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Quantifying the relevance of intraspecific trait variability for functional diversity

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Summary

1. Intraspecific trait variability is a crucial, often neglected, component of functional diversity (FD) in ecological communities. In particular, uncertainty remains as to the importance of intraspecific variability in the quantification of FD.

2. To explore this uncertainty, we propose two methods addressing two critical and complementary, but largely unexplored, questions: (i) what is the extent of within- vs. between-species FD in different communities? and (ii) to what extent is the response of FD to environment because of compositional turnover vs. intraspecific trait variability across habitats? The methods proposed to address these questions are built on a variance partitioning approach and have the advantage of including species relative abundance, therefore taking into account species dominance and rarity. For each of the questions, we illustrate one dedicated case study in semi-natural grasslands with associated sampling strategies.

3. The decomposition of total community variance into within- vs. between-species effects can be implemented in a manner similar to the decomposition of quadratic entropy on pairwise individual dissimilarity. The approach can be applied with single and multiple traits, although it proves more informative for single traits. It can prove particularly useful when assessing the role of different sources of trait variability in the assembly of communities.

4. The assessment of the relative contribution of intraspecific trait variability and species turnover to the response of FD to environment is based on a variance partitioning comparing FD indices computed (i) either using individuals measured in a specific habitat alone (*FDhabitat*) or (ii) all individuals measured across different habitats (*FDfixed*). This approach provides a more complete understanding of the response of FD to environment.

5. We further propose a guide to apply these two methods and to choose the most suitable method for intraspecific trait measurements. Assessing the role of intraspecific trait variability should allow a more comprehensive understanding of the processes that link species and ecosystems.

Key-words: biodiversity, community dynamics, grassland management, mass-ratio hypothesis, repeated measures, trait convergence and divergence, trait intraspecific variability, variance partitioning

Introduction

Functional diversity (FD) has emerged as a critical aspect of biodiversity that strongly influences species coexistence, species dynamics and ecosystem functioning (Tilman *et al.* 1997; Diaz & Cabido 2001). Various indices and mathematical frameworks have been developed to estimate FD, defined as the extent of trait dissimilarity in a given community (Petchey & Gaston 2006; Villeger, Mason, & Mouillot 2008; de Bello *et al.* 2009a). Among the different existing methods to estimate FD, it is remarkable that within-species (or 'intraspecific') trait

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variability is often neglected in FD calculations (but see Lepš *et al.* 2006; Mason *et al.* 2007; Cianciaruso *et al.* 2009). The importance of intraspecific trait variability (i.e. phenotypic differences among individuals within a species) has been long recognized by evolutionary ecologists (Fox, Roff, & Fairbairn 2001). More recently, various studies have shown the important effects of intraspecific trait variability on community assembly, habitat selection and ecosystem functioning (Callaway, Pennings, & Richards 2003; Boege & Dirzo 2004; Lecerf & Chauvet 2008; Funk 2008; Berg & Ellers 2010; Cornwell & Ackerly 2009; Gross *et al.* 2009; Thuiller *et al.* 2009).

However, when calculating FD, traits are in practice generally estimated using only one mean trait value per species and trait (either measured in the field or extracted from available databases). Such an approach implicitly ignores the extent of intraspecific trait variability (Cornwell & Ackerly 2009). Potential technical solutions of varying complexity have been proposed to incorporate intraspecific trait variability in FD calculation (Mouillot *et al.* 2005; Lepš *et al.* 2006; Mason *et al.* 2007; Lavorel *et al.* 2008). Cianciaruso *et al.* (2009) have also recently highlighted the importance of integrating intraspecific trait variation into calculations of FD indices. They demonstrated that FD indices computed either on a species basis (i.e. using only a mean trait value per species) or on an individual basis (using the trait values of each individual) are potentially different. Taken together, these preliminary works highlight that more detailed investigations of the relevance of intraspecific variability in the quantification of FD are necessary.

This task, however, requires methodological advances to efficiently account for intraspecific trait variability and understand to which extent and under which conditions it plays an important role in the assembly and dynamics of communities (Mouillot *et al.* 2005; Mason *et al.* 2007; Lavorel *et al.* 2008). In particular, two main ecological questions remain largely unexplored (Table 1): (i) what is the extent of within- vs. between-species FD in different communities? and (ii) to what extent is the response of FD to environment because of intraspecific trait variability vs. species turnover? Regarding the first question, it is striking that comparisons of FD across communities in the literature are performed almost exclusively in terms of between-species diversity (i.e. where only species mean trait values are considered in FD calculations; Petchey & Gaston 2006; de Bello *et al.* 2009a,b), usually linked to data availability. It is largely unknown what is the formal contribution of within-species diversity to total community FD (but see Westoby *et al.* 2002). Regarding the second question, FD response to environment can result from both changes in species composition (species turnover) and intraspecific trait response to environment. For example, a hypothetical decrease in plant FD with fertilization could be the result of either a change in species composition alone (e.g. the height of individuals is constant within a species, but taller species dominates fertile habitats) or to intraspecific variability alone (e.g. species composition remains unchanged, but most individuals are taller in fertile habitats). Very often, however, FD response results from the combination of both. Decomposing their relative contribution can be essential in understanding how communities react to environmental changes.

Here, we propose two related methods, based on the partitioning of variance, to address these two complementary questions. The two different ecological questions lead, however, to different types of variance partitioning, i.e. the two methods, and as a result, each of them imposes their own constraints on sampling individuals for trait measurements. We illustrate each of the methods using dedicated examples from different semi-natural grasslands based on pre-existing data sets. Although either of the two methods could be potentially applied to any given data set, particular approaches for measuring intraspe-

Table 1. Questions that can be asked with the methods proposed in this study and the different options that need to be considered when applying these methods. FD, functional diversity

Questions	Method	Species relative abundance	Single or multiple traits	Selection of individuals for trait measurements
1. Partitioning of trait diversity within- vs. among-species	Variance or quadratic entropy partitioning*	Equal or unequal‡	Single and multiple 'normalized' traits§	Random individuals
2. Separating the effect of species turnover vs. intraspecific trait variation	Repeated measures ANOVA and variance partitioning†	Depending on the FD index applied		Fully developed individuals¶

*For size-related traits, variance is expected to be linearly dependent on the mean, leading to higher variance for larger species. This is not because of higher intraspecific trait variability but rather to scaling in measurement units. In such cases, the log-transformation is an appropriate solution as it results in the independence of mean and variance as opposed to the use of the coefficient of variation which precludes additivity of within and between FD.

†FD values calculated, for each plot, both considering and not considering intraspecific trait variations among samples/habitats. Other mixed models could be also applied.

‡Species relative abundance can to be considered. 'Equal', here implies $p_i = 1/Nsp$ for all species.

§Variance partitioning can be compared across traits or computed with multiple traits together after trait normalization. Trait normalization can be obtained via different solutions (see text). For both methods, it is suggested to begin with assessing single traits and, only very carefully, then combining them into integrated FD indices.

¶For example, mature individuals and growing in optimal conditions.

cific trait variability should be preferred for each question (as highlighted in last section 'The selection of individuals'). Based on these examples, we propose a set of guidelines to be considered when sampling individuals for intraspecific trait variability assessments.

What is the extent of within- vs. between-species FD in different communities?

To answer this question, we propose the first of the two methods (Table 1), which aims at partitioning total community diversity into 'within-species FD' (extent of trait dissimilarity in a community because of intraspecific trait variability) and 'between-species FD' (extent of trait dissimilarity in a community because of differentiation between coexisting species). This can be accomplished in a manner similar to the decomposition of variance or, which is equivalent, with the decomposition of the quadratic entropy diversity (Pavoine & Dolédec 2005; Rao 2010). We formalized these calculations and apply them to a data set from an experiment on two Central European meadows (Table S1 & Fig. 1).

THE METHOD

As proposed by Lepš *et al.* (2006), the 'total community variance' can be decomposed into the sum of 'between-species variance' and 'within-species variance'. The equation below formalizes this approach, for the first time. Let us take a community composed by *i*-th species, with species richness (i.e. number of species) expressed as *Nsp*. Within each species *i*, quantitative trait values (x_{ai}) have been measured for several individuals a_i, for a total of $Nind_i$ individuals belonging to each species *i* (the total of individuals sampled, across all species, being *Nind*). The left hand of the eqn 1 represents the total community trait variance, while on the right hand of the equation corresponds to the between-species variance and within-species variance, respectively (from left to right).

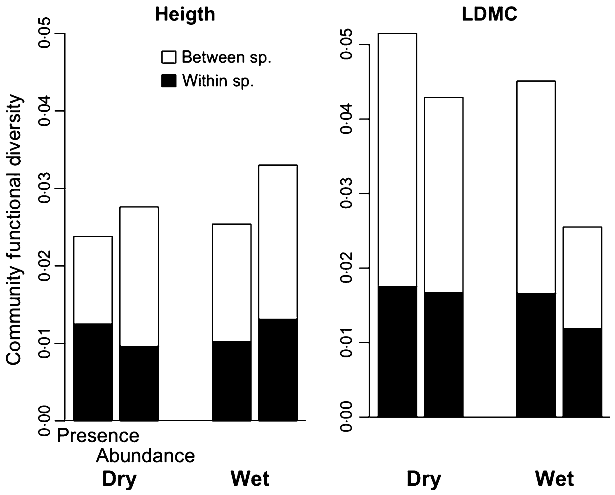

Fig. 1. Partitioning of total functional diversity (FD) (total FD; whole bars) into between- and within-species FD (white and black elements, respectively) into two different meadows (dry vs. wet; see methods). Results from case study 1. The FD is expressed as trait variance for height and leaf dry matter content (LDMC). The variance can be computed using only species presence/absence data or it can be weighted by the species relative abundance (see separate columns 'Presence' and 'Abundance').

$$\sum_{i=1}^{Nsp}\frac{1}{Nsp}\sum_{a_i=1}^{Nind_i}\frac{1}{Nind_i}(x_{a_i}-x_{com})^2=\sum_{i=1}^{Nsp}\frac{1}{Nsp}(x_i-x_{com})^2+\sum_{i=1}^{Nsp}\frac{1}{Nsp}\sum_{a_i=1}^{Nind_i}\frac{1}{Nind_i}(x_{a_i}-x_i)^2 \qquad \text{eqn 1}$$

where

$$x_i=\sum_{a_i=1}^{Nind_i}\frac{1}{Nind_i}x_{a_i}$$

and

$$x_{com}=\sum_{i=1}^{Nsp}\frac{1}{Nsp}x_i$$

This formulation implies that the contribution of each species to the variance decomposition is identical (i.e. differences in sampling efforts across species are ignored), as all factors are weighted by the number of species *Nsp*. In case one would consider that the number of individuals sampled corresponds to the abundance of species in the community (i.e. differences in sampling efforts across species do count), then the parameter *1/Nsp* should be replaced by $Nind_i/Nind$. It should be noted that with this replacement the total and within-species sum of squares are actually divided by the total number of individuals (see below for the equivalence with PERMANOVA – Anderson 2001, 2005 – and the Appendix S1 for a practical example). However, as discussed in the last section of this work ('The selection of individuals'), very often the individuals sampled for trait measurements are only part of the population of a given species in a community. Note also that the formulation of the x_{com}, also depending on how the relative abundance of species is considered, corresponds to the community trait mean, as applied e.g. by Garnier *et al.* (2007) and Lavorel *et al.* (2008).

Although this kind of variance partitioning should be rather intuitive for most ecologists, the equation could be written in more general terms. Indeed, any form of variance could be expressed in terms of the mean dissimilarity between pairs of observations. For example, as demonstrated by Champely & Chessel (2002), for the dissimilarity between pairs of species:

$$\sum_{i=1}^{Nsp}p_i(x_i-x_{com})^2=\frac{1}{2}\sum_{i=1}^{Nsp}\sum_{j=1}^{Nsp}p_ip_jd_{ij}^2 \qquad \text{eqn 2}$$

where $d_{ij} = ||x_i\text{-}x_j||$ expresses the trait dissimilarity between each pair of species *i* and *j*, i.e. the Euclidian trait value distance between pairs of species, and p_i expresses the relative abundance of species in a community (with $\sum_{i=1}^{Nsp}p_i=1$). Generally, if all observations have the same abundance, then $p_i = 1/Nsp$, otherwise p_i, can express different measures of species relative abundance (based on species cover, biomass, number of individuals etc.; see Lavorel *et al.* 2008). As a matter of fact, the right term of eqn 2 corresponds to the Rao quadratic entropy index of diversity, for the case that the Euclidian distance between individuals is squared and divided by two (Rao 1982, 2010; Pavoine & Dolédec 2005). This important equivalence between variance and the diversity in terms of quadratic entropy has several interesting implications.

First, the partitioning of variance expressed earlier (eqn 1) can be articulated as the partitioning of quadratic diversity with the Rao index (eqn 3 below). The partitioning of diversity with the Rao index is often calculated to decompose total regional diversity into between communities and within communities (Champely & Chessel 2002;

de Bello *et al.* 2010). Here, building in Pavoine & Dolédec (2005), we propose to use the partitioning of the quadratic entropy to decompose total community diversity into between-species and within species:

$$\sum_{a=1}^{Nind}\sum_{b=1}^{Nind} P_a P_b \frac{d_{ab}^2}{2} = \sum_{i=1}^{Nsp}\sum_{j=1}^{Nsp} p_i p_j \frac{d_{ij}^2}{2} + \sum_{i=1}^{Nsp} p_i \sum_{a_i=1}^{Nind_i}\sum_{b_i=1}^{Nind_i} \frac{1}{Nind_i}\frac{1}{Nind_i}\frac{d_{ab\,i}^2}{2} \qquad \text{eqn 3}$$

Where

$$P_a = p_i \frac{1}{Nind_i}$$

As in eqn 1, the total diversity is represented by the left hand of eqn 3, while the right hand of the equation corresponds to between-species diversity (defined in eqn 2) and within-species diversity. The total diversity is represented by the average dissimilarity between each pair of individuals (*a* and *b*) weighted by the relative abundance of the species they belong to. The within-species diversity, for each species, is represented by the average dissimilarity between each pair of individuals (*a* and *b*) within that species. The contribution of the within-species diversities to the total diversity is also weighted by the relative abundance of the species they belong to. This partitioning of diversity corresponds exactly to the decomposition of the Rao index into within- and among-samples diversity (see details in Pavoine & Dolédec 2005; Ricotta 2005; de Bello *et al.* 2010). The only difference is that the 'samples' in analyses are not represented by a species x communities matrix but by an individuals x species matrix (so that species are formally considered as 'samples' in the analyses).

Although this formulation might be less intuitive than variance, it has several advantages. For example, in the case that $p_i = 1/Nsp$, the eqn 3 corresponds to the 'unweighted' decomposition of diversity (which equates eqn 1), the within-sample diversity averaged over the number of samples (Ricotta 2005; note again that 'samples' are represented by species in eqn 1). However, this established formulation also allows for $p_i \neq 1/Nsp$, which corresponds to the 'weighted' decomposition of diversity (Rao 1982; Ricotta 2005; de Bello *et al.* 2010), which in our case implies that the diversity within each species is weighted by a factor indicating the contribution of species to the overall diversity. Therefore, this allows to include a parameter which takes into account that species have different abundances in the field and not only how much intensively each species has been sampled for trait measurements. Also, as briefly above-mentioned, when $p_i = Nind_i/Nind$, then the approach corresponds to the decomposition of mean sum of squares as in AMOVA and PERMANOVA (see worked example in the Appendix S1), which is also equivalent to the weighted diversity decomposition approach proposed by Villeger & Mouillot (2008). It should be reminded that the total diversity also includes the parameter p_i in its calculation. Overlooking this parameter in the calculation of total diversity, or in the contribution of within-species diversity, might lead to negative between-samples diversity (see de Bello *et al.* 2010). For the correspondence between the variance vs. the Rao partitioning, and the application with different p_i values, see a worked example in the Appendix S1. Different R functions decomposing total community diversity into within- and between-species diversity are also available in the Appendix S1 (based on the functions in de Bello *et al.* 2010; The function 'RaoRel.r' can be used for the equivalence between variance and Rao, both when considering or not species abundances in the field, i.e. using, respectively, parameter 'weight = T' or 'weight = F'; the function 'RaoAdo.r' can be used for the equivalence with PERMANOVA, where the a proper weight, i.e. $p_i = Nind_i/Nind$, is applied by the option 'weight = T'; see more details in the example).

An interesting consequence of the analogy between variance and the Rao index is that this approach, implicitly or explicitly, is already applied in different existing algorithms. Therefore, the method can integrate already existing approaches. In particular with our approach, similarly to PERMANOVA (Anderson 2005; which algorithm, as mentioned, can lead to eqn 3), it is possible to test the effect of within- and among-group diversity against null expectations (because of permutations of the dissimilarity between observations). Note also that PERMANOVA analysis of variance based on a distance matrix, with the corresponding randomization procedures, is actually equivalent to the one described by Pillar & Orloci (1996). Our approach allows, nevertheless, a more flexible use of the relative abundance of species than PERMANOVA, which is fundamental for the question being asked. A similar principle is also used into the test of homogeneity of dispersion from the group centroids into PERMDISP (Permutational analysis of multivariate dispersion; Anderson 2006; available at http://www.stat.auckland.ac.nz/~mja/Programs.htm or in *R*, as the function 'betadisper' in the package 'vegan'). In our case, the species mean trait values (x_i) represent the centroids for each species, and the dispersion of traits around this centroid (as calculated with PERMDISP) represents within-species diversity. Hence, when applying the PERMDISP algorithms to our case, the test indicates whether the extent of the within-species diversity changes across species (in essence, one calculates an *F*-statistic to compare the average distance of observation units to their group centroid). It should be noted that, to the best of our knowledge, the existing PERMDISP algorithms do not allow users, at present, to consider different species relative abundances (p_i) in the calculation (see help of the fdisp function, package 'FD', in R).

Another interesting result from the equivalence between variance and the diversity in terms of quadratic entropy is that different measures of trait dissimilarity could be used in this equation, not only for quantitative traits. This implies that the approach can be applied for categorical, fuzzy, circular traits etc. Also, the trait dissimilarity can be computed based on several traits together, even different types of traits. For example, Botta-Dukat (2005) and Pavoine *et al.* (2009) proposed a standardized approach to compute trait dissimilarity based on multiple traits as appropriate for the computation of the Rao index. In these established approaches, each quantitative trait is standardized by dividing the trait value by the range of possible values for this trait (which corresponds to the Gower distance based on one trait; see Botta-Dukat 2005 and Pavoine *et al.* 2009 also for standardizations based on other type of traits). After this standardization, the Euclidian distance between pairs of individuals is calculated, for each single trait, ranging between 0 and 1. While using more traits, the Euclidian distance calculated based on several traits will not be bounded to the upper limit of one. We applied this approach in the first case study.

Several alternatives exist to calculate dissimilarity between observations based on multiple traits, whose description is outside the scope of the present work. Dividing by the range of trait values could be unsafe in the case of having several outliers, which should be removed manually or by standardizing by quantiles instead of trait range (with these two solutions driving to roughly the same results with the second involving some arbitrary decision of the quantiles applied). Ideally the range of traits considered for this standardization should reflect the whole range existing in nature, but this information is often not available for most of the existing traits (see Botta-Dukat 2005 for further discussion). Other approaches based on multivariate analyses, for example based on principal coordinate analysis on trait matrices (Villeger, Mason, & Mouillot 2008; Laliberté & Legendre 2010), could be applied. Although a consensus on calculating trait dissimilarity based on multiple traits is far from being achieved in the literature, we believe that the decomposition of community diversity into within- and between-species components should be first analysed based on single traits and, only very cautiously, then compared across traits (see case study below).

CASE STUDY 1

Field site and measurements

This first case study involves FD calculations for two meadows in the Czech Republic (see Lepš 2004; Klimeš *et al.* 2001 for details) – one 'dry' characterized by lower soil moisture (Čertoryje, south Moravia) and one 'wet' characterized by higher soil moisture (Ohrazení, South Bohemia). In both meadows, individuals were selected randomly for trait measurement. As discussed in the following paragraphs (see 'The selection of individuals'), random selection of individuals guarantees that the variability of our sample correctly reflects the variability of the sampled population, and thus also the potential effect of intraspecific trait variability on community structure.

A total of 22 species were sampled in the dry meadow and 18 species in the wet meadow by randomly selecting 15–25 individuals per species at the beginning of the growing season (for a total of 863 individuals). These species represent on average > 80% of the total biomass in both meadows (see Garnier *et al.* 2007). Species relative abundance used to compute the indices was based on average species frequency in 50 × 50 cm quadrats divided into 25 10 × 10 subquadrats, randomly placed in these mown meadows. We measured vegetative height, as the distance between the top of the photosynthetic tissues of each individual and the soil surface, at the end of the growing season (in total, we measured 785 individuals). We expected variance in plant height to be linearly dependent on the mean, i.e. with higher variance for larger species not being because of greater intraspecific trait variability, but rather to scaling in measurement units. We therefore applied a log-transformation to vegetative height measurements, as it results in independence of mean and variance (not shown) while keeping the additivity of within- and between-species FD. We also measured leaf dry matter content (LDMC), the dry mass of a leaf divided by its fresh mass (Cornelissen *et al.* 2003) expressed in mg g^{-1}, for 658 individuals at the end of the growing season (avoiding individuals with damaged leaves). Log-transformation for LDMC in this case was required to improve normality (even if trait mean and variance per species were independent, not shown; the results for LDMC did not change considerably without using log-transformations).

Results and implications

In this section, following Table S1, we first highlight the results regarding total community diversity partitioning for height, LDMC alone and the potential of linking both traits together. Then, we comment on the importance of species relative abundance in the partitioning of diversity and conclude discussing the results observed against null expectations.

For both traits, the extent of within-species FD tended to be lower than between-species FD. As expected (Cornelissen *et al.* 2003), within-species FD was generally greater for height (reaching up to 52% of total FD in the dry sites when not considering species abundance), reflecting size differences between individuals under varying growth microsite conditions. For *height*, the total and between-species FD were slightly greater in the wet site (compare the absolute observed diversity values across sites), where vegetation was taller (the community mean for height was around 37 cm vs. 27 cm when using species abundance and 26 cm vs. 19 cm when not). These patterns of height highlight different community vertical structures in the two different meadows. At the dry site (lower and less dense vegetation), smaller plants such as rosette and prostrate species dominate. At the wet site (with a taller and denser vegetation, suggesting higher competition for light), smaller and taller species tend to coexist more frequently indicating differences in light acquisition strategies.

For *LDMC*, the extent of within-, between-species and total FD was higher in the dry site. This suggests higher differentiation in resource acquisition patterns generally linked to LDMC (Cornelissen *et al.* 2003), which could be explained by a more heterogeneous, patchy, resource availability in the dry site (Klimeš *et al.* 2001). Considering *both traits* together, one should note the additivity of the diversity of single traits on the combined FD. For example, without considering species relative abundance, the between-species diversity in the dry site was 0·0453, which is the sum of the between-species diversities based on height and LDMC alone, i.e. 0·0113 and 0·034, respectively (Table S1).

This additivity of FD based on single traits has some key implications. First, in our case, one would be inclined to consider the differentiation in terms of LDMC, and its contribution to the total diversity with both traits, to be higher than in terms of height. Such comparisons should be however carefully avoided as, in fact, the absolute value of diversity of single traits depends strongly on the distribution of traits values across individuals (although both traits were standardized as above-mentioned, a more even distribution leads to a higher dissimilarity, as in the case of LDMC here). As a consequence, the decomposition of absolute diversity based on multiple traits has a lower interest when compared to the decomposition based on single traits. On the contrary, the comparison of the percentage variance explained by within- and between-species effects can be more safely compared across traits.

Another key observation highlighting the risks of combining multiple traits is given by the results including species relative abundances in FD estimation. As a matter of fact, the effect was opposite for height vs. LDMC (Fig. 1). For height, the observed total diversity values increased, in both sites, using relative abundances when compared to using only species presence/absence. For LDMC, the total diversity decreased. This implies, first, that at both sites dominant species are more dissimilar among themselves than are all species taken together in the case of height and *vice versa* in the case of LDMC. Second, this incongruence across traits suggests, again, that combining different traits together should be performed very carefully as these opposite effects on single traits will be overlooked.

It is also interesting to note that the use of abundance-weighted diversity modified the extent of within- and between-species trait variability (especially concerning the absolute values of variance; Table S1 and Fig. 1). This suggests different patterns of differentiation among dominants and subdominant species. A higher between-species FD when considering species relative abundance (as for height in the dry site) suggests, in principle, that different strategies between dominants can be important for coexistence and resource acquisition (Stubbs & Wilson 2004). A lower between-species variability when considering species relative abundance (as for LDMC) can suggest convergence in traits between dominants. Comparing variance partitioning with and without considering species relative abundance could, therefore, be applied to test the relevance of different sources of trait variability on community assembly (Mason *et al.* 2007; Swenson & Enquist 2009). Also, these results highlight the importance of methods that allow the inclusion, or not, of species relative abundance in calculations of FD.

Finally, as in PERMANOVA (Anderson 2001, 2005), we randomized dissimilarity across observations (individual trait values in our case) and assessed how many times the observed values were lower, or higher, than expected by chance. In all cases, the observed diversity between-species was higher than expected by chance (with 99 randomizations), while the within-species diversity was lower than expected by chance (Table S1). More heterogeneous patterns were found for the total diversity. Most importantly, these results advocate for the importance of between-species differentiation in community assembly. Second, we used PERMDIST tests (Anderson 2006), indicating here a significant difference in within-species diversity across different species (i.e. some species have higher dissimilarity within them than others). This, as discussed later, has clear important implications on the way individuals are selected for trait measurements.

To what extent is the response of FD to environment because of intraspecific trait variability vs. species turnover?

To answer this question, we developed the second of the two methods proposed here (Table 1). As for the first method discussed earlier, this method is also based on a variance partitioning approach. We illustrate here the application of the method using an example from a set of meadows under different land use in the French Alps (Table 2 & Fig. 2).

THE METHOD

This method requires, first, that individuals of each species are sampled across the different habitats within a study (e.g. treatments, environmental gradients etc. – see Albert *et al.* 2010). Having collected

Table 2. Results from case study 2. (a) Results of the repeated measures ANOVA. The within factors indicate the relevance of intraspecific trait variability and its effect on the response of functional diversity (FD) to the environment (a significant 'Habitats * Intraspecific' interaction indicates that the response of FD to treatments in not only because of species replacements but also intraspecific trait adjustments). Significant ($P < 0{\cdot}05$) results are in bold. (b) ANOVA results for FDfixed, FDhabitat values and their differences. Using covariation in the sum of squares of these three models, it is possible to assess the relative effect of species turnover and intraspecific trait variability

	Height				LDMC		
	df	SS	*F* value	*P*-value	SS	*F* value	*P*-value
(a)							
Between factors							
Habitat (5 land uses)	4	120·73	3·75	**0·052**	3710·70	1·12	0·410
Residuals	8	64·38			6581·30		
Within factors							
Intraspecific	1	0·93	3·89	0·084	17·83	7·63	**0·024**
Habitat* Intraspecific	4	4·01	4·17	**0·040**	388·09	41·53	**< 0·001**
Residuals	8	1·92			18·69		

	df	Turnover (FDfixed)	Intrasp. variability (FDhabitat minus FDfixed)	Covariation	Total (FDhabitat)	Turnover (FDfixed)	Intrasp. variability (FDhabitat minus FDfixed)	Covariation	Total (FDhabitat)
(b)									
Decomposition SS									
Habitat	4	48·00	8·02	20·72	76·75	2835	776·174	−2347·28	1263·9
Residuals	8	31·77	3·84	−1·08	34·53	3209·6	37·371	143·39	3390·4
Total		79·77	11·87	19·65	111·28	6044·60	813·55	−2203·89	4654·30
P-value		0·084	**0·041**	-	**0·034**	0·229	**< 0·001**	-	0·587
Relative contribution (in %):									
Habitat		43·14	7·21	18·62	68·97	60·91	16·68	−50·43	27·16
Residuals		28·55	3·45	−0·97	31·03	68·96	0·80	3·08	72·84
Total		71·68	10·66	17·65	100·00	129·87	17·48	−47·35	100·00

LDMC, leaf dry matter content.

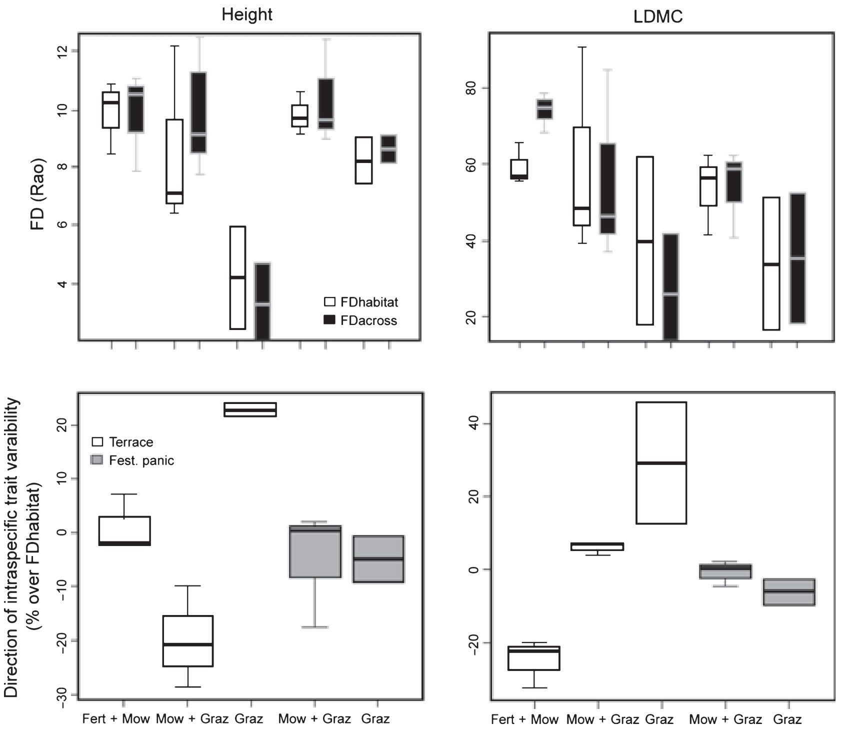

Fig. 2. Results from the case study 2 comparing *FDfixed* against *FDhabitat*. For each trait, the impact of intraspecific trait variability is understandable as the differences between *FDfixed* against *FDhabitat* (with *FDfixed* computed using the same trait value per species in all treatments and *FDhabitat* computed using the trait values per species measured in different habitats). The direction of intraspecific functional diversity (FD) variation is shown here as a percentage of FDhabitat, i.e. (FDhabitat-FDfixed)*100/FDhabitat. Positive values indicate that intraspecific variability increase the dissimilarity between species compared to the FD because of species turnover alone, negative values indicate the opposite pattern. This index indicates, also, the error estimates performed by neglecting intraspecific trait variability in FD calculation.

such data, FD can be computed using two approaches. First, FD can be computed using only the individuals measured in each habitat (see below for formulas). We can call this sort of measure a '*FDhabitat*'-based index. Alternatively, FD can be calculated using the same trait values for all plots (independent of the habitat conditions, the species experiences in different plots). We can call these measures '*FDfixed*' indices, as all individuals measured across habitats are used together.

The variation in *FDhabitat* indices can result from both differences in species composition and from within-species trait variation expressed under different habitats. On the contrary, the difference in *FDfixed* values among sampling units can only be the result of differences in species composition (both in terms of species occurrence and relative proportions), because in *FDfixed*, species are considered as having the same trait values in all samples. Then, the differences between *FDfixed* and *FDhabitat* values for a given sample are logically only because of intraspecific trait variability across habitats.

Intraspecific trait variability = *FDhabitat* − *FDfixed*

In particular, the differences between *FDfixed* and *FDhabitat* are caused by the fact that, for given species, the trait values in a given habitat can differ from the mean of trait values across all habitats (e.g. taller plant individuals are found in fertilized conditions and shorter ones in unfertilized ones). This implies that if *FDhabitat* is systematically higher than *FDfixed* in some habitat types, it must be systematically lower in other habitats reflecting systematic variations in trait values within species in different habitats. Overall, *FDhabitat* > *FDfixed* indicates that FD is increased by intraspecific trait differentiation (with respect to the effect because of species turnover), while *FDhabitat* < *FDfixed* indicates that FD is decreased by intraspecific trait differentiation.

Here, we first propose a way to test for the significance of intraspecific trait variability effects on FD, i.e. performed by assessing how the differences between *FDfixed* and *FDhabitat* change under different environmental conditions. This can be measured by estimating the interaction between environment and the type of FD index used (*FDfixed* vs. *FDhabitat*) with univariate repeated measures model of ANOVA (which is identical to the split plot ANOVA; Table 2). Different linear mixed effect models could also be applied. In this approach, the habitat characteristics represent the 'between' factors in the repeated measures model of ANOVA, and the two FD values (i.e. those based on *FDfixed* values vs. those based on *FDhabitat* values) represent the repeated measures (or 'within' factors). It should be noted that the results of the interaction between habitat and intraspecific logically corresponds to those obtained, e.g. with univariate ANOVA, for the effect of habitat on the intraspecific trait variability (i.e. the difference between *FDfixed* and *FDhabitat*).

Second, whereas the rejection of null hypotheses is often an expected outcome of the analysis, researchers are often interested in the quantification of variability explained by individual factors. To quantify the effect of turnover against that of intraspecific response to environment, the responses of three separate univariate analyses can be analysed and compared, as proposed for community trait means by Lepš *et al.* (in revision). Parallel ANOVAS should be run (a) one on *FDfixed* (characterizing the effect of species composition), (b) one on intraspecific trait variability (the differences between *FDfixed* and *FDhabitat*) and (c) one on *FDhabitat* (characterizing both turnover and intraspecific trait variability). The total sum of squares (SS) within each ANOVA corresponds to the total variability explained by each of these three components. As the SS are additive, the SS of individual effects within each ANOVA can be decomposed into the amount of variability explained by individual terms of the model (treatments and their interactions) and the unexplained variability (error) in the analysis. For instance, for an experimental design with two factorial treatments each individual analysis: SStotal = SSfactor1 + SSfactor2 + SSfactor1*factor2 + SSerror. It should be noted that quantitative explanatory variables could be also included in the method, as with the decomposition of SS in General Linear Models.

As above-mentioned, the variation in *FDhabitat* results from the addition of *FDfixed* and intraspecific variability effect. Consequently, we take the total variation in *FDhabitat* as 100%. When the turnover and intraspecific effects vary independently, then (df are the same for all the three quantities):

$$SS_{FDhabitat} = SS_{\mathrm{FDfixed}} + SS_{\mathrm{intraspec.variability}}$$

If the two effects are positively correlated (i.e. when high *FDfixed* values are accompanied by increased intraspecific variability, and *vice versa*), $SS_{FDhabitat}$ will be higher than expected under the independence of the two effects. When the two effects are negatively correlated (i.e. when high *FDfixed* values are accompanied by negative intraspecific variability, and *vice versa*) $SS_{FDfixed}$ will be lower than expected. In analogy with covariance (varA + B = varA + varB + covarA,B, i.e. covarA,B = varA + B − varA − varB) therefore, we can define the effect of 'covariation' on the total variability ($SS_{\mathrm{FDhabitat}}$) as:

$$\mathrm{covSS} = SS_{\mathrm{FDhabitat}} - SS_{\mathrm{FDfixed}} - SS_{\mathrm{intraspec.variability}}$$

As mentioned, this term is negative when the two effects are negatively correlated and *vice versa* is they are positively correlated. Then, the total variation ($SS_{\mathrm{FDhabitat}}$) can be decomposed into parts explained by the effects of *FDfixed*, of intraspecific variability and of their covariation. This reasoning can be applied for each individual term of the model and for the total variation (SStotal) of, in this case, ANOVA models for *FDfixed*, intraspecific effect and *FDhabitat* (Table 2).

This approach could be applied with different mathematical formulations of FD. To remain consistent with the first method proposed earlier, the following case study uses the same mathematical framework as for the first case study, i.e. the Rao index. Contrary to the first method above-mentioned, however, the squaring of distances (and therefore of the FDindex) is performed by definition during the calculation of sum of squares in ANOVA. Therefore, to avoid a double squaring, there is no need to square the distances before the calculation. Using squaring distances to centroids would result in overemphasizing the difference between *FDhabitat* and *FDfixed* (e.g. if the difference between-species trait values and community centroid is increased twice by using the habitat-specific traits values, then the diversity is increased four times). With the Rao index, the *FDhabitat* and *FDfixed* could be expressed as:

$$FDfixed = \sum_{i=1}^{Nsp}\sum_{j=1}^{Nsp} p_i p_j dfixed_{ij} \text{ and}$$

$$FDhabitat = \sum_{i=1}^{Nsp}\sum_{j=1}^{Nsp} p_i p_j dhabitat_{ij}$$

where $dfixed_{ij}$ is the dissimilarity between each pair of species *i* and *j*, with the same (fixed) trait value used in all sampled communities (here based on the mean trait of all individuals measured per species). On the contrary, for $dhabitat_{ij}$, the dissimilarity used is different in different communities (here based on the mean trait of all individuals measured, for each species, in the habitat corresponding to each community). Finally, this approach can be used for single and multiple traits; however, we again suggest first assessing results on single traits, as the relative effect of intraspecific trait variability can be considerable different across traits.

CASE STUDY 2

Field site and measurements

We illustrate this method by reanalysing data from Lavorel *et al.* (2008) where various grassland types in the French Alps were sampled (Villar d'Arène, 45·04°N, 6·34°E; see Quétier, Thebault, & Lavorel 2007 and Lavorel *et al.* 2008 for site details). Species traits were measured, for the same species, under different environmental conditions. Compared to the previous example (case study 1), many same species were present in different habitats, and therefore intraspecific trait variability for any given species could be assessed across several habitats (contrary to case study 1, where the two sites have almost no overlap in the pool of species and intraspecific trait variability was only expressed within a given habitat).

In this data set, the various sampled grasslands are differentiated by current land use and their location on either former cultivated terraces or uncultivated fields. There are five different land use types represented by 13 plots: (i) three fertilized hay meadows on previously cultivated terraces; (ii) three unfertilized hay meadows on previously cultivated terraces; (iii) two unfertilized and unmown terraces lightly grazed by sheep; (iv) three never ploughed hay meadows; and (v) two former hay meadows that are no longer mown but lightly grazed in summer. These communities are perennial, resprout every year after snowmelt mostly from buried buds and are dominated by graminoids (45–80% total cover). Un-terraced pastures are dominated by *Festuca paniculata*, a late succession perennial grass tussock. Replicate plots per each habitat were sampled by choosing a 15 × 15 m visually homogenous area. Species relative abundances were estimated using BOTANAL, a calibrated method for visual estimates of species biomass (Tothill *et al.* 1992; see Lavorel *et al.* 2008 for more details). The traits considered for this example are (as for case study 1) plant height and LDMC (a minimum of 10 individuals, well-developed mature plants, of each species per treatment were sampled).

Results and implications

FDfixed and *FDhabitat* were strongly correlated (for both traits $R > 0{\cdot}90$). Despite this strong correlation, the repeated measures ANOVA shows that intraspecific trait variability had an important effect on the response of these communities to environment (Table 2a, e.g. significant interaction of habitat type × intraspecific trait variability; the interaction was significant both when considering five different habitats or when considering land use intensity and terrace vs. nonterraced meadows; not shown).

Overall, these results show that the response of FD to land use is not only because of changes in species composition but also, to a non-negligible extent, to intraspecific trait variability. For height, the effect of intraspecific trait variability in FD response to environment accounted for around 11%, while species turnover accounted for 72% of total variability. With an overall correlation between *FDfixed* and intraspecific variability of $R = 0{\cdot}31$, both effects covaried accounting together for 18% of FD response. Both *FDfixed* and *FDhabitat* responded to land use, with higher responsiveness of *FDhabitat* (in univariate ANOVA, $P = 0{\cdot}084$ and $P = 0{\cdot}034$, respectively for *FDfixed* and *FDhabitat;* Table 2b). The higher responsiveness of *FDhabitat* compared to *FDfixed,* together with the positive covariation of species turnover and intraspecific trait variability, indicates a similar effect of turnover and intraspecific variability on the response of FD (i.e. the increase in *FDfixed* is accompanied by an increase in intraspecific trait dissimilarity). Under the effect of land use, however, in some conditions, the intraspecific trait variability compensated the effect of species turnover. For example, in only grazed terraces, the FD values were among the lowest but *FDfixed* < *FDhabitat*. This suggests that grazing alone increased height variability between species, as because of intraspecific trait adjustments.

For LDMC, neither *FDfixed* and *FDhabitat* responded to land use (in univariate ANOVA, $P = 0{\cdot}587$ and $P = 0{\cdot}229$, respectively; Table 2b) while intraspecific trait variability did ($P < 0{\cdot}001$). This indicates that considering only results on *FDfixed* or *FDhabitat* would lead to conclude that there is no response of FD to environment. Overall, the effect of intraspecific trait variability on FD response to environment accounted for around 17% of total variability alone. The negative correlation between *FDfixed* and intraspecific variability ($R = -0{\cdot}49$), with the corresponding negative covariation of their effects, suggests that the effects on FD of intraspecific trait variability strongly compensate the effects of species turnover. For example, in the fertilized and mown terraces, *FDfixed* > *FDhabitat* which suggests that fertilization decreases dissimilarity between species, probably by inducing most species to have more fleshy leaves with lower dry matter content as a consequence of nutrient addition. However, because of species turnover only, fertilized and mown terraces tended to show the highest FD values. Taken together, these patterns advocates for compensatory effects by species turnover and intraspecific trait variability. Similarly, in grazed terraces, *FDfixed* showed among the lowest FD values, indicating a sorting effect of grazing on species with highest LDMC (Quétier, Thebault, & Lavorel 2007). However, *FDfixed* < *FDhabitat* which reflects, as for height, the fact that herbivores tend to prefer some plants to others, thereby increasing the dissimilarity between individuals in a community (therefore compensating again the effect of species turnover).

Finally, the results interestingly show that only assessing the extent to which *FDfixed* and *FDhabitat* are correlated (i.e. *sensu* Cianciaruso *et al.* 2009) is not sufficient to give insight into the relevance of intraspecific trait variability to FD (Table 2). To better understand in which treatments and in which direction intraspecific trait variability plays a stronger role in shaping FD response to environment, the results of *FDfixed* vs. *FDhabitat* need to be compared for the different habitat types (Table 2 and Fig. 3, as an illustration).

The selection of individuals

Although the two questions discussed here can be potentially combined, each method requires dedicated sampling and imposes different constrains on the selection of individuals for trait measurements. The approach chosen has important consequences on the nature of the information to be collected and consequent patterns in FD variation. First of all, FD calculations should be based on realistic field measurements, i.e. we should expect that all the individuals sampled are selected from an assumed trait value distribution within species. Ideally, all individuals in a community should be measured (Cianciaruso *et al.* 2009). However, measuring multiple traits for all of the species in each plot could prove to be prohibitively laborious (Cornelissen *et al.* 2003; Baraloto *et al.* 2009). For a moderately sized study, with 25 plots, and an average of 30 species per plot, each characterized by 10 measured individuals, we would need 7500 measurements for a single trait. A less intensive solution (as used here) is to consider the traits to be fixed (or less variable) under a set of environmental conditions (for example the treatments in an experimental approach) and sample only a part of the existing population of each species. It has also been suggested that a similar sampling intensity across species is necessary for reliable population trait values (Hulshof & Swenson 2010).

Overall, we can distinguish two main strategies for selecting which individuals to include for measuring intraspecific trait variability (Table 1). The first approach (here applied in case study 1) is based on a *quasi-random selection* of individuals (see Albert *et al.* 2010). The second approach (here applied in case study 2) relies on a *nonrandom selection* of individuals, e.g. selecting well-developed individuals within a population (Cornelissen *et al.* 2003). Underlying the second approach is the premise that trait variation between well-grown individuals of various species is generally more important than within species (Fig. 1 and Garnier *et al.* 2001; Westoby *et al.* 2002). Generally, nonrandom selection of individuals often leads to the (conscious or unconscious) selection of larger and more developed individuals (Gaucherand & Lavorel 2007) because they are more easily detected.

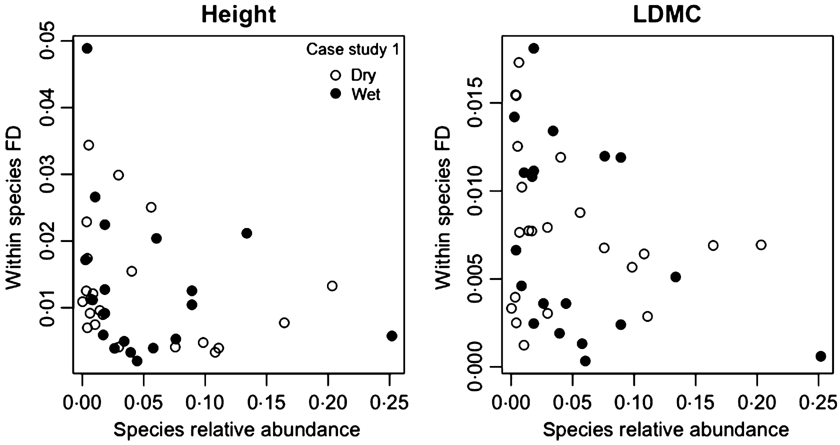

Fig. 3. Results (from case study 1) showing the most dominant species tend to have lower within-species functional diversity.

The random selection of individuals (first approach) is not, however, an easy task to achieve in the field (both for plants as for other organisms). For example, true random sampling insures that each individual has an equal chance of being selected, independent of the selection of other individuals. Selecting an individual closest to a random point is not a true random sample, as individuals in clumps have lower probability of being selected than solitary individuals. Similarly, selecting all individuals in randomly located quadrats violates the independence of individuals, as the probability of selecting one individual is dependent on the selection of its close neighbours (which can be particularly important in the case of clonal plants). Probably, the closest approach to random sampling is random location of sampling plots so small that they are unlikely to contain more than one individual. This would, however, result in very high number of plots, most of which could be empty, and so the procedure would be extremely time-consuming. To achieve a close to random selection, it could be sufficient to have a selection approach that is independent of the measured trait (i.e. not only large individuals are selected because they are easier to detect). For herbaceous species, selecting individuals at the beginning of vegetation season (at a time when all the individuals are small as for case study 1) according to the rule 'what we are able to find' is likely to approach a true random selection of individuals (even though, in early spring, larger individuals are probably more apparent that the small ones and dominant species more frequent that rare species). At the same time, this approach is also not always feasible. For animal studies, measurements are usually carried out on those individuals that can be trapped, which is not a random sample as it was noted many years ago in the classical textbook of Southwood (1978). This textbook's caveats should also be seriously considered in context of sampling for FD.

For both approaches, also, intraspecific trait variability will inevitably be significantly different between dominant and rare species. For abundant species at a site, it may be easy to find several individuals to be measured. In contrast, for rare species, the choice will likely be more limited (as they may be represented by few individuals in a community). In particular, individuals growing in optimal conditions could be more easily found for abundant species than for rare species, simply because there is a larger selection pool. Therefore, within-species FD may be found to be greater in rare species because a mixture of well-developed and less well-developed individuals are likely to be sampled (increasing the extent of intraspecific trait variability; Cornelissen *et al.* 2003). These patterns are clearly illustrated by an example from the first case study (Fig. 3) where, despite a quasi-random selection of individuals, most abundant species (i.e. having higher relative abundance) tended to show lower intraspecific trait variability (note that PERMDIST tests indicated differences in within-species diversity across different species; Table S1).

Also, we believe that the question of which individuals to measure will also largely depend on the available measures of species proportions in a community, i.e. p_i. If p_i is measured as a proportion of the number of individuals, then the decision is rather straightforward and random-like approaches should be preferred. In many cases, however, the role of a species in a community is related to its biomass (Grime 1998), so that p_i is estimated as a biomass proportion (Lavorel *et al.* 2008). In this case, it should be noted that total population biomass can be dominated by large individuals, even though their number is usually low when compared to small individuals. For example, if randomly selecting oak individuals in a forest, oaks may represent 90% of the forest biomass, largely because of those individuals that are large trees, and despite the fact the majority of oak individuals may be seedlings or saplings. In the case of biomass-based proportions, the nonrandom selection of individuals should thus be preferred.

Finally, given the aforementioned considerations, we suggest that 'random-like' sampling of individuals should be generally preferred whenever dealing with the question of the extent of within- vs. between-species FD (question 1, Table 1). Such an approach, given sufficient sampling intensity, should better reflect the variability of the sampled population for both dominant and rare species. To answer the second question, a nonrandom sampling of individuals might be preferable. In fact, if the aim of the sampling is to measure a given species in different environmental conditions, the sampling strategy will need to be (to a greater or lesser extent) always based on the nonrandom selection of individuals. Answering both questions with a single sampling approach could be possible, but results should be interpreted knowing the potential effects of each sampling approach. Possibly, the random-like approach could present more advantages for answering both questions with a single sampling although, as discussed earlier, multiple factors needs to be considered when designing a specific sampling design aiming to estimate intraspecific trait variability effects within and across communities.

Conclusions

The two complementary questions addressed here quantify the effects of intraspecific trait variability for FD, both within communities and in the response of communities to environmental changes. For both questions, a variance partitioning approach can be applied based on the decomposition of FD into the different sources of variation. The analyses can be based on single and multiple traits; however, we strongly suggest to calculate first FD values based on single traits alone. In the context of intraspecific trait variability, in fact, combining multiple traits together could lead to overlooking and mask potential markedly different patterns across traits. The two different ecological questions lead also to different types of variance partitioning, i.e. the two methods, and as a result, each of them imposes their own constraints on sampling individuals for trait measurements. Ultimately, it is possible to use a single sampling method to answer both questions. However, this solution should be applied carefully, assuming that each sampling approach can enhance or decrease the effect of intraspecific trait variability effects across rare and dominant species. Our results finally stress the important role of intraspecific trait variability in community dynamics within and across habitats.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Results of the partitioning of total community diversity into within- and between-species diversity in two sites (dry vs. humid).

Appendix S1. R functions for the first method proposed together with a corresponding example (also showing the equivalence with the variance partitioning).