type of trait × number of traits	3	0.006	5,176
Type of trait × number of species	3	0.005	4,354
Number of traits × number of species	1	< 0.001	269

Outputs of the linear model testing the influence of four variables and of their six pairwise interactions on the mean squared deviation of the simulated functional spaces.

Table 2 Effects of the number and type of functional traits and of the total number of species functionally described on the identity of the best functional space.

Type of traits	Number of traits	Number of species	Functional dendrogram	Multidimensional functional space					
				2D	3D	4D	5D	6D	7D
Categorical	5	50	0	0	0	0	0	0	**100**
		100	0	0	0	0	0	0.5	**99.5**
		200	0	0	0	0	0	1.5	**98.5**
		400	0	0	0	0	0	0.9	**99.1**
Ordinal	5	50	0	0	0.3	**66.3**	32.7	0.7	0
		100	0	0	0	**54.7**	43.5	1.7	0.1
		200	0	0	0	41.5	**57.7**	0.8	0
		400	0	0	0	19.9	**80.1**	0	0
Continuous	5	50	0	0	0	0.1	42.0	**49.6**	8.3
		100	0	0	0	0	26.7	**58.1**	15.2
		200	0	0	0	0	15.4	**65.9**	18.7
		400	0	0	0	0	6.0	**71.2**	22.8
Mixed	5	50	0	0	0	0.7	10.9	**45.4**	43.0
		100	0	0	0	0	1.9	24.0	**74.1**
		200	0	0	0	0	0.6	8.8	**90.6**
		400	0	0	0	0	0.2	2.6	**97.2**
Categorical	10	50	0	0	0	0	0	0.2	**99.8**
		100	0	0	0	0	0	0.2	**99.8**
		200	0	0	0	0	0	0	**100**
		400	0	0	0	0	0	0	**100**
Ordinal	10	50	0	0	0	0	0.9	25.4	**73.7**
		100	0	0	0	0	0.9	12.7	**86.4**
		200	0	0	0	0	0.2	9.6	**90.2**
		400	0	0	0	0	0	7.8	**92.2**
Continuous	10	50	0	0	0	0	0	0	**100**
		100	0	0	0	0	0	0.1	**99.9**
		200	0	0	0	0	0	0.5	**99.5**
		400	0	0	0	0	0	0.4	**99.6**
Mixed	10	50	0	0	0	0	0.1	0.5	**99.4**
		100	0	0	0	0	0	0.8	**99.2**
		200	0	0	0	0	0	1.9	**98.1**
		400	0	0	0	0	0.1	3.4	**96.5**

Values are the percentages of times each type of functional space was the best (i.e. has the minimum mean squared deviation) over the 1000 datasets simulated for each of the 32 situations. The highest value for each situation is in bold type.

Table 3 Effect of the type of functional space on functional diversity patterns in local communities.

Variable	Degree of freedom	Functional richness		Functional entropy	
		SES niche filtering	SES limiting similarity	SES niche filtering	SES limiting similarity
Type of representation	6	**0.312**	**0.243**	**0.083**	**0.152**
Type of trait	3	0.010	**0.232**	**0.098**	**0.420**
Number of traits	1	0.007	0.002	0.014	0.001
Number of species in the regional pool	1	**0.122**	0.036	**0.316**	0.022
Number of species in the local assemblage	1	**0.222**	0.005	**0.246**	**0.087**
Type of representation × type of trait	18	0.035	**0.084**	0.010	**0.062**
Type of representation × number of traits	6	0.005	0.000	0.002	0.003
Type of representation × no. of species in the regional pool	6	**0.091**	0.024	0.002	0.005
Type of representation × no. of species in the local assemblage	6	0.009	0.002	0.005	0.006
Type of trait × number of traits	3	0.004	0.029	0.005	0.034
Type of trait × no. of species in the regional pool	3	0.003	0.049	0.005	0.040
Type of trait × no. of species in the local assemblage	3	0.003	0.007	0.002	0.024
Number of traits × no. of species in the regional pool	1	0.000	0.000	0.001	0.000
Number of traits × no. of species in the local assemblage	1	0.003	0.001	0.001	0.000
No. of species in the regional pool × no. of species in the local assemblage	1	0.030	0.005	0.035	0.007

Values are relative sum of squares explained by each variable and pairwise combination of variables, obtained with a Type II ANOVA on a linear model testing the effects of the type of functional space, type and number of functional traits and species richness at the regional and local scales on the deviation in functional diversity (richness or entropy) of assemblages simulated under a niche filtering (or limiting similarity) process and assemblages sorted randomly. Deviation was measured as standardized effect size (SES). Relative sum of squares higher than 0.05 are in bold type.

We found no rule to choose a priori the best functional space. Spaces with higher dimensionality (i.e. with at least four dimensions) performed the best on average (Table 2, Fig. 2) and the difference in quality between four- and seven-dimensional spaces was relatively low, except when only categorical traits were used to describe species. Importantly, the quality of the best multidimensional functional space for a given situation could be several times higher than the quality of the best functional dendrogram or than the quality of a two-dimensional space (Figs 2 & 3). More precisely, a functional dendrogram tended to artificially increase the functional distance between species while low-dimensionality Euclidean spaces tended to underestimate the actual functional distances between species (Fig. 3). For instance, in the study case 13% of pairs of species with a low Gower's distance (i.e. lower than 0.3) were twice as distant on the functional dendrogram (standardized co-phenetic distance higher than 0.6; Fig. 3).

A good functional space has to allow us to discriminate the different functional strategies, i.e. combinations of trait values present in the species pool. For instance, in a k-dimensional space, there are 2^k corners available to discriminate contrasted functional strategies. Functional dendrograms, which could be considered as one-dimensional diagrams representing a hypothetical hierarchy in functional dissimilarity of species, are thus less efficient than multidimensional spaces for faithfully representing a high diversity of functional trait combinations. Indeed, even if species with high dissimilarity in their trait values are represented far from each other on a functional dendrogram, a high proportion of species with similar trait values are also far

from each other (Fig. 3). Functional dendrograms thus bias the initial distribution of functional distances towards overestimating the functional dissimilarity between some species pairs. The importance of dimensionality for representing distances between objects described with multiple variables has already been pointed out for community ecology analyses (Legendre & Legendre, 1998). In contrast, the potential biases of using a dendrogram on the quality of data representation have until recently been ignored in functional ecology (but see Chao *et al.*, 2014).

Categorical traits tended to decrease the quality of the best functional space (Fig. 2). Indeed, discrete coding of categorical traits led to a higher proportion of species with high functional distance, and hence more dimensions are required to faithfully represent these unique functional strategies. Increasing the number of traits and/or the number of species described also tended to increase the number of dissimilar functional strategies and thus to decrease the quality of the best functional space (Fig. 2).

As it is hard to predict a priori the identity of the optimal functional space based on the characteristics of the study case, we recommend using a pragmatic approach consisting of computing the quality of all the possible spaces. Towards this end, we provide an R function ('quality_funct_space' in Appendix S1) that computes the mSD for all possible spaces (i.e. dendrograms and multidimensional spaces up to more than 10 dimensions). This tool will prevent the computation of functional diversity indices in a functional space of poor quality and will help to select an optimal functional space. This pragmatic choice does
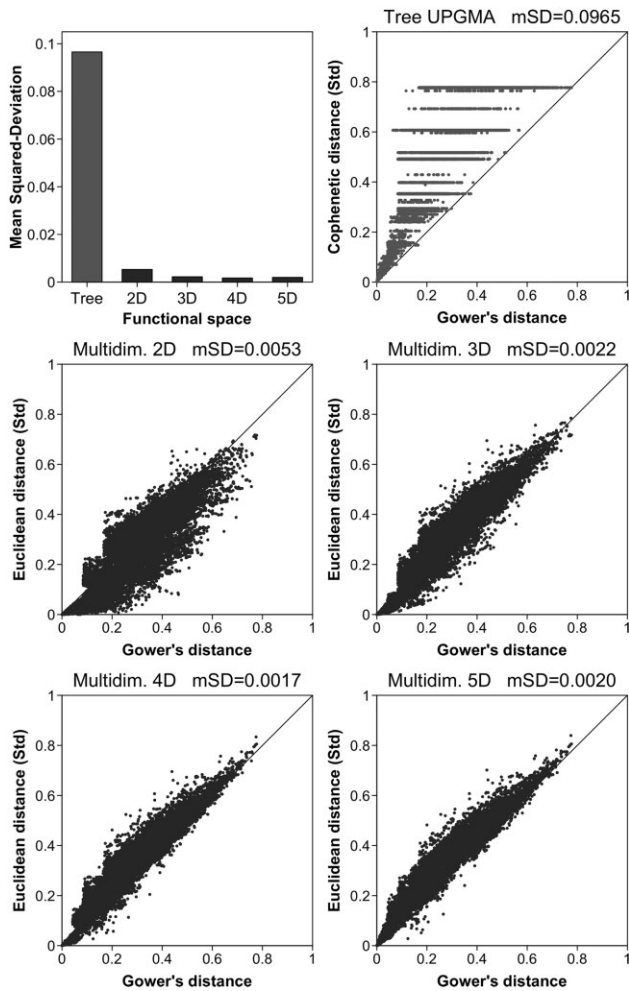
Figure 3 Illustration of the difference in the quality of functional space between dendrogram and multidimensional spaces. The top-left panel shows the quality (assessed using mean squared deviation, mSD) of five functional spaces computed for a dataset of 246 native fish species described with six functional traits (two continuous, one categorical, three ordinal): one dendrogram built using the unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm, and four multidimensional spaces (from two to five dimensions) built using a principal coordinates analysis. The correlation between pairwise distance computed on species traits (the Gower's distance) and standardized distance in each of the five functional spaces are illustrated on the five remaining panels. Each point represents a pair of species.

not necessarily imply the selection of the functional space with the highest quality, as a functional space with a quality close to this optimum but with a lower dimensionality can have practical advantages, such as computing time for functional diversity indices (e.g. functional beta-diversity indices; Villéger *et al.*, 2014) or feasibility of graphical outputs (e.g. visualizing the distribution of species in four-dimensional functional space required only six biplots while with a seven-dimensional space it required 21 biplots).

As distances between species on the functional dendrogram or on low-dimensionality spaces often do not accurately reflect the dissimilarity in traits values between species (Fig. 3), computing an index of diversity using these functional spaces of poor quality can lead to biased estimates of functional diversity. Functional diversity indices measured in simulated local assemblages of species revealed that the identity of the functional space significantly affects the detection of non-random assembly processes (Table 3). Indeed, deviations of functional diversity between scenarios were not congruent (i.e. they differ in both intensity and ranking among assemblages) between indices measured on dendrogram and in spaces with at least four dimensions (Table 3, Fig. S2). The quality of the functional space built for the regional pool of species thus affected the assessment of functional diversity in the set of local assemblages studied and can ultimately lead to biased estimation of the strength of the ecological processes that structured assemblages.

In the study case of the functional biogeography of European freshwater fish, the three-dimensional functional space had a quality very close to that of the optimal four-dimensional space, but the quality of the best functional dendrogram was more than 50 times lower than that of the optimal functional space. This magnitude of difference in quality was congruent with that found using simulated data for 200 species described using five mixed functional traits (Fig. 2). As a consequence of these differences in quality of functional space, the biogeographical patterns of functional diversity obtained using the three-dimensional space were congruent with those obtained using the optimal four-dimensional space while the patterns obtained using the two-dimensional functional space or the functional dendrogram were opposite (Figs 4, S2 & S3).

From a biogeographic point of view, the spatial distribution of fish assemblages across Europe has been shaped by the Quaternary glaciation events (Hewitt, 1999). During glaciations, the

Figure 4 (On following page) Consequences of difference in the quality of functional dendrogram and multidimensional functional spaces when testing biogeographic patterns. Panels on the left show the correlation between species richness and functional richness in 137 fish faunas. The best functional space according to the mean squared deviation criterion is the four-dimensional space (see Figure 3). Black dots show observed species and functional richness: the functional diversity (FD) index for the unweighted pair group method with arithmetic mean (UPGMA) dendrogram and functional richness (FRic) index for two-, three- and four-dimensional spaces. Grey areas and dark-grey squares represent the mean and 95% confidence interval of expected functional richness given species richness under the null hypothesis that species were randomly selected among the regional pool. Central and right columns of panels show correlation between the standardized effect size (SES) of functional richness (under the null hypothesis of random assemblage of species) and the geographical position of fish assemblages. Correlation values at the top of each panel are Pearson's coefficients (ns not significant, * P < 0.05, ** P < 0.01, *** P < 0.001).
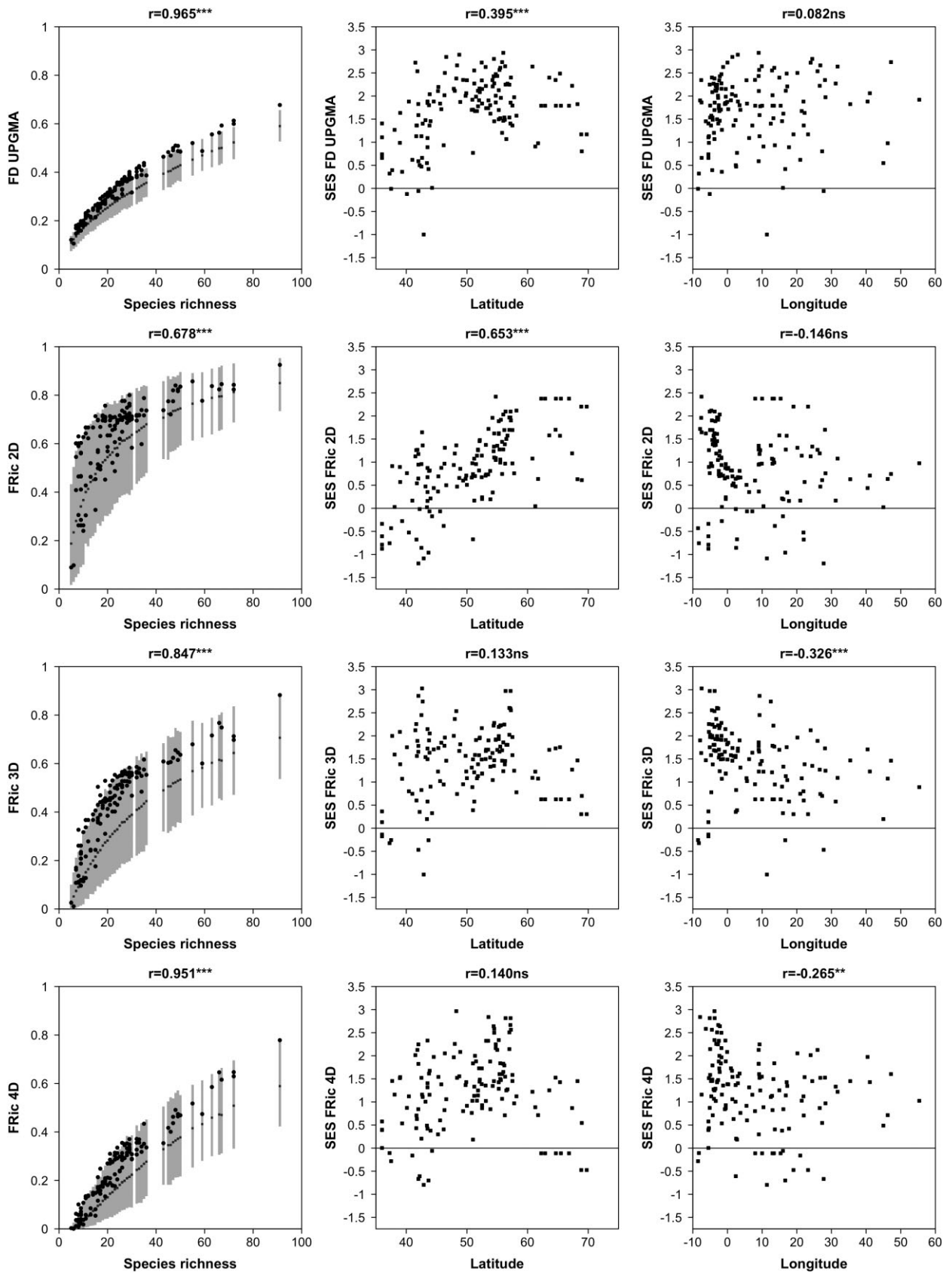
Danube (and to a lesser extent some Mediterranean coastal rivers) acted as the main refuge for freshwater fishes, and hence it now hosts most of the European fish fauna (Griffiths, 2006; Reyjol *et al.*, 2007). Post-glacial fish recolonization from the Danubian refuge was accompanied by species radiation in some genera, which produced several congeneric species with similar functional strategies (e.g. Ketmaier *et al.*, 2008), explaining why most fish functional strategies are present in all faunas across Europe (Griffiths, 2006; Reyjol *et al.*, 2007). There is thus a slight latitudinal gradient in species and functional richness across Europe and a longitudinal decrease in functional richness from east to west but of lower magnitude than the decrease in species richness (Villéger *et al.*, 2013). A significant effect of longitude and a non-significant effect of latitude on functional overdispersion are hence expected. These patterns are verified for functional richness indices computed in three- or four-dimensional functional spaces. On the contrary, dendrogram-based functional richness showed an undue significant latitudinal increase and did not detect the longitudinal gradient (Fig. 4). These biases are due to the overestimation of functional richness in north-eastern European assemblages and to the underestimation of overdispersion in south-western assemblages (Fig. S3), while these assemblages have a similar low species richness (Fig. 4). Thus, the low quality of the functional dendrogram, because of the overestimation of functional dissimilarity between some species that are actually functionally similar, resulted in biased estimates of functional richness, especially in species-poor assemblages, which ultimately biased the detection of biogeographical patterns.

To conclude, the analysis of simulated datasets and a real study case revealed that a poor representation of the functional distances between species using dendrogram and two-dimensional functional spaces might lead to erroneous ecological conclusions, and we therefore advise using them with caution. Moreover, although functional ecology and functional biogeography have developed very quickly over the last decade (Violle *et al.,* 2014), there is still no objective way to decide how to represent the functional distances between species, despite it is a critical step in the accurate assessment of functional diversity. The framework we propose here offers a consensual method to select an optimal functional space using a relevant index of quality. We recommend using it to prevent the computation of biased estimates of functional diversity and hence misinterpreting functional diversity patterns.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Figure S1 Summary of simulated datasets used in the study.
Figure S2 Effects of functional space type on functional diversity patterns.
Figure S3 Impact of functional space quality on functional richness patterns.
Table S1 Functional traits used to describe fish functional strategy.
Appendix S1 R script to compute the quality of functional spaces.

BIOSKETCH

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