Integrating intraspecific trait variability in functional diversity: an overview of methods and a guide for ecologists

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Abstract. The variability in traits within species (intraspecific trait variability; ITV) has attracted an increased interest in functional ecology, as it can profoundly influence the detection of functional trait patterns, calculation of functional diversity (FD), and assessments of ecosystem functioning. This renewed focus stems from the recognition that species are not homogeneous entities but rather mosaics of individuals with varying traits. Researchers dealing with FD have increasingly recognized this issue, and consequently, multiple methods have emerged to explicitly incorporate ITV into FD calculations. Some methods treat individuals as the unit of analysis, while others characterize trait distributions around their means. Ecologists navigating this landscape of methods may face challenges in selecting the most appropriate approach to address their research questions. This synthesis provides an overview and guidelines on how and when to use the different methods available to quantify ITV in biological assemblages and integrate it into FD. As a case study, we computed correlations on simulated assemblages with varying degrees of trait variability. Our findings suggest that the choice of FD metric should be guided primarily by the ecological question being addressed and, to a lesser extent, by the number and types of traits. Simulations revealed strong correlations among FD metrics that account for ITV, particularly those indicating the size of the occupied functional trait space. As evidence accumulates and shows how ITV is key to shaping species' fitness and distribution as well as affecting ecosystem functioning, this synthesis will serve as a conceptual and practical tool inspiring and guiding researchers to integrate ITV in functional diversity analyses.

1 Introduction

Functional diversity (FD), defined as the extent of trait differences among organisms within a unit of study (e.g. population, community, biome), is a core eco-evolutionary concept [122]. Biodiversity is indeed a multifaceted notion, and FD constitutes one of its key components (together with taxonomic, genetic and phylogenetic diversity), which can have a significant impact on how individuals assemble to form a population or species a community as well as ecosystem functioning ([40, 65, 78]). Although the study of FD has fueled major conceptual, empirical, methodological, and philosophical research and advances ([93, 122]), the vast majority of studies on FD has heavily relied on averaging individual trait values of the same species. Using mean trait values per species bears the implicit assumption that intraspecific differences are negligible compared to those among species ('mean field approach') ([41, 39, 67]). However, although within species trait variability has been found often lower than between species differences, its extent and effects are definitely not minor; for example, there is, on average, 25% variability in functional traits within species in plant communities ([56]). The assumption of intraspecific trait variability (ITV) being relatively small has been also the foundation of standardized sampling protocols to collect trait data (e.g. [13, 66, 72, 89]) focused on the creation of large scale databases which, in turn, have contributed to further focusing on interspecific trait differences. By contrast, ITV, defined as the variation in a trait between individuals of the same species (e.g. forming a local population or across populations along gradients), has been often overlooked in FD analyses [33, 108]. In a recent review of 1655 articles on trait-based animal ecology, [119] found that 94.6% of the articles ignored ITV. Despite there has been an increase in the number of articles published on FD accounting for ITV in recent years, they are still vastly outnumbered by those that do not include ITV (Fig. 1). This reveals that ITV is a significantly understudied aspect in trait-based functional ecology, and highlights the need for more research on this topic to better understand the role that ITV can have in ecological systems.

More recently, ITV has attracted an increasing interest among ecologists [34, 68, 91, 108]. ITV is increasingly considered key in functional ecology and evolutionary biology because it can 1) promote species' coexistence, 2) allow populations to cope with changing environmental conditions, and, in the long-term, 3) lead to speciation and adaptive evolution [57, 118]. Growing evidence also shows that the magnitude of ITV can, under specific circumstances such as small spatial scales or low species richness assemblages (Box 1), be equal to or surpass that attributable to interspecific trait variation [91, 92]. Furthermore, intraspecific effects on ecological responses, as those associated with changes in a species' abundance and community composition, are often comparable to, and sometimes even stronger than interspecific effects linked to species turnover [79]. As a result, the 'mean field approach' [41] may underrate possible consequences on FD effects [27, 35, 109].

Overlooking ITV can therefore underestimate FD by downplaying the range and variety of resources a species can use and thus its ecological role or ability to cope with environmental changes [46, 91]. For instance, in a simulation experiment on alpine plant assemblages, [39] found that the relationship between FD and environmental factors was altered when accounting for ITV. Specifically, the relationship between leaf dry matter content and temperature varied between negative, positive or null, depending on ITV's quantity and structure [39]. Larger ITV may also lead to a higher uncertainty in FD estimation, with some metrics (e.g. functional richness and Rao's quadratic entropy) more sensitive to the inclusion of ITV than others [39]. Similarly, [109] found that ITV largely affected some metrics of FD in plant and ant assemblages, and that neglecting ITV distorted relationships between FD and environmental covariates. Accounting for ITV can deepen our understanding of ecological dynamics, as ITV may 1) enhance the stability of populations and communities [75, 80, 93], 2) affect species' responses through abiotic and biotic constraints along environmental gradients [54, 70], 3) shape biotic interactions [84], and ultimately 4) influence ecosystem functioning by increasing the range of functional roles and the contribution to ecosystem services [49, 98].

Aiming to consider these reasons and address these challenges (Box 1), several methods have been developed to explicitly incorporate ITV in FD calculations. Some methods consider individuals as the unit of interest (i.e. observations) when including ITV (e.g., 'individual-level FD' –iFD– [27, 62]), whereas others characterize a given trait distribution either parametrically (e.g., [41, 35]) or non-parametrically ([59, 76, 90]). In this context, a useful concept when considering ITV is the Hutchinsonian niche, defined as a multidimensional hypervolume (see 'n-dimensional hypervolumes' below) of ecological variables in which a species maintains a viable population [4]. In particular, the mean trait value and its variance are descriptors for niche position and breadth, respectively [23, 28, 82]. Therefore, functional traits can describe species niches within assemblages, and intraspecific functional diversity can be thought of as the distribution of species' niches (in terms of position and breadth) in an assemblage. The greater the distance between niche positions and/or the smaller the niche breadth of species, the higher the iFD. Although the niche concept has been recently applied in trait-based ecology to represent the functional trait space of assemblages [59, 76, 77] and quantify functional diversity [97], the volume of this space strictly measures the variation in niche position among species forming an assemblage, not the Hutchinsonian niche per se (e.g., [47, 105]. A more direct link between the niche concept and FD can be established when moving from species to individuals because these can intrinsically and better capture the variability of trait values in populations [28].

Ecologists delving into the many approaches available for integrating ITV into FD analyses are therefore facing the challenge of selecting the most appropriate method to address their research question. Here, we aim to provide an overview and guidelines on how and when-to-use the different methods capable of quantifying ITV in biological assemblages, with a special focus on those that allow integrating ITV into FD. In the following sections, for each of these methods, we identify and describe 1) their main characteristics, 2) their strengths and drawbacks, 3) examples of which and how ecological insights can be gained and inferences made, and 4) redundancy in the ecological patterns they capture. The sections and methods below are organized based on the number of species and traits that a given metric can accommodate, regardless of the way it is applied (e.g. the coefficient of variation is computed on a single species, yet it is often used to compare multiple species). Method notation is summarized in Table 1. Finally, to further assist researchers in their selection of which approach to implement when deciding to integrate ITV in FD analyses, we examine the impact of ITV on the different methods presented below with a simulated case study comparing how a variable degree of ITV affects different FD metrics. All the metrics and approaches used are available as an R tutorial at https://github.com/facuxpalacio/intraspecific-trait-variability-and-functional-diversity/tree/main/tutorial

2 One species, a single trait

The simplest and most widely used measure to quantify ITV for species i and trait j is the coefficient of variation (CV):

$$CV_{ij} = \frac{S_{ij}}{\overline{X}_{ij}} \tag{1}$$

where S and $\overline{X}$ are the standard deviation and the mean of species i and trait j ($\overline{X} \neq 0$), respectively. The CV quantifies the degree of variability relative to its mean; it is dimensionless, and thus can be used to compare traits, species, and studies [102, 107]. Although it does not require a priori assumption about the underlying distribution of traits, it is a biased estimator of the population CV because the sample standard deviation tends to be smaller than the population standard deviation, underestimating the true population CV [7, 12]. In a recent comparison of different CV metrics, [102] found that the CV underestimated ITV up to nearly 50%. This bias can be largely reduced by log-transforming trait data and using the Bao's CV or CV4 estimator (CV_B) [26, 102]:

$$CV_B = CV_{ij} - \frac{CV_{ij}^3}{n} + \frac{CV_{ij}}{4n} + \frac{CV_{ij}^2\gamma_{1ij}}{2n} + \frac{CV_{ij}\gamma_{2ij}}{8n} \tag{2}$$

where n is the number of individuals, and γ_1 and γ_2 are Pearson's measures of skewness and kurtosis of the trait sample distribution, respectively. This is because the bias of the sample CV depends on the skewness and kurtosis of the distribution [26]. Moreover, this procedure allows to use a minimum sampling size (to achieve a ±5% accuracy) of 20 individuals for most species and traits (at least in plants), in contrast to the CV, whose minimum sampling size can range from 10 to 295 individuals, depending on the skewness of the trait distribution [102]. Despite its historical use in biology, it suffers from sensitivity to excess zero values and/or irrelevant low mean values, so the CV should not be applied to traits with these characteristics. More recently, a modified version of the CV, termed KCV [71], has shown better properties than the CV, particularly when there is high variability (e.g. CV > 1.0), such as in the case of phenological traits [121].

This measure is bounded between 0 and 1, has increased statistical power, and can be computed directly from the CV as:

$$^{K}CV = \sqrt{\frac{CV^2}{1+CV^2}} \tag{3}$$

It should also be noted that the CV and its variants account for ITV of a single species, not of the overall assemblage. However, it represents a first step to describe and explore ITV of a set of traits and species.

3 One species, multiple traits

Although a univariate metric such as the CV provides a rapid, descriptive measure of ITV, it neglects the fact that organisms comprise a multivariate set of correlated traits [8, 14]. Therefore, a multivariate variant of the CV can be more informative than the univariate CV about the diversity of entire multifunctional phenotypes (if the selected traits capture the main functions [11]), while accounting for the correlative nature of traits. However, deriving a multivariate CV (MCV) is not straightforward, and several generalizations of the MCV have been introduced in the literature [29, 52]. For the sake of simplicity, we only provide the Voinov and Nikulin's CV (γ_{VN}), which has an intuitive definition, is scale invariant and has shown a better performance than other MCVs [52]:

$$\gamma_{VN} = \sqrt{(\mu^T \Sigma^{-1} \mu)^{-1}} \tag{4}$$

where μ is the mean vector ($\mu \neq 0$) and Σ is the variance-covariance matrix. To our knowledge, MCVs have not been applied in trait-based ecology, and thus offer new opportunities to disentangle how multiple, correlated traits respond to environmental gradients. It should be noted, however, that considering multiple traits reflecting different functions may convey a 'blurred' signal, obscuring main ecological patterns [40].

A major caveat of the CV is that it is computed on each individual population and cannot be partitioned between different populations, overestimating ITV across environmental gradients[114]. For example, imagine a plant species growing in two different environments, one found at higher altitudes and the other at lower altitudes. The plants in the high-altitude environment may be smaller on average than the plants in the low-altitude environment. Now suppose that both populations have the same CV (different mean and standard deviation, but proportional). If we combine the individuals from the two populations and calculate the CV for the entire species, the CV will be larger than the CV for either population individually, as we now incorporate substantial variability spanning individuals from both environments. To overcome this limitation, [114] modified the relative distance plasticity index (RDPI; [22]) to quantify ITV across environmental gradients accounting for within-population trait variability (PhD_K):

$$PhD_S = \frac{\sum_{s>m}^{S} PhD_{sm}}{\frac{S(S-1)}{2}} \tag{5}$$

where PhD_S is the phenotypic dissimilarity index for S ecological units (e.g., populations, individuals), and PhD_{sm} is the normalized version of the RPDI, which represents the expected trait dissimilarity across S ecological units (s, m = 1, 2, ..., S) in the range 0-1 [48]:

$$PhD_{sm} = \frac{RDPI - 0.5(D_s + D_m)}{1 - 0.5(D_s + D_m)} \tag{6}$$

The RDPI is represented by:

$$RDPI = \sum_{i}^{N_s} \sum_{j}^{N_m} \frac{d_{ij}}{N_s N_m} \tag{7}$$

where N_s and N_m are the number of individuals i and j in ecological units s and m, respectively, and $d_i j$ is any dissimilarity metric between the trait values of individuals i and j with the constraints $d_{ij} = d_{ji}$ and $d_{ii} = 0$ ([22]). D_s and D_m represent the expected trait dissimilarity between two individuals i and j drawn at random from ecological units s and m, respectively:

$$D_s = \frac{1}{N_s^2} \sum_{i,j}^{N_s} d_{ij} \tag{8}$$

$$D_m = \frac{1}{N_m^2} \sum_{i,j}^{N_m} d_{ij} \tag{9}$$

Essentially, the RDPI computes the mean trait pairwise dissimilarity among all the individuals from different populations (without accounting for within-population variation). Therefore, the PhD_S index generalizes the RDPI by computing the mean value of PhD_{sm} for all possible $\frac{K(K-1)}{2}$ pairs of ecological units and accounting for within-population trait variation [114].

4 Multiple species, one trait

4.1 Community-weighted intraspecific trait variance

From an ecological perspective, it is often of interest to describe functional diversity or composition of a species in a given local or regional assemblage, using a single trait. One of the most widely used functional composition metrics which does not incorporate ITV is the community-weighted mean (CWM), representing the average trait distribution (weighted by species abundances) of a given assemblage k [16]:

$$CWM_k = \sum_{i=1}^{N} p_{ik} \overline{X}_i \tag{10}$$

where p_{ik} is the relative abundance of species i in assemblage k and $\overline{X}_i$ is the mean trait value of species i. A simple extension to account for ITV of this metric consists of computing the community-weighted variance (CWV) as the dispersion of a functional trait around its mean, weighted by relative abundances [32]:

$$CWV_k = \sum_{i=1}^{N} p_{ik}(\overline{X}_i - CWM_k)^2 = \left(\sum_{i=1}^{N} p_{ik}\overline{X}_i^2\right) - CWM_k^2 \tag{11}$$

The CWV does not account for ITV per se, but for between-species variability (i.e., it is a measure of interspecific functional diversity). A further step is to expand this metric to account for ITV in a community-weighted intraspecific trait variance (CWITV; [87]):

$$CWITV_k = \left[\sum_{i=1}^{N} p_{ik}(\overline{X}_i^2 + S_i^2)\right] - CWM_k^2 \tag{12}$$

where S_i^2 is the variance of the trait for species i. From this, it follows that intraspecific variability increases functional diversity; if $S_i^2 = 0$ for every species in the assemblage (i.e., when ignoring ITV), then CWITV = CWV.

4.2 Linear mixed effects models

To describe the extent of ITV and decompose it by different organizational levels (e.g., community, species, individuals), several approaches have been proposed based on variance partitioning methods [35, 41]. These provide an intuitive way to quantify both the degree of overall ITV within a given organizational level and the relative contribution of ITV to FD of different levels in a hierarchical manner. The simplest method is based on linear mixed effect models (LMMs), also called variance component analysis. Although LMMs have been traditionally applied in quantitative genetics to estimate genetic variances and covariances of phenotypic traits [5, 10, 60], their use in trait-based ecology is more recent [29, 31, 91]. The response variable is represented by a functional trait, whereas random effects are represented by individuals, species or populations, nested one into another. Then, the relative contribution of a level is estimated as the ratio between the variance of a given level and total variance [15]. The species random effect is typically of most interest, because it allows assessing whether between-species trait variance is larger than within-species trait variance, the key assumption of the mean field approach [41]. Fixed effects (biotic and abiotic predictors) can also be included, but these are usually only represented by the intercept, because the primary aim is often to describe the relative contribution of main sources of ITV to overall variation. As many as different ecological levels can be included to account for any other context-specific sources of variation like plots, forest patches, or vegetation strata (e.g., [41, 91]). The main drawback of LMMs is that these assume a normal distribution of errors, so that these are restricted to continuous symmetric trait distributions (but see expansions for other error distributions in [60]), and that these do not include information about multiple functional traits within and between species. The first limitation can be overcome by using generalized linear mixed models (GLMM) to select the most appropriate error distribution based on the nature of the trait under study. For instance, since most continuous traits cannot take negative values in nature, a gamma distribution may often be a more suitable choice than a Gaussian distribution (which is typically used in a LMM). Similarly, for dummy-coded traits, a Bernoulli distribution can be employed to effectively model the frequency of zeros and ones in the trait's distribution.

4.3 T-statistics

Another approach to partition functional trait variance across organizational levels is based on T-statistics [41]. These represent variance component ratios considering four organizational levels: individual, population, community, and regional pool. Based on [6] findings that the relative importance of intra- and interspecific phenotypic variation is key for species coexistence, these variance components test for internal and environmental filtering of a given assemblage at different organizational levels [41]. As a consequence, T-statistics, in contrast to LMMs, rely strongly upon species competition notions to improve our theoretical understanding about the importance of ITV for assemblage structure. This framework identifies six variance components based either on individuals or on the mean of a set of individuals of each respective ecological level. From these components, a set of three ratios, termed T-statistics, are computed to estimate the magnitude of external and internal filtering based on ITV: $T_{IP/IC}$, $T_{IC/IR}$, and $T_{PC/PR}$. $T_{IP/IC}$ is the ratio of population-level variance (σ^2_{IP}) to community-level variance (σ^2_{IC}), and measures the strength of internal filtering or niche packing (e.g., microenvironmental heterogeneity or density-dependent processes) among the species of the community; $T_{IC/IR}$ is the ratio of community-level variance (σ^2_{IC}) relative to the total variance in the regional pool assessed at the individual level (σ^2_{IR}), and measures the strength of external filtering (e.g. abiotic constraints or external interactions) when accounting for individuals; and $T_{PC/PR}$ is the same ratio as TIC/IR, but without accounting for ITV, and measures the strength of external filtering at the species level. To quantify T-statistics, therefore, researchers must clearly define what a community and a population represent under their context-specificity of each study (e.g., [88]).

4.4 Trait overlap

Another potential solution to account for ITV is to characterize the trait distribution within each species assuming some particular shape (e.g. fitting a Gaussian probability distribution with trait mean and variance or using kernel density estimation), and computing the overlap between curves ([21, 36, 93]). The larger the overlap, the lower the trait dissimilarity between species. These distributions represent probability density functions that integrate to one, so that the dissimilarity between species i and j is given by 1 - overlapij ([21]). The dissimilarity between each species pair for a single trait can be computed ([36]), and then any FD metric based on dissimilarities can be used (see 'Trait probability density functions' below). Using trait dissimilarities based on ITV is biologically more meaningful than trait dissimilarities based on species means, as it links traits with the Hutchinsonian niche concept and trait overlap with niche overlap ([43, 64]). The biggest issue with this approach is that two non-overlapping trait distributions may be very close or very far from each other, so that an overlap equal to zero cannot tell how different the traits of each species are.

5 Multiple species, multiple traits: functional diversity and the inclusion of intraspecific variability

Despite the importance of ITV in functional and community ecology, the vast majority of FD methods rely on using a single mean trait value per species, and thus assuming that ITV equals zero (see [106, 93] for reviews). However, as we have seen above, ITV is not always negligible and can be substantial under certain circumstances, so the question is: How can we account for ITV when estimating FD for multiple species and multiple traits simultaneously?

5.1 Individual-level functional diversity

The simplest approach to account for ITV in FD consists of using an individual-by-trait matrix instead of a species-by-trait matrix, and then computing FD metrics straightforwardly. For instance, a functional dendrogram (representing the functional distances among individuals) can be built with a hierarchical clustering algorithm, and used to compute the sum of branch lengths connecting individuals present in a given assemblage (dendrogram FD) [27]. Alternatively, functional ordination can be applied on the individual-by-trait matrix and used to calculate functional richness, regularity and divergence indices (e.g., [91]). This approach has usually low computation time, yet its main limitation is the requirement of exactly the same individuals in both the individual-by-trait and the assemblage-by-individual matrices in order to obtain conformable matrices. This can require considerable sampling effort, particularly for large assemblages. When individual trait data are not available, a common situation when working with trait databases, trait values can be simulated by drawing random values from a theoretical probability distribution (e.g., [74]). Nevertheless, this approach should be taken with great caution, as ecological processes are not expected to result in purely random ITV values under environmental change, but the opposite [33, 39, 41]. In particular, there is no theoretical justification to include ITV into FD as random draws from a single probability distribution if the hypothesized mechanism structuring ITV is environmentally-driven [33, 39]; in those cases it would be preferable to estimate a probabilistic distribution for each set of environmental conditions [103, 109].

5.2 Variance decomposition

To assess the impact of ITV on FD, [35] based on [37] proposed two methods based on variance partitioning, using a multivariate expression of variance based on dissimilarity between species. The first method aims at partitioning FD into within- and between-species FD (a variance partitioning recalling the quadratic entropy partitioning), whereas the second method aims at separating the effects of species turnover from ITV (repeated measures ANOVA and variance partitioning) [35]. The first method decomposes total community trait variance into between- and within-species variance:

$$\frac{1}{N}\sum_{i=1}^{N}\sum_{l=1}^{n_i}\frac{1}{n_i}(x_{il}-\overline{X})^2=\frac{1}{N}\sum_{i=1}^{N}(\overline{x}_i-\overline{X})^2+\sum_{i=1}^{N}\sum_{l=1}^{n_i}\frac{1}{n_i}(x_{il}-\overline{x}_i)^2 \tag{13}$$

Therefore, this approach is conceptually similar to a linear mixed effect model, but all the traits are simultaneously used and a measure of overall FD is obtained [35, 37]:

$$\frac{1}{2}\sum_{k=1}^{n}\sum_{l=1}^{n}P_kP_ld_{kl}^2=\frac{1}{2}\sum_{i=1}^{N}\sum_{j=1}^{N}p_ip_jd_{ij}^2+\frac{1}{2}\sum_{i=1}^{N}p_i\sum_{k_i=1}^{n_i}\sum_{l_i=1}^{n_i}\frac{1}{n_i^2}d_{kl_i}^2 \tag{14}$$

where d_{kl}, is the functional distance between individuals k and l, $P_k = p_i/n_i$, d_{ij} is the functional distance between species i and j, and p_i and p_j are the relative abundances of species i and j, respectively (with $\sum_{i=1}^{N} p_i = 1$ and $\sum_{j=1}^{N} p_j = 1$). This corresponds to the Rao quadratic entropy (with Euclidean distance and divided by two), a widely used metric of FD [17]. Total diversity encompasses both between-species diversity and within-species diversity. On the left-hand side of the equation, total diversity is quantified as the weighted average dissimilarity between all pairs of individuals, where the weights represent the relative abundances of the species to which the individuals belong. In contrast, between-species diversity, represented by the first term in the right-hand side of the equation, solely considers the average dissimilarity between individuals from different species. On the other hand, within-species diversity (second term in the right-hand side) focuses on the average dissimilarity between individuals within the same species. The contribution of each species' within-species diversity to the overall total diversity is also weighted by the species' relative abundance. As a result, multiple traits for individuals belonging to multiple species can be analyzed simultaneously, in addition to single traits like in LMMs or T-statistics.

Functional diversity responses to the environment cannot only result from changes in ITV, but also from species composition (species turnover). For instance, a hypothetical decrease in bird body mass variability with elevation could stem from changes in species composition alone (the body mass of individuals is constant within species, but larger species dominate at lower elevations) or to ITV alone (species composition is constant across elevations, but larger individuals dominates at lower elevations; [122]). Thus, [35] proposed a method to dissect the effects of ITV and species turnover on FD, in which two FD measures are computed [35]: an FD metric calculated using only the individuals in each assemblage or sampling unit ('FDhabitat'), and an FD metric computed using the same (mean) trait values across all assemblages ('FDfixed'). Differences in FDfixed values among sampling units can only be due to species composition since FDfixed is constant for every individual, whereas differences in FDhabitat can be due to changes in either ITV or species composition [35]. Thus, the difference between FDhabitat and FDfixed is only due to ITV across assemblages:

$$ITV = FD_{habitat} - FD_{fixed} \tag{15}$$

5.3 Trait onion peeling and trait even distribution

Functional richness (defined as the amount of functional trait space occupied by an assemblage; [18, 25]) is a key functional diversity dimension that has been extensively used in FD studies [81]. Operationally, it is quantified as the volume of the minimum convex hull containing all species in an assemblage [20]. It is strongly related to species richness, and it is only sensitive to species loss at the edges of the distribution, ignoring gaps and inner species within the functional space. To account for ITV, consider all the individuals within functional trait space, and minimize the amount of filled functional space, [62] proposed an index representing the sum of all successive convex hull areas touching all individuals within functional trait space, termed trait onion peeling (TOP) index. This index is more sensitive to disturbance agents or other environmental constraints (e.g. ecological filtering of individuals due to land use transformation) that impact the extreme trait values species than those species with common trait values, since the volume represented by the convex hull of the extreme trait values is larger [81]. By contrast, the TOP index detects changes that affect the center of the trait distribution better than other functional richness metrics [62, 81].

Additionally, [62] proposed a functional regularity metric to measure how evenly distributed are individuals within the functional trait space, termed trait even distribution (TED) index. Based on n-dimensional sphere with evenly distributed points, a distance matrix is obtained as a distribution reference and compared with the distance matrix of observed individuals as $1 - log_{10}(KL + 1)$, where KL is the Kullback-Leibler divergence between the two probability distributions of distances [62]. If the two distributions are equal, then $KL = 0$ and TED = 1 (maximum regularity). Through simulations, [62] showed that TED outperformed classical metrics in detecting changes in functional regularity. For instance, [85] found that two bumblebee species showed higher TOP and lower TED indices in urban than in rural populations based on morphological traits, suggesting some degree of phenotypic and functional divergence.

5.4 Trait probability density functions

Along the same line of the trait overlap approach, an improvement in incorporating ITV into functional diversity was the integration of the probabilistic nature of trait distributions with the Hutchinsonian niche concept of a multidimensional n-hypervolume, termed trait probability density (TPD) approach [59, 84]. Again, the trait distribution of a species can be described by a trait probability density function TPD_i, which is summed for a given assemblage and weighted by species abundances to give the trait probability density of the assemblage k, TPD_k:

$$TPD_k = \sum_{i}^{N} p_i TPD_i \tag{16}$$

There are several alternatives to estimate TPDs. Kernel density estimators (KDE) are the most widely used and are suitable in most situations, having the advantage of not assuming an a priori shape of the trait distribution. Given a set of n observations of R traits measured on species i, the KDE of the species is:

$$TPD_i(x; \mathbf{H}) = \frac{1}{n} \sum_{l}^{n} K_{\mathbf{H}}(x - X_l) \tag{17}$$

where $x = (x_1, x_2, ..., x_R)^t$ is an R-dimensional functional trait space, $X_l = (X_{l1}, X_{l2}, ..., X_{lR})^t$, $K(x)$ is the kernel, and $\mathbf{H}$ is the bandwidth matrix.

The most important feature of this framework is that TPD functions can be aggregated to characterize any spatial scale or ecological unit, from individuals (if several measurements per individual are available) to the global scale [84, 2, 104], so that any other level of intraspecific trait variability beyond the individual can be accounted for (e.g., [91]). Because TPD functions are probability density functions, probabilistic overlap between TPD functions can be estimated in a similar way as explained above in the one-dimensional case. When applied at the species level this approach provides an estimation of species dissimilarity. In addition, TPD functions from different spatial scales can be compared to obtain an estimation of functional distinctiveness at any scale [69]. Once TPD functions have been established for each species, functional richness, regularity and divergence can be computed as the amount of functional space occupied by the TPD function, the overlap between the TPD and a uniform trait distribution occupying the same part of the functional space, and the abundance-weighted distance to the center of gravity of the TPD, respectively [59, 84]. The most important caveat of this approach is that the sample size per species needed to characterize the underlying probability density function accurately increases exponentially with the numbers of dimensions considered (known as the ‘curse of dimensionality’). As a result, multiple traits may require a prohibitively large amount of trait data [77]. Alternatively, assumed probability density functions can be used if a low number of individuals are sampled or only means and variances are known (e.g., normal distribution). Because of this, current software implementation may be unreliable if considering more than four traits, although dimension reduction techniques can be used to previously extract a lower set of dimensions as surrogates for functional traits [59, 104]. Finally, the use of probability functions with non-zero density everywhere and no defined thresholds to delimit their edges could lead to hypervolumes with infinite boundaries (which also applies to hypervolumes and trait overlap for a single dimension) [58].

5.5 n-dimensional hypervolumes

In the same vein of the TPD approach, [45] described the Hutchinsonian niche with an n-dimensional hypervolume by delineating a geometrical shape around a set of occurrences on environmental axes. The idea is constructing a hypervolume function based on species data located in environmental space. More recently, the hypervolume concept was extended to build functional trait spaces [47, 90, 94, 97], in which a set of species are located on functional trait axes. Essentially, a n-dimensional hypervolume is delineated around species trait centroids, and its size is considered a measure of functional diversity. The general procedure to build an n-hypervolume consists of (1) generating random points around each species, (2) resampling down to uniform density, (3) computing a KDE at each random point, and (4) defining some threshold τ to retain points that will characterize the hypervolume (see Box 1 in [77]). The random points delineating the hypervolume can be generated using different algorithms (Gaussian KDE, box KDE, and support vector machines [76], yet the Gaussian KDE is often the recommended choice under most situations, as the other two methods assume a constant probability density across the distribution [77, 97]. A detailed mathematical description of these algorithms can be found in [45, 76], and we refer to [77] for technical description of hypervolumes, and to [97] for their extension to FD analyses.

Although hypervolumes based on species data establish an analogy with the niche concept, functional trait spaces do not describe niches strictly speaking, because the niche is typically a property of a species and often estimated by environmental variables [28, 122]. However, if intraspecific trait variability is accounted for to characterize the functional trait space, a straightforward link between niche and the hypervolume can be made. For example, this idea has been applied to quantify and compare hypervolumes at the intraspecific level by using individuals by trait matrices of Darwin's finches [94] and cave spiders from Europe ([97, 120]). In this case, random points are generated around each individual (instead of species) to build the hypervolumes, and then multiple species-level hypervolumes can be combined to perform assemblage-level analyses (see specific function in [110]). By definition, the volumes of these shapes will always increase after accounting for ITV, so excluding ITV will underestimate functional richness. However, since functional diversity analyses accounting for ITV using this approach has been little implemented, the degree of bias on functional richness, as well as the impact of ignoring ITV on other FD facets, remains to be tested.

Similar to the TPD approach, major drawbacks of hypervolumes include the difficulty of handling categorical traits (although dimension reduction methods can be used) and highly correlated traits (which produce degenerate results), as well as the need for a high number of data to accurately represent KDEs [45]. In addition, input parameters (kernel bandwidth and threshold

τ) influence the results, with smaller bandwidths and/or a larger threshold producing smaller hypervolumes (because each observation is now more separated from each other) and vice versa. Moreover, KDE may under- or overestimate hypervolumes, depending on the dimensionality of the data (overestimation in lower than four dimensions, overestimation in larger than six) and the number of species considered [73]. It only recognizes clusters and holes when data are numerous (100 and 1000 respectively; [73]). This is problematic for community ecology, wherein most assemblages have less than 1,000 species, but this could be potentially overcome with the use of a large sample of individuals when accounting for ITV (e.g. for biogeographic studies examining the role played by ITV in shaping species distributions; see Point 2 in Box 1). More importantly, hypervolumes are built with nonparametric methods, so that the integration of empirical niches with niche theory is limited [105]. To address this caveat, [105] proposed a method to quantify and partition niche volume and dissimilarity based on the assumption of multivariate normal (MVN) distribution of traits, and termed this framework multivariate normal hypervolumes (MVNH). One major advantage of this method is that it provides parametric measures of the size (determinant of Σ of a MVN distribution) and dissimilarity (Bhattacharyya distance) of niche hypervolumes [105]. The determinant of the trait covariance matrix (hypervolume size) is a measure of functional richness [105]. Niche size can be further decomposed into univariate variances and a correlation component measuring hypervolume dimensionality, whereas niche dissimilarity can be decomposed into the Mahalanobis distance between hypervolumes and a size difference of niche volumes [105]. This approach had been already proposed to estimate the TPDs of species using the mean and variance (and eventually covariance) of traits when information on individuals is not available (function TPDsMean in the TPD package; [59]). Under the TPD framework, Bhattacharyya distance should give an estimation of dissimilarity similar to this approach (overlap between probabilistic distributions). Although this method is useful when individual trait data is not available (but there is information on trait means and variances), overcomes the limitations of nonparametric methods described above, and provides a partitioning framework for understanding different questions in ecology, it computes only one FD metric (functional richness, but see [59]) and has the strong assumption of a MVN trait distribution. In the case of functional diversity, there is no a priori reason to expect a MVN distribution of functional traits, which are indeed often quite irregular, due to the occurrence of species with extreme trait values and areas of high and low species densities (e.g., [61, 91, 104]). However, when moving from species to individuals, normal trait distributions to describe species fundamental niches are more theoretically and empirically justified [101, 99]. To our knowledge, there have been no applications of this method to account for ITV in FD analyses, which however holds great potential.

6 A simulated case study comparing different functional diversity metrics accounting for intraspecific trait variability

To compare different methods accounting for intraspecific trait variability, we simulated assemblages with varying levels of ITV, internal and external filtering (see below). Since we were interested in the effects of ITV on different FD metrics, the same parameters for a given assemblage were used throughout (number of assemblages = 10, species richness per assemblage = 10, number of individuals per species = 10). For the sake of simplicity, we used only two continuous traits following normal distributions. For the first trait, the distribution of assemblage means was simulated with a sequence of equally distributed mean values (between 10 and 50) along the trait gradient. For the second trait, the distribution of assemblage means was simulated with normal distributions with $\mu = 50$ and σ ranging from 5 to 20. This has the purpose of reducing the correlation between (and thus the redundancy of) both traits. For a given trait, the variance of each assemblage was simulated with a sequence of CV values (ranging between 0.3 and 0.6) representing the assemblage trait variance. The broader the range and the lower the assemblage variance, the higher the external filtering. In turn, for each assemblage, we created a sequence of CV values (ranging between 0.05 and 0.4) representing species trait variances (i.e. ITV). The lower the ITV, the higher the internal filtering. Therefore, every possible pair of combinations of overall trait range, assemblage variance and ITV resulted in a total of 1,000 simulation scenarios. With the resulting values, we fitted linear mixed effect models between each pair of metric including the number of simulations (which represents a set of 10 assemblages resulting from the same random process) set as a random effect to account for differences between simulated contexts. Metrics were standardized to mean 0 and variance 1 to allow model coefficient comparison. Given the large samples used, we use Cohen's heuristics to interpret standardized partial model coefficients: ±0.1 for a small effect size, ±0.3 for a moderate effect size, and ±0.5 for a large effect size [9].

Table 2 summarizes the different methods presented to account for ITV in trait-based ecology and functional diversity calculations, along with their strengths and drawbacks, and type of ecological questions that can be addressed.

Simulations on assemblages with varying degrees of between-assemblage, between-species and intraspecific trait variability showed that some intraspecific functional diversity metrics are strongly correlated. For instance, metrics describing the functional trait size (functional richness based on functional dendrograms, trait onion peeling, trait probability density functional richness and hypervolume functional richness) were strongly, positively associated, with standardized model coefficients ranging between 0.50 and 1.14 (Fig. 2). By contrast, most metrics were weakly correlated or not correlated at all (Fig. 2), indicating the measurement of different FD facets or the inherent different nature of each method. Other less clear patterns emerge, including strong associations between hypervolume functional divergence and metrics describing the functional trait size, with standardized model coefficients ranging between 0.79 and 1.05, and a strong association between hypervolume functional regularity and functional richness based on functional dendrograms (standardized model coefficient = 0.79).

7 Discussion

Species are not homogeneous entities, but rather mosaics of individuals with traits values which can vary greatly [1]. This understanding, known as intraspecific trait variability (ITV), is having a significant impact on the field of functional ecology, which is moving forward along the path to incorporate ITV more routinely [33, 34, 108, 109]. However, the presence of many methods available to potentially account for ITV in FD analyses combined with the absence of a methodological synthesis have prevented full application of these methods, much like has happened in 'classical' diversity approaches [19, 83, 113]. Here, we performed a review of the methods available to account for ITV in ecological research and, more specifically, in functional diversity analyses, with the aim of aiding ecologists to select the most suitable approach based on the questions of interest and the context-dependency of each study.

7.1 When and why considering ITV is important?

Although accounting for ITV has been a concerning issue in trait-based functional ecology, it is critical to decide when ITV should be considered [33]. It should be stressed that examining ITV is not always necessary nor desirable. For instance, if the aim is to assess changes in FD at a continental or the global scale (e.g. [2, 104], mean traits alone should be sufficient to capture changes in functional traits or FD, and ITV would potentially represent random noise. For instance, using bird responses to urbanization across the continental United States, [116] found that interspecific variability was greater than intraspecific variability, and that using traits to predict urban tolerance did not change patterns using mean traits alone vs. mean traits accounting for ITV. In other words, the spatial scale of analysis becomes highly relevant when deciding whether or not to incorporate ITV in any FD analysis (Box 1).

The decision to include ITV should also be motivated by theoretical justifications or previous knowledge, ideally forming conceptual frameworks wherein specific hypotheses can be tested. If one's objective is to quantify changes in FD along an environmental gradient, it is sensitive to account for ITV, because gradients usually reflect different local abiotic conditions in which pools of species or individuals exhibit different trait values matching these conditions [70, 112]. For example, in woody plant species of mediterranean-type ecosystems, ITV and functional traits related to resource use and conservation in more water-limited conditions tended to be more constrained than those in more environmentally relaxed conditions [23, 82], aligning with predictions of the physiological tolerance hypothesis [50]. If one is unsure whether to include ITV in the analysis, we recommend testing its contribution to FD beforehand, whenever possible. One first, rapid step to achieve this is to collect 5–10 individuals per species of a given assemblage, measure some functional traits and assess the contribution of ITV to overall variation (e.g. through linear mixed models). Previous literature should be scrutinized when deciding whether including ITV is relevant. Some taxonomic groups, such as invertebrates, have shown low levels of ITV (1–6% of intraspecific trait variance; [63, 86], whereas others, such as fungi, have shown large ITV (more than twice as variable as interspecific variation; [96]). In turn, some traits can be highly variable within a given taxonomic group (e.g., body mass CV in birds = 593%; [51]), while others can be much less variable (tail length CV in birds = 71%; [115]. Therefore, researchers should be careful when studying organisms known to be highly variable in functional traits, as well as when including traits that largely differ in their variance.

7.2 How to integrate ITV into FD estimations?

After deciding that ITV may be of interest or relevant to estimate FD, the next step is to consider how it will be obtained. Ecologists may be tempted to retrieve data from public databases (when available), yet one should be cautious about this practice, especially if species occurrence data are gathered at small spatial scales. This is because global or regional trait variation is unlikely to accurately reflect variation at small scales [42, 91]. Therefore, a more appropriate decision to quantify ITV is to take on-site measurements, or at least measure traits on natural history specimens from the same study area – although this assumes that ITV does not significantly change over time. In this sense, the use of standardized protocols to measure functional traits is strongly encouraged (e.g. [66, 72, 89]), ensuring that traits are measured in comparable ways across studies. This makes it possible to integrate data from different sources, taxa and regions, while minimizing measurement errors. In addition, standardized protocols often offer guidelines to obtain adequate sample sizes to properly estimate and report not only trait means but also variances [72]. If the number of species is extremely large (e.g. tropical regions or microbiomes), a lower number of species can be sampled, but the proportion of species that needs to be sampled ultimately should depend solely on interspecific trait variability; the larger the trait variability between species, the larger the fraction of species that should be sampled [24]. These considerations about individual sampling and ITV measurement should be kept in mind to properly obtain unbiased estimators of ITV [102].

After quantifying ITV, proper identification of the FD metric to be used is crucial. Our review shows that this is not a straightforward task, because different frameworks aim to solve different questions (Table 2). For instance, if the main objective is to dissect ITV from other sources of variation at the assemblage level, then (G)LMMs, T-statistics and quadratic entropy partitioning constitute viable options. However, researchers should decide if their interest is in filtering processes (T-statistics), species turnover (quadratic entropy partitioning) or in specific ecological scales (LMMs). If the aim is to quantify the role of ITV on FD, then approaches such as individual-level FD, TPD functions or n-dimensional hypervolumes can be used. However, this decision will depend on the main research interest; n-dimensional hypervolumes and TPD functions are suitable to gain insights into a geometrical representation of the functional niche space occupied by assemblages, whereas individual-level FD would serve well for a description of the functional dissimilarity between individuals. These choices also depend on whether all the individuals within an assemblage have been sampled (individual-level FD) or not (n-dimensional hypervolumes and TPD functions), which is usually constrained by the taxon under analysis (mobile vs sessile organisms). Further, one should also keep in mind that metrics differ in the number of species and traits that can be included. In an attempt to guide ecologists in selecting the most appropriate metric according to their needs, we summarized the methods described in our review in Table 2, emphasizing the key question each approach tries to answer, as well as the number of traits and species each method can handle. Our simulations are consistent with recommendations provided above related to the decision-making procedure of the most appropriate FD metrics to use. For example, whether one aims to assess how some predictors, such as biogeographic and environmental factors (e.g. latitude, elevation, aridity, disturbance regimes), influences the size of the functional trait space, any of the approaches described above (functional dendrograms, TPD, n-dimensional hypervolumes) can be applied as they capture the same dimension, namely functional richness. We also caution the reader that our simulations encompass a limited range of ecological scenarios and employ a reduced set of traits following specific distributions, so that it is essential to validate the observed correlations against real-world data. More importantly, as [113] have pointed out, researchers are advised to employ a single trait space representation for all estimations to ensure consistency in FD calculations across multiple dimensions. While the correlation between some metrics may tempt their application across different frameworks, it is crucial to recognize that these frameworks vary fundamentally in their conceptual underpinnings.

7.3 Concluding remarks and future directions

We emphasize the importance of properly reporting ITV results after quantification and publication. In this sense, reporting all sources of variation, including between- and within-species variability, along with their values and some metric of confidence (standard errors, confidence/credible intervals), is desirable and highly encouraged. Whenever possible, we encourage reporting and depositing data and analytical codes in public repositories. This enhances transparency and reproducibility of trait-based studies [95, 113], and allows these sources of variability to be included into meta-analyses (e.g. meta-analysis of variation; [55, 100]).

As there is growing recognition of the importance of ITV in functional ecology, we expect a bright future of ITV and FD. However, to further advance the study of ITV, we still need to address some gaps. First, we require more studies describing ITV across spatiotemporal scales and taxa. In particular, the temporal dimension of FD incorporating ITV is poorly understood. Therefore, we stress the importance of quantifying FD accounting for ITV and identifying the factors that drive changes in FD over time. Second, ITV should be included in global databases as a standard practice, since most taxonomic groups need this information. Finally, theoretical and modeling attempts so far cannot fully capture empirical patterns [123], revealing the need for more predictive theory.

Overall, and despite the importance of ITV in ecology, we are far from a comprehensive and multifaceted understanding of the underlying mechanisms driving ITV in biological communities. Empirical and theoretical models that describe biological trends across space and time can provide insights into how ecological traits change. There is still much that we have to learn about ITV; we envision this synthesis both as a trigger and a step forward towards developing a more accurate trait-based ecology, by assisting ecologists when working with ITV.

8 Box 1. Why only a fraction of functional diversity analyses accounts for intraspecific trait variability?

It is now well-understood that ITV can largely influence or alter the detection of FD patterns. So why only a small portion of functional diversity analyses accounts for ITV (Fig. 1)? Some of the possible reasons and challenges are:

1. Data limitation: ITV databases are costly and time-consuming to assemble. While high-resolution trait data are increasingly available for groups such as birds (e.g. AVONET) and plants (e.g. TRY, DiasMorph), these are still lacking for other groups, such as most invertebrates and fungi. Two additional issues complicate the use of databases. First, if most of the trait measurements for a species come from similar conditions, then the ITV estimate for that species will be biased towards those conditions. This is because ITV can vary largely depending on the environment, so an estimate based on a limited sample of observations may not be representative of the entire species. Second, it is possible to think that one has a good estimate of ITV for a species because there are measurements from different studies. However, these measurements are often the average of many individual measurements, which can lead to an underestimation of ITV. Hopefully, the increased availability of standardized protocols for collecting traits designed to consider ITV (e.g. [72]) constitutes a first, yet decisive step forward to overcome this hurdle and achieve this long-term goal.

2. Nature of traits and data handling: Conceptually, ITV can be applied to any variable (i.e. trait) type. Working with continuous traits certainly represents the most straightforward way. However, most traits (especially for some taxonomic of the neglected groups above), are coded as categorical, ordinal, or binary variables, which may require some data handling (e.g. functional ordination) so as to make them suitable to integrate ITV in FD analyses. Importantly, categorical traits can sometimes accommodate ITV directly, such as in the case of the same species distinguished by individuals with different life histories (e.g. annual, biennial, perennial).

3. Scale of the study: The influence of ITV on biodiversity patterns is assumed to decrease with increasing geographic scale (see Box 2 for more details). While for the majority of continental-to-global-scale analyzes the inclusion of ITV may be trivial and/or impractical, at these broad scales some key questions in ecology and biogeography can be tackled, such as testing for pair-

wise differences in FD accounting for ITV for a set of widespread vs. geographically restricted congeners, expecting higher FD for widespread species.

9 Box 2. Methodological aspects matter for quantifying the impact of intraspecific trait variability on functional diversity

Although ITV represents an important aspect for the fields of ecology and evolution, different methodological choices may alter its estimation, resulting in mathematical artifacts that may bias their interpretation and conclusions. To showcase these possible issues (points 1–3 below), we consider three examples: 1) 216 individuals of 25 bird species of the genera Turdus (Turdidae) and Patagioenas (Columbidae) and nine morphological trait data ('bird dataset 1'; see full details in [115]), 2) 3550 individuals of 30 random species of European diaspore traits ('fruit dataset'; see full details in [117]), and 3) 5179 individuals of 29 bird species widely distributed (occurring in six or more countries) and beak length ('bird dataset 2'; [115]).

1. The number of traits: As the number of traits used to compute ITV increases, species will differentiate more. Consequently, the proportion of variation due to differences within species will decrease. Essentially, when many traits are considered simultaneously, the opportunities for species to differentiate will increase. For a given metric, dissimilarity may increase, decrease or remain unchanged with a larger number of traits, depending on their mathematical properties, as shown in the next point.

2. The metric employed: When estimating dissimilarity between species accounting for ITV, different metrics may either increase or decrease species dissimilarity. For instance, Euclidean distance increased with the number of traits considered in both datasets, while Gower dissimilarity remained relatively constant (Fig. 3). In the first case, adding traits increases the chance of finding subtle differences between species, until each species becomes unique. This is a long-held notion in mathematics termed the 'curse of dimensionality': each new dimension adds a non-negative term to the sum in the equation, so that the distance increases with the number of dimensions ([3]). Gower dissimilarity, in contrast, scales absolute distances to the overall trait range, so that species dissimilarities depend on trait variability. However, as this is computed as an arithmetic average, it is expected that this metric will eventually reach a plateau as more traits are added. When using trait overlap metrics to assess dissimilarity (e.g., hypervolumes, TPD functions), an increase in the number of traits is expected to reduce trait overlap, because the potential number of neighbors in niche space increases more or less geometrically as the

effective number of dimensions rises ([38]). In the bird dataset, niche overlap computed using kernel density functions ([36]) and dynamic range boxes ([64]) showed a negative trend between trait overlap and the number of traits. By contrast, niche overlap computed using kernel density functions showed a positive relationship between overlap and the number of traits (Fig. 3), indicating that an increase in the number of traits does not always lead to lower trait overlap.

3. The scale of analysis: The relative magnitudes of ITV relative to interspecific variation varies across species and is scale-dependent [33, 30, 44, 70]. Yet, predicting how spatiotemporal scaling structures trait variation (i.e. variance scaling) remains a challenging and unsolved question in ecology [53, 111]. As a general trend, the ‘spatial variance partitioning’ hypothesis states that the amount of intra- and interspecific variations are low at fine spatial scales and increase with scale [33]. At coarse scales, the entire species’ ranges are included, and ITV tends to reach an asymptote. At fine scales, however, this model cannot predict the relative magnitudes of ITV and interspecific trait variation, since it depends on species ranges, gene flow, environmental heterogeneity and the traits under analysis [33]. When comparing the coefficient of variation (as a metric of ITV) in beak length against the number of countries a species occurs in (as a measure of spatial extent), a similar pattern emerges across species. As the number of countries a species occurs in increases, the CV of beak length increases as well. This trend continues until the entire distribution is included, at which point the ITV reaches a plateau (Fig. 3). Only one species showed a clear negative trend between ITV and scale increase (Anthus similis), which could be the result of high variation within a large country (India) and among disjunct populations (Tanzania, Malawi, Yemen).

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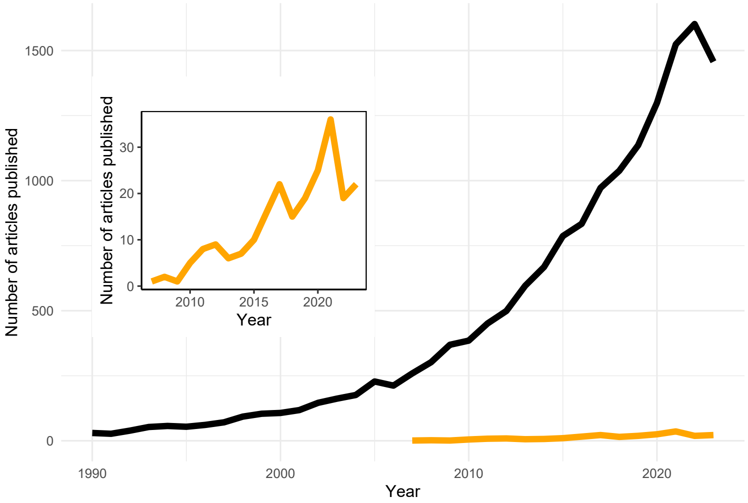

Fig. 1. Annual (1990–2023) number of published articles using the term ‘functional diversity’ (black line) compared to ‘intraspecific trait variability’ (orange lines). For enhanced visualization, the inset zooms in the curve of FD studies that includes ITV, which increased markedly in recent years, although still constituting a small fraction of the entire scientific production focused on FD. Articles were retrieved from Scopus on 14 November 2023, using the queries: ‘functional diversity’ and ‘functional diversity’ AND ‘intraspecific trait varia*’.

Table 1. Table for notations

Symbol	Description
i	Species
j	Trait
k	Site or community
l	Individual
N	Number of species
R	Number of traits
n	Number of individuals
p	Species relative abundance
s, m	Ecological units
S	Number of ecological units
$\mathbf{C}$	Community by species matrix
$\mathbf{T}$	Species by trait matrix
CV	Coefficient of variation
CV_B	Bao's coefficient of variation
^{K}CV	Kvålseth's coefficient of variation
MCV	Multivariate coefficient of variation
RDPI	Relative distance plasticity index
PhD	Phenotypic dissimilarity
$\overline{X}$	Trait mean
S^2	Trait variance
μ	Mean vector
$\varSigma$	Variance-covariance matrix
γ_{VN}	Voinov and Nikulin's coefficient of variation
γ_1	Sample sknewness
γ_2	Sample kurtosis
CWM	Community-weighted mean
CWV	Community-weighted variance
CWITV	Community-weighted intraspecific trait variance
TOP	Trait onion peeling
TED	Trait even distribution
KL	Kullback-Leibler divergence
σ^2_{IC}	Community-level variance
σ^2_{IP}	Population-level variance
σ^2_{IR}	Region-level variance
$T_{IP/IC}$	$\frac{\sigma^2_{IC}}{\sigma^2_{IC}}$
$T_{IC/IR}$	$\frac{\sigma^2_{IC}}{\sigma^2_{IR}}$
$T_{PC/PR}$	$\frac{\sigma^2_{PC}}{\sigma^2_{PR}}$
TPD	Trait probability density function
KDE	Kernel density estimator
$K(x)$	Kernel density function
$\mathbf{H}$	Bandwidth matrix
MVNH	Multivariate normal hypervolume

Table 2. Methods describing species trait variation in functional diversity studies indicating the number of traits and species allowed, primary research aim, example of ecological questions that can be tackled, strengths and drawbacks of each method, and key references.

Method	Number of traits	Number of species	Aim	Example of ecological question that can be addressed	Strengths	Drawbacks	Key references
Coefficient of variation	1	1	Assessing species-specific variability of a single functional trait	How leaf dry matter content vary across a latitudinal gradient?	Dimensionless. Useful to compare species, traits and studies	It is a biased estimator of the population coefficient of variation and does not account for within-population trait variability and multiple correlated traits	[71, 102, 121]
Trait overlap	1	2	Computing trait diffrerences between species accounting for intraspecific trait variability	How similar two mice species are in their tail length accounting for intraspecific trait variability?	A simple metric of trait similarity between species	Zero overlap does not provide a definitive measure of similarity between two distributions	[21, 36, 93]
Multivariate coefficient of variation	n	1	Assessing species-specific variability in multiple traits simultaneously	Does morphological variation (represented by a multivariate trait space) respond to altitude?	Dimensionless and scale invariant. It accounts for correlation among traits	Traits are assumed to follow normal distributions	[52]
Phenotypic dissimilarity index	1	1	Quantifying intraspecific trait variability across environmental gradients accounting for within-population trait variability	How trait matching between a flower and their pollinators vary across populations in their geographic range	It can partition intraspecific trait variability between populations	The accuracy and completeness of the trait data significantly impact its reliability	[114]
Community-weighted intraspecific variance	1	n	Estimating functional diversity of a single trait accounting for intraspecific trait variability	Does butterfly functional diversity ignoring intraspecific variability substantially underestimates that accounting for trait variability?	It quantifies functional diversity at the community level	It quantifies diversity of a single trait, which is assumed to follow a normal distribution	[87]
Linear mixed models	1	n	Quantifying the contribution of interspecific and intraspecific variability to overall trait variance	Is body mass variability larger at the community, species or individual level?	It can account for multiple different nested factors and fixed effects (predictors)	They assume a multivariate normal distribution	[56, 91]
T-statistics	1	n	Quantifying the contribution of assembly processes (external and internal filtering) to overall trait variance	Is environmental heterogeneity more important than species interactions for foraging behavior variability?	It relies on species competition theory, relating variances to ecological processes	Appropriate only for continuous traits. It considers only four organizational levels	[41]
Quadratic entropy partitioning	n	n	Quantifying the contribution of interspecific and intraspecific trait variability to functional diversity	Is intraspecific trait variability more important than interspecific trait variability for functional diversity?	It dissects the effects of interspecific trait variability from intraspecific trait variability at the community level	It considers only one functional diversity metric (i.e., Rao's quadratic entropy)	[35]
Repeated measures ANOVA and variance partitioning	n	n	Quantifying the contribution of intraspecific trait variability and species turnover to functional diversity	Is species turnover more important than intraspecific trait variability for fungi functional diversity?	It dissects the effects of species turnover from intraspecific trait variability at the community level	It considers only one functional diversity metric (i.e., Rao's quadratic entropy)	[35]
Individual-level functional diversity	n	n	Estimating functional diversity of multiple traits accounting for intraspecific trait variability	Does freshwater fish functional diversity accounting for trait variability relate to water salinity and depth?	Low computation time. Ability to compute metrics through simulations when data on individuals are not available	Need of conformable community and trait data matrices. Abundance is not accounted for. Random simulations when ITV not available is not appropriate when assessing environmental-driven changes	[27, 74]
Trait onion peeling	n	n	Estimating functional diversity of multiple traits accounting for intraspecific trait variability	Does freshwater fish functional diversity accounting for trait variability relate to water salinity and depth?	It detects changes that affect the center of the trait distribution better than other functional richness metrics	It describes only one facet of functional diversity (functional richness). Computationally demanding	[62, 81]
Trait evenness distribution	n	n	Estimating functional diversity of multiple traits accounting for intraspecific trait variability	Does freshwater fish functional diversity accounting for trait variability relate to water salinity and depth?	It detects changes in functional regularity better than functional evenness metrics	It describes only one facet of functional diversity (functional regularity)	[62, 81]
Trait probability density functions	n	n	Estimating functional diversity of multiple traits accounting for intraspecific trait variability	Does freshwater fish functional diversity accounting for trait variability relate to water salinity and depth?	They implement the Hutchinsonian niche concept as a probabilistic hypervolume. Scale independent metrics	A high number of observations is needed for accurate delineation of hypervolumes. Computationally demanding for large datasets	[59, 84]
n-dimensional hypervolumes	n	n	Estimating functional diversity of multiple traits accounting for intraspecific trait variability	Does freshwater fish functional diversity accounting for trait variability relate to water salinity and depth?	They implement the Hutchinsonian niche concept as a probabilistic hypervolume. Non-parametric methods account for disjunctions and holes (if data are sufficiently large). Parametric methods allow dissecting hypervolume size into univariate variances and a correlation component (dimensionality), and dissimilarity into size and distance shifts	Non-parametric methods need a high number of observations for accurate delineation of hypervolumes. Computationally demanding for large datasets. Dependency on input values (kernel bandwidth, quantile threshold in kernel-density hypervolumes). The inclusion of species abundances is restricted to Gaussian kernels. Parametric methods only estimate functional richness, and rely on the assumption of normally distributed traits	[45, 76, 97, 105]

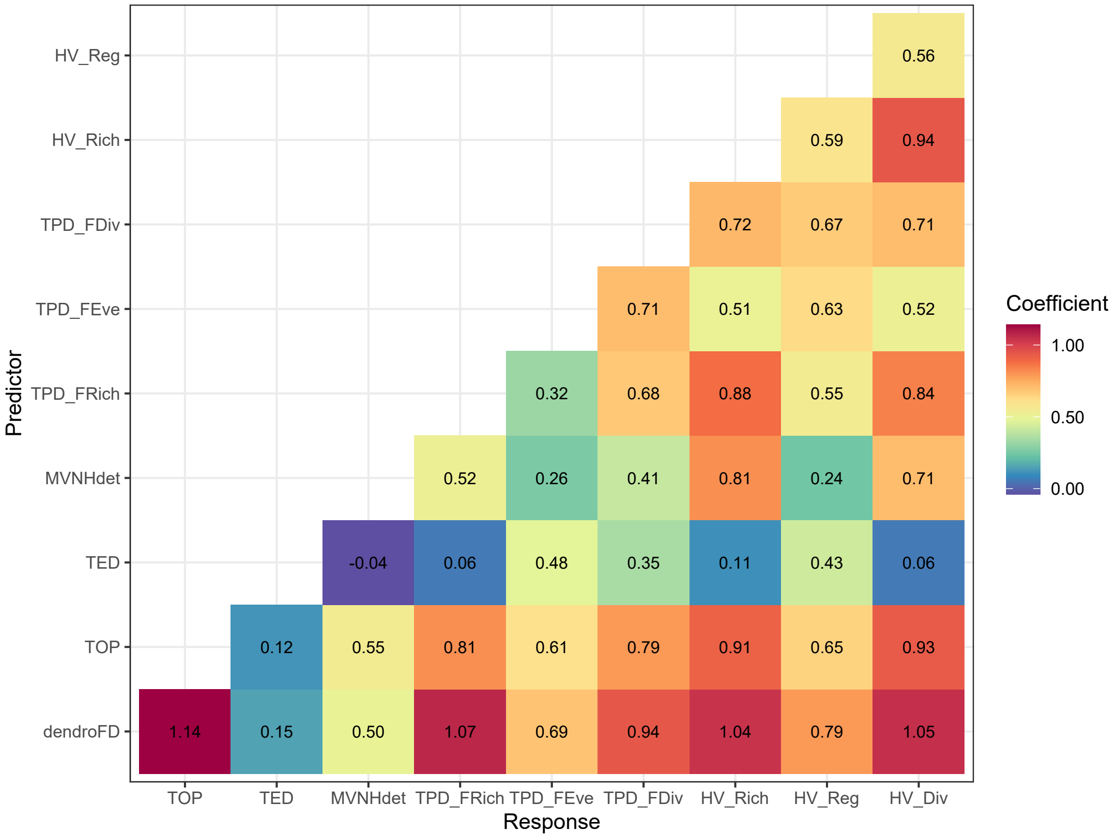

Fig. 2. Relationships between functional diversity metrics accounting for intraspecific trait variability. Results are based on simulating 1000 communities with varying degrees of between-assemblage, between-species and intraspecific trait variability (see main text). Standardized model coefficients between a predictor (first column) and a response (first row) derived from linear mixed models are reported (see main text). FD: functional diversity, FRich: functional richness, FReg; functional regularity, FDiv: functional divergence, FTOP: trait onion peeling, TED: trait even distribution, MVNH: multivariate normal hypervolume determinant, TPD: trait probability density, HV: hypervolume.

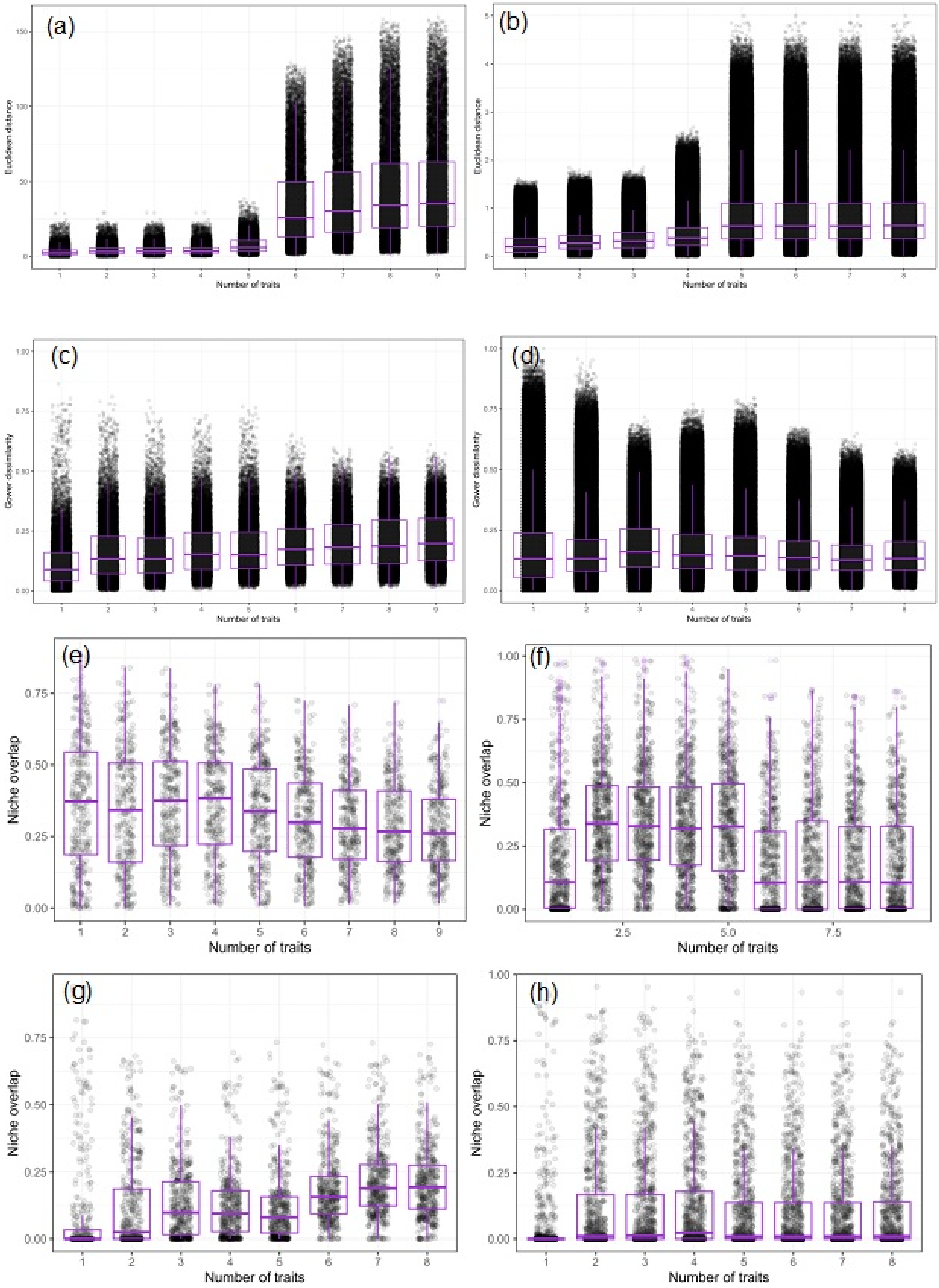

Fig. 3. Influence of the number of traits on metrics accounting for intraspecific trait variability. Points represent individuals (in the case of Euclidean and Gower distances) or trait overlap (in the case of dynamic range boxes and kernel density functions). Data retrieved from AVONET (a, c, e-f) and DiasMorph (b, d, g-h). See Box 2 for details.

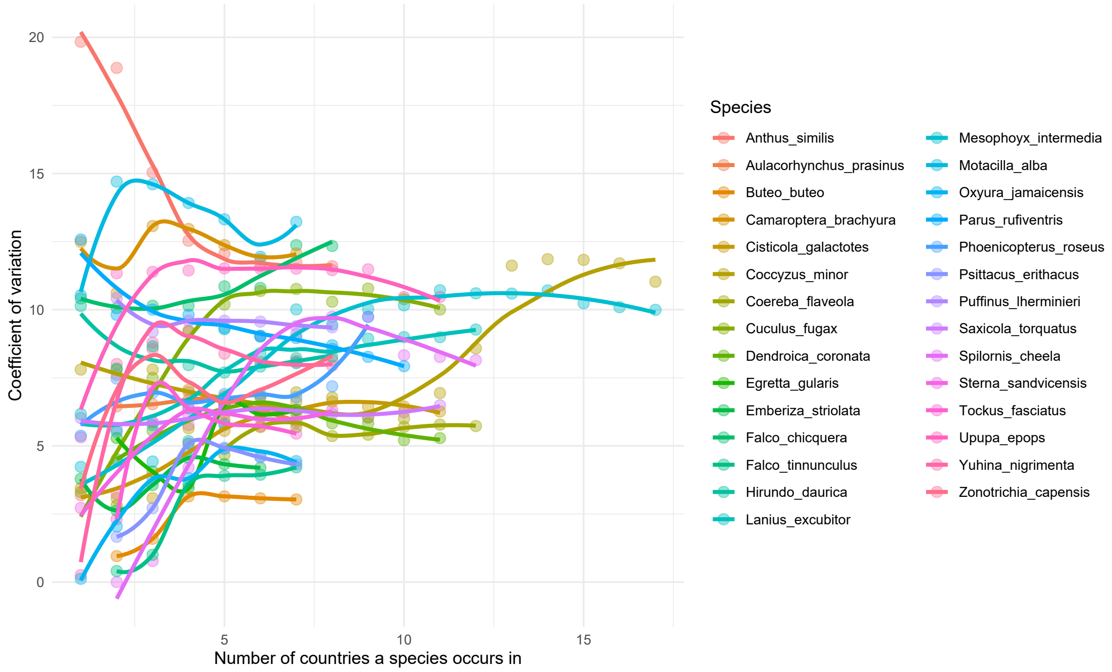

Fig. 4. Effect of spatial scale on intraspecific trait variability. Points depict bird species widely distributed and lines are loess smoothers representing the relationship between the geographic extent and the coefficient of variation (CV) in beak length.