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More for less: sampling strategies of plant functional traits across local environmental gradients

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Running headline: Strategies for functional traits sampling

SUMMARY

1. Ecologists use approaches based on plant functional traits to tackle several fundamental and applied questions. Although a perfect characterization of functional trait structure requires the measurement of all the individuals in communities, this is prohibitively resource-consuming. Consequently, the general practice is to average the trait values of a reduced number of individuals per species. However, there are different alternatives regarding the number, identity and spatial location of the individuals chosen to calculate species-averaged trait values.
2. In this study, we compared different strategies for sampling functional traits, using community-weighted mean trait values (CWM) and the Rao index of functional diversity (FD). We intensively sampled the functional trait structure along a topographical gradient in a Mediterranean grassland, obtaining accurate estimations of the 'real' values of these indices (CWM_I and FD_I) for three traits (height, leaf area and specific leaf area).
3. We simulated three different sampling strategies differing in the spatial location of the individuals used to estimate species-mean trait: i) average of the whole gradient (GLO), ii) average of the sampling unit in which the abundances of species maximize (MAX) and iii) average of a reduced number of individuals per species and sampling unit (LOC). For each strategy, we simulated different sampling intensities (number of individuals sampled).
4. For each trait, we examined the ability of each strategy and sampling intensity to accurately estimate CWM_I and FD_I, as well as their ability to detect changes in functional trait structure along the topographical gradient.
5. LOC outperformed the other strategies in terms of accuracy and bias, and was much more efficient to describe changes along the gradient, regardless of the traits and indicators considered. Furthermore, LOC was the only strategy that improved consistently as sampling intensity increased, especially at low levels of intensity.
6. Our results indicate that the impact of considering intraspecific variability in trait values can be greater than commonly assumed. Strategies that neglect this source of variability can result in inaccurate or biased estimations of the

44 functional trait structure of plant communities. Most importantly, we show
45 that intraspecific variability can be taken into consideration without any
46 increases in the total number of individuals measured.

47 **Keywords:** Community weighted mean; functional diversity; intraspecific variability;
48 Mediterranean grasslands; plant height; specific leaf area.

INTRODUCTION

Ecologists are increasingly relying on plant functional traits as a means to tackle some of the most fundamental and applied questions in ecology, because trait-based approaches can help to disentangle the effect of ecological processes on communities (McGill et al. 2006; Díaz et al. 2007; Mason & de Bello 2013). By providing a more mechanistic point of view than the use of species identities alone, functional trait approaches can help to test hypotheses about the existence of assembly processes operating in plant communities (Cornwell & Ackerly 2009; Spasojevic and Suding 2012; Mason et al. 2012), to predict the abundance of species (Shipley, Vile & Garnier 2006; Laughlin et al. 2012) or to understand the influence of organisms on ecosystem functioning (Díaz & Cabido 2001; Lavorel & Garnier 2002). One of the most critical steps in these studies is to scale-up from the traits measured in individual plants to the community level.

Estimations of the functional trait structure of plant communities, which is often characterized by the simultaneous consideration of functional composition and diversity (Ricotta & Moretti 2011), rely on accurate measurements of the trait values of the individuals that compose them. This entails that a perfect characterization of the functional trait structure of a community would require the measurement of the traits of all its individuals. Such level of detail is extremely time-consuming and hardly ever executed (Baraloto et al. 2010; Messier, McGill & Lechowicz 2010). Instead, and given that conspecifics display similar trait values, the most common way to solve this problem is to sample a reduced number of individuals of each species present in the community. Subsequently, the trait values of the sample can be averaged, assigning that average to all the individuals of the corresponding species (Lavorel et al. 2008; Lepš et al. 2011). There are, however, several alternatives in the selection of the number, identity, maturity status (i.e. selecting only mature individuals or all individuals; Cornelissen et al. 2003; de Bello et al. 2011), and spatial location of the individuals selected to calculate species-averaged trait values. For instance, for a given sampling intensity (estimated here as the number of individuals in which traits are measured), one could select some individuals (among either all individuals or only mature ones) in each sampling unit in which the species is present and assign, for each sampling unit, a local-averaged trait value (e.g. Mason et al. 2012; Gross et al. 2013). Alternatively, a similar total number of individuals could be randomly selected among

all the individuals from all the sampling units, assigning a global-averaged trait value to each species (e.g. Bernard-Verdier et al. 2012). Furthermore, one could select and measure the traits of the individuals from the sampling unit(s) in which the relative abundance of the species maximizes, assigning the average of those measurements to all the individuals of the species across all the sampling units (e.g. Carmona et al. 2012).

Each of these approaches entails a series of assumptions that have to be carefully considered, because the election of the sampling strategy can have a great influence on the resulting indicators of functional trait structure (Baraloto et al. 2010). For instance, the approaches that use a single average trait value for each species in all the sampling units assume that intraspecific variability in trait values play a minor role compared to interspecific differences. Although this assumption might be appropriate in many occasions, several recent studies indicate that considering intraspecific variability can have a considerable influence in the power to detect changes in functional trait structure along environmental gradients (Hulshof & Swenson 2010; de Bello et al. 2011,2013; Violle et al. 2012). This influence can be critical at local scales, where the relative importance of intraspecific variability is expected to maximize, and be reduced as the studied scale widens (Albert et al. 2010). Therefore, local differences in ecological factors such as productivity can have a great influence on the functional trait structure of plant communities (Pakeman, Lennon & Brooker 2011; Carmona et al. 2012), not only because they foster species turnover but also because they promote differences among conspecifics. For instance, at the species level, foliar traits such as leaf area (LA) and specific leaf area (SLA) increase when water availability is higher (Cornwell & Ackerly 2009). Therefore, considering intraspecific variability in trait values should be especially important for the estimation of the shifts in functional trait structure across environmental gradients (Messier, McGill & Lechowicz 2010; Kichenin et al. 2013).

In spite of its potential relevance, very little is known about the consequences of adopting different approaches in the selection of individuals for the measurement of functional traits. The few studies that have addressed these questions have not considered the effects of environmental features on plant traits (Baraloto et al. 2010), or have focused on spatially large environmental gradients (Messier et al. 2010; Gross et al. 2013; Kichenin et al. 2013), but not on local scales, where the relative

importance of intraspecific variability should be highest. In this paper, we assess the impact that different strategies for sampling functional traits have on two of the most commonly used indicators of the functional trait structure of plant communities, community-weighted mean trait values and the Rao index of functional diversity (hereafter referred to as CWM and FD, respectively). We study functional trait structure along a topographical gradient in a Mediterranean grassland, using a very intensive sampling design that should very closely reflect the 'real' functional trait structure of the different sampling units. Afterwards, we simulate different sampling strategies, and compare the resulting CWM and FD values with the ones obtained following the most intensive approach. We finally discuss the results provided by each sampling strategy, focusing on two specific questions: 1) the accuracy and bias of each strategy, and 2) the ability of each strategy to reliably detect changes in functional trait structure across the topographic gradient.

MATERIAL AND METHODS

Study area

The study area is situated 20 km north of Madrid, in central Spain (40° 36' N 3° 45' W; elevation ca. 700 m). It is under a Mediterranean climate, with an average temperature of 13.5 °C, with cold winters and hot summers. The average annual rainfall is 560 mm, and is mainly concentrated in spring and autumn, with very dry summers. Soils are composed of Arcosic sands. The sampled grassland is mainly composed of annual species, such as *Plantago lagopus* L., *Echium plantagineum* L., *Trifolium cherleri* L., *Anthemis arvensis* L., *Bromus hordeaceus* L., *Hordeum murinum* L. or *Biserrula pelecinus* L.

Vegetation and functional traits sampling

We selected a slope (average inclination 25%) that encompassed significant differences in productivity within a reduced spatial framework. This topographic gradient went from the upper slope, characterized by shallow soils and reduced nutrient and water availability, towards the bottom of the slope, characterized by deeper and much more humid soils (Appendix S1 in Supporting Information). Starting from the highest part of the slope, and following the direction of the maximum inclination, we situated 40 sampling quadrats (20 x 20 cm) separated 2 m from each other. We surveyed these quadrats in the spring of 2013, categorizing the cover of the

present species according to eight classes: (0) absent; (1) cover <1%; (2) 1% < cover ≤ 5%; (3) 5% < cover ≤ 12%; (4) 12% < cover ≤ 25%; (5) 25% < cover ≤ 50%; (6) 50% < cover ≤ 75% and (7) cover >75%. Afterwards, we assigned to each species in each quadrat the median value of its cover class. We found a total of 89 species in the survey.

On each quadrat, we measured the traits of the most abundant species, ensuring that they represented at least 90% of the cover of the quadrat. This included 51 different species (between 3 and 9 species per quadrat, with an average of 6.4). We randomly selected 10 individuals of each of these species within each quadrat; in the cases in which they were less than 10 individuals of a given species in one quadrat, we selected the closest individuals outside the quadrat up to a distance of 0.5 m, always at the same elevation of the quadrat. Although we did not specifically selected mature individuals, the great cover of annual species in the sampled grasslands, and the time of the year in which the sampling took place (late spring), practically ensured that all the selected individuals were mature ones. In all, we measured the traits of for a total of 2540 individuals. On each of those individuals we measured data on three of the most commonly used functional traits, all of which were expected to vary across gradients of productivity: plant height, leaf area (LA) and specific leaf area (SLA). Plant height, expressed in cm, is defined as the distance between the highest photosynthetic leaf and the plant's base (Cornelissen et al. 2003). Height is an important determinant of the competitive ability for light, and is influenced by resource availability (Westoby et al. 2002). LA, expressed in mm², was measured on two randomly selected leafs of each of the selected individuals of each species and quadrat; LA influences the water and energy balance of the plants, and is related to nutrient availability (Cornelissen et al. 2003). The same leaves were used to calculate SLA, which is the ratio between the surface of the leave (including the petiole) and its oven-dry weight (Cornelissen et al. 2003), expressed in mm² mg⁻¹. SLA is an indicator of resource-use strategies, with low SLA values being associated with resource-retaining strategies, which are favored when resource availability is low (Westoby et al. 2002; Cornwell & Ackerly 2009). Prior to analyses, LA values were log-transformed to attain normality.

Functional trait structure calculations and simulations

The exhaustive nature of the strategy followed to collect functional traits allowed us to have a very good characterization of the variability in trait values of the different species across and within quadrats (Fig. 1A). Using the average trait values of each species on each quadrat, we estimated the community weighted mean for the three traits (CWM). CWM, which is frequently used as an indicator of functional composition, reflects the average trait values of the dominant species (Díaz et al. 2007). For each species and quadrat, we calculated the average trait values and used Gower distances to calculate the species dissimilarity matrix. With these values, we calculated for each trait and quadrat the Rao index of functional diversity (FD; Rao 1982):

$$Rao = \sum_{i=1}^s \sum_{j=1}^s d_{ij} p_i p_j ,$$

where d_{ij} is the dissimilarity in trait values between each pair of coexisting species i and j and p_i and p_j indicate the relative abundances of species i and j . Finally, we expressed FD in equivalent numbers, using the correction proposed by Jost (2007). The Rao index combines functional richness and functional divergence (Mouchet et al. 2010) and is frequently used to analyze changes in assembly processes across gradients of productivity (Mason et al. 2012, 2013).

The CWM and FD values calculated for each quadrat and trait should be an accurate approximation of the 'real' CWM and FD values of each quadrat (de Bello et al. 2011). Subsequently, we compared these 'intensive' values (CWM_I and FD_I onwards) with the values obtained following three alternative strategies, which were intended to encompass a range of realistic alternatives on the sampling of individuals plants for the measurement of functional traits across environmental gradients:

Global mean (GLO): With this strategy, we simulated the case in which the traits of each species are estimated using the average trait values of randomly chosen (R) individuals situated along the gradient. Therefore, the trait value T assigned to a species j in all the quadrats is:

$$T_j = \frac{\sum_{R=1}^N T_R}{N} ,$$

where the sampling intensity (N) is the number of individuals chosen at random among all the individuals of the species j sampled across the gradient (Fig. 1B). We simulated five different sampling intensities, depending on the number of individuals

used to estimate the average value of each species ($N = 3, 5, 10, 15$ and 20 individuals per species). We performed 1,000 simulations on each of these sampling intensities, which differed in the identity of the selected individuals. For each simulation we calculated the CWM and FD of each quadrat.

Maximum abundance mean (MAX): With this strategy, we simulated the case in which the traits of each species are estimated using the average trait values of the individuals (R^*) from the location (the quadrat) in which the relative abundance of the species maximizes. In this case, the trait value T assigned to a species j in all the quadrats is:

$$T_j = \frac{\sum_{R^*=1}^N T_{R^*}}{N},$$

where the sampling intensity (N) is the number of individuals chosen at random among all the individuals of the species j sampled in the quadrat in which the relative abundance of the species maximizes (Fig. 1C). We simulated eight different scenarios, which differed on sampling intensity (from 3 to 10 individuals per species). We performed 1,000 simulations for each of these sampling intensities, except for the last one (10 individuals per species), because it encompasses all the measured individuals on the corresponding quadrat. For each simulation we calculated the CWM and FD of each quadrat.

Local mean (LOC): For this strategy, we simulated the case in which the traits of each species at each quadrat are estimated using the average trait values of the individuals from each individual location (R_i). In this case, the trait value T assigned to a species j in quadrat i is:

$$T_{ij} = \frac{\sum_{R_i=1}^N T_{R_i}}{N},$$

where the sampling intensity (N) is the number of individuals chosen at random among all the individuals of the species j sampled in the quadrat i (Fig. 1D). Therefore, the only difference between this approach and the one used to estimate CWM_i and FD_i is the number of individuals used to calculate the local average of the traits, i.e. the sampling intensity. We performed 1,000 simulations for each sampling intensity (1 to 9 individuals per species and plot); in each simulation we randomly selected the corresponding number of individuals per species and quadrat and averaged their trait values. Finally, we calculated the CWM and FD of each quadrat.

Statistical analyses

Following Messier et al. (2010), we performed a variance component analysis using the functions 'varcomp' and 'lme' of R (version 2.15.3; R Core Team 2013), using bootstrapping (1000 repetitions) to estimate the 95% intervals for the variance components. This procedure allowed us to partition the variance of the three traits across the nested levels of the study (i.e. among individuals within species within quadrats, among species within quadrats and among quadrats).

Afterwards, we examined the effect of the different sampling strategies on two closely related questions. The first one is which of the sampling strategies can more accurately predict the CWM and FD values attained with the most intensive strategy, and how does these accuracies covary with sampling intensity. For each resampling event, we calculated the ratio between the CWM and FD values obtained following the different sampling strategies and the CWM_I and FD_I of each quadrat. Afterwards, we computed the absolute difference between 1 and the aforementioned ratio, thus obtaining an estimate of the error (which we expressed as a percentage) of each sampling strategy and intensity. A strategy that predicts the values attained by the most intensive method accurately will present values close to 0 for this index. For each of the 1,000 resampling events, we performed a repeated-measures ANCOVA analysis (using the quadrat as the repeated measures) to study the proportion of variance of this error explained by each of the studied factors (trait, strategy, intensity and their pairwise interactions). Subsequently, we calculated the averages and standard errors of the percentages of variance explained by each factor across the 1,000 ANCOVAs. Additionally, we plotted the values attained by the most intensive method against the simulated ones and examined whether the different strategies provided biased (i.e. systematically higher or lower CWM/FD values than observed) or unbiased results, and whether these biases depended on the CWM_I and FD_I values.

Second, we assessed the ability of the different sampling strategies to detect changes in functional trait structure across environmental gradients. We tested the effect of the position in the gradient on CWM and FD for each of the proposed strategies, and compared it with the response of CWM_I and FD_I across the gradient. We first fitted a linear and a quadratic model for each trait and the real indicators of functional trait structure (CWM_I and FD_I):

*Linear model: CWM_I or $FD_I = \beta_{I0} + \beta_{I1} * Gradient + \varepsilon$;*

*Quadratic model: CWM_I or $FD_I = \beta_{I0} + \beta_{I1} * Gradient + \beta_{I2} * Gradient^2 + \varepsilon$,*

where *Gradient* refers to the position of the quadrat across the sampled slope (1 being the most uphill quadrat and 40 the lowest one). To improve the interpretability of the coefficients and reduce the correlation between the predictors, *Gradient* was centred (by subtracting its mean) before squaring (Schielzeth 2010). With this transformation of the input variable, β_{I1} expresses the linear effect, i.e. whether the studied indicator increases or decreases along the gradient, whereas β_{I2} indicate whether the extremes of the gradient have higher ($\beta_{I2}>0$) or lower ($\beta_{I2}<0$) values on top of the linear relationships (Schielzeth 2010). Between the two models, we selected the one with the lowest AIC value. Such model should reflect the 'real' variation in the indicator along the studied gradients, estimated by means of the most labor-intensive strategy. Afterwards, for each simulation of each sampling strategy, we fitted the same model (linear or quadratic, depending on the selected 'real' model), and stored its parameters (β_0 , β_1 and β_2). We considered that an approach adequately described the variation in functional trait structure across the gradient if β_{I0} , β_{I1} and β_{I2} were within the 95% confidence intervals of the β_0 , β_1 and β_2 calculated in its 1,000 resampling events.

RESULTS

Differences among species accounted for most of the variance in all the studied traits, but there were important differences between traits in the proportion of variance found at this level, which ranged between 70.7% for LA and 49.1% for Height (Table 1). Similarly, the different traits differed in the proportion of variance due to differences between quadrats, with Height being the trait for which this level displayed a bigger importance (35.3%). By contrast, differences among individuals within plots accounted for a higher proportion of the variability of SLA than differences along the gradient (Table 1).

Question 1: Error and bias

The simulations revealed important differences between strategies in their power to detect the functional trait structure of plant communities and its variability across the studied slope (Table 2). LOC consistently outperformed the other strategies, regardless of the studied trait, and both for CWM and FD (Fig. 2). For a similar sampling intensity, LOC produced results with a much smaller error than GLO and

MAX (Fig. 3). In addition, LOC was the method that experienced the greatest improvements in its performance when sampling intensity increased, although these increases varied between traits and indicators of functional trait structure (Fig. 3). In fact, even for the lowest sampling intensity (1 individual per species per plot), LOC generally yielded much more reliable results than the highest sampling intensities simulated for GLO and MAX (Fig. 3).

Among the other strategies, MAX was a better predictor of CWM than GLO, both in terms of error and of bias (Figs 2 and 3). Although both strategies behaved poorly for extreme CWM and FD values —overestimating for small CWM_I values and underestimating for high CWM_I values—, this problem was considerably more important for GLO than for MAX (Fig. 2). Increasing sampling intensity did not substantially reduce the error of these methods (Fig. 3). Conversely, FD values calculated using GLO were more similar to FD_R than those calculated using MAX (Fig. 3). Interestingly, FD values based on MAX were consistently higher than FD_I , regardless of the studied trait, whereas the direction of the bias of the FD values based on GLO depended on the value of FD_I (Fig. 2).

Question 2: Patterns of variation across the topographical gradient

LOC was the method that most closely estimated the parameters of the models for the variations in CWM_I and FD_I across the gradient for all the studied traits (Table 3). The regression parameters estimated with LOC were always similar to the true parameters, and this similarity increased with sampling intensity (Table 3; Appendix S2).

The patterns of CWM across the gradient were not satisfactorily described when GLO was used. This was especially evident for LA, for which the GLO strategy failed to detect any relationship between the position in the gradient and this trait, despite the underlying pattern of increased CWM_I across the gradient (Table 3; Appendix S2). In contrast, the MAX strategy was able to detect such positive relationship (Table 3). In addition, both GLO and MAX detected poorly the patterns of FD_I across the gradient. Again, LA was the trait for which the use of GLO and MAX had most important implications, with both strategies indicating a decrease of FD along the gradient, when actually there was not a significant effect of the position in the gradient on FD_I (Table 3; Appendix S2). For the other two traits considered (Height and SLA), both GLO and

MAX generally underestimated the values of β_{11} . GLO tended to underestimate β_{12} and MAX to overestimate it. Increases in sampling intensity of GLO and MAX resulted in narrower confidence intervals for the parameters of the regressions estimated with these methods, i.e. a higher precision, but not in a better correspondence with the parameters of the 'real' regression, i.e. accuracy did not improved with sampling intensity (Table 3).

DISCUSSION

Although ecologists are increasingly assessing the variation of functional trait structure patterns across environmental gradients, very little is known about the optimal strategy for functional traits sampling. Our results indicate that the impact of considering intraspecific variability in trait values can be greater than commonly assumed. As a result, strategies that neglect this source of variability can result in inaccurate or biased estimations of the functional trait structure of plant communities, which in turn can significantly impact the predictions and conclusions drawn from such studies. However, we show that the accurate and unbiased results attained when intraspecific variability is taken into consideration can be achieved without any increases in the total number of individuals measured.

Variability in indicators of functional trait structure across environmental gradients is the combined result of intraspecific variability and of changes in the identity and abundance of species across the gradient (Lepš et al. 2011; Kichenin et al. 2013). We found that, in spite of the reduced spatial scale of our study, interspecific differences were the main source of variability in trait values, with a relatively small contribution of intraspecific variability. In fact, the proportion of variance explained by differences among species (ranging between 49% and 71%, depending on the trait) was comparable to those reported in previous studies (see review in Auger and Shipley 2013). Notwithstanding the moderated importance of intraspecific variability, the LOC strategy, which considers both inter- and intra-specific variability, was remarkably better than GLO and MAX, which only consider interspecific variability. Notably, even in the case of LA, which presented the highest proportion of variability due to interspecific differences, the LOC strategy was the only one that correctly described the changes along the gradient (Table 3). This result shows that intraspecific variability has an important and non-negligible effect on the functional trait structure of the studied community, regardless of traits and indicators of functional trait

structure. In relation with this, in their study of a much larger aridity gradient (more than 350 km of length and ca. 300 mm in precipitation), Gross et al. (2013) found that, although intraspecific variability had a significant effect in the relationship between the FD of SLA and Height and environment (aridity), this effect was negligible for the FD of Leaf Area and Thickness and for the CWM of all the traits considered. The contrast between those results and the ones presented here is in agreement with the notion of a decrease in the relative importance of intraspecific variability in trait values as the spatial scale widens, as changes in species composition between sampling units account for a higher proportion of the variability in trait values (Albert et al. 2010; Auger and Shipley 2013).

Differences between sampling strategies and between traits accounted for the highest proportion of the variability in the error of CWM and FD values, with the other factors being much less important (Table 2). Interestingly, a similar study identifying the most suitable procedure for characterizing the functional trait structure of tropical rainforests (Baraloto et al. 2010) found that intensity and the interaction between trait and strategy played much more important roles. The small effect of the trait:strategy interaction indicates that the level of accuracy of the different strategies did not greatly varied among traits. Further, we consider that the reason for the lack of any effect of sampling intensity in our study is twofold. First, the GLO and MAX strategies were in general rather inaccurate, and increases in intensity (i.e. a higher number of individuals measured to estimate average values of the traits) resulted in more precise, but not more accurate estimations (Table 3; Fig. 3). Second, higher intensities resulted only in minor improvements in the accuracy of the LOC strategy, and such improvements were mostly irrelevant beyond a relatively low level of intensity (Appendix S3). This last result suggests that the differences among conspecifics under different environmental conditions (i.e. environmental plasticity; Lavorel et al. 2008) is the greatest source of ITV in our study, whereas differences among conspecifics within quadrats played a more modest role.

The high similarity of coexisting (at the quadrat level) conspecifics entails an important consequence from the point of view of the applicability of the LOC strategy: average trait values of each species at each quadrat can be accurately estimated with a reduced number of observations. In fact, in our study, even the selection of a single individual per species and quadrat led to much better estimations

of the functional trait structure at the quadrat level in terms of accuracy and bias (Figs 2 and 3), as well as to a much better description of the changes in functional trait structure across the environmental gradient (Table 2) than higher sampling intensities of the other strategies. Interestingly, the ONE_PER_SP strategy of Baraloto et al. (2010) in tropical forests, analogous to our LOC strategy in grasslands –one individual per species and sampling unit–, was acknowledged as the best compromise in terms of its cost-benefit relationship (Baraloto et al. 2010). However, whereas the accuracy of this strategy was similar regarding CWM, for FD (Variance in Baraloto et al. 2010), it yielded much better results in our study. In this regard, it is important to consider the different spatial scales of the two studies. Whereas our sampling units are 20x20-cm quadrats, the study of Baraloto et al. (2010) uses 1-ha plots. Indeed, a greater scale can be associated with a much greater variability in environmental conditions within sampling units, which in turn should lead to greater differences in the trait values of conspecifics within plots (Albert et al. 2011; de Bello et al. 2011). Logically, the greater the variability of the trait values of coexisting conspecifics, the higher the number of individuals that should be selected for adequately estimating the local trait value of the species.

Once established that the LOC strategy outperforms strategies that use a single whole-gradient average per species, one may wonder about the effect of species abundances on the optimum number of individuals that should be randomly selected per sampling unit and species. First of all, it is important to consider that, in the presented case, the main objective was to characterize the CWM and FD of the sampling units, and that, in the studied indices, the trait value of the species was weighted by its relative abundance. Therefore, the impact of inaccuracies in the estimations of local average trait values must be proportional to the relative abundance of the species. This reasoning is in agreement with the mass ratio hypothesis (Grime 1998; Díaz et al. 2007), which states that the traits of the dominant species are the most important determinants of ecosystem functioning, whereas those of subordinate species are of much lesser importance. Keeping this in mind, we propose a strategy that seeks to improve the accuracy of the estimations of functional trait structure while not greatly increasing the required effort in terms of selected individuals. This strategy, which we will term EFF (from 'efficient'), simply consists in the selection of a higher number of individuals of the species that are more abundant in each sampling

unit. To illustrate this point, we performed 1,000 simulations of the case in which we collected 2 individuals per species and quadrat if the cover of the species in the quadrat is <25% and 4 individuals otherwise. This strategy entails only an 11.4% increase in the total number of individuals measured compared with collecting 2 individuals per species and quadrat (from 514 to 580). However, this little extra effort reduced the average error of the estimations of CWM and FD by an average of 19.9% and 8.6%, respectively, substantially improving the expected results for a similar intensity (Appendix S4). One of the main advantages of this strategy is that it can be easily performed at the same time as the vegetation surveys, thus minimizing the total amount of time invested. We want to stress that we provide this example simply as an illustration, but we do not pretend to suggest that this is the most efficient strategy in our dataset, an issue that we have not specifically explored in this paper. Indeed, similar strategies have been used before (for instance, Le Bagousse-Pinguet et al. 2014 selected between 1 and 5 individuals per site and species), but the question about the optimum strategy in relation with the abundances of species in the sampling units or the type of plant community considered requires further research.

In spite of the aforementioned advantages of selecting a reduced number of individuals per species and sampling unit, ecologists should be aware of the limitations of this strategy. For instance, while it seems appropriate for characterizing changes in functional trait structure across environmental gradients, studies analyzing the overlap in functional traits among coexisting species by means of species' probability density functions (e.g. Mason et al. 2011; de Bello et al. 2013) should generally require sampling a higher number of individuals per species and sampling unit. Furthermore, it is important to stress that the results presented here cannot be directly generalized to other systems. For example, if within a sampling unit the contribution of intraspecific variability to the total variance in trait values is relatively great compared to that of interspecific differences, a greater number of individuals per species should be selected to adequately estimate the local average trait values.

In conclusion, our results indicate that neglecting intraspecific trait variability can lead to substantial underestimations of the functional trait structure response along local environmental gradients. In fact, we show that, when a single average value per species is considered for the whole gradient, greater sampling intensities (i.e. calculating species means based on a great number of individuals) result in a higher

precision, but not in a higher accuracy. Therefore, caution is needed when deciding the strategy for measuring functional traits, especially when there is low species turnover along environmental gradients. Although this result might at first glance seem as a constrain for the advance of trait-based ecology, we show that accurate characterizations of the average trait values of species do not require greater investments in terms of the total number of individuals selected for trait measurements. We hope that this paper will stimulate more research aimed at the characterization of the most adequate strategies for estimating the functional trait structure of biological communities in other ecological systems.

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DATA ACCESIBILITY

The data used in this manuscript will be archived in figshare. Given that we are currently preparing other manuscript using part of this data, we prefer to embargo access to the data for a period of one year after publication.

REFERENCES

- Albert, C.H., Thuiller, W., Yoccoz, N.G., Douzet, R., Aubert, S. & Lavorel, S. (2010) A multi-trait approach reveals the structure and the relative importance of intra-versus interspecific variability. *Functional Ecology*, **24**, 1192-1201.
- Auger, S., Shipley, B. (2013) Inter-specific and intra-specific trait variation along short environmental gradients in an old-growth temperate forest. *Journal of Vegetation Science*, **24**, 419-428.
- de Bello, F., Carmona, C.P., Mason, N.W.H., Sebastià, M.T. & Lepš, J. (2013) Which trait dissimilarity for functional diversity: trait means or trait overlap? *Journal of Vegetation Science*, **24**, 807-819.

498 de Bello, F., Lavorel, S., Albert, C.H., Thuiller, W., Grigulis, K., Dolezal, J., Janeček, Š.
 499 & Lepš, J. (2011), Quantifying the relevance of intraspecific trait variability for
 500 functional diversity. *Methods in Ecology and Evolution*, **2**, 163-174.

501 Baraloto, C., Timothy Paine, C.E., Patiño, S., Bonal, D., Hérault, B. & Chave, J. (2010)
 502 Functional trait variation and sampling strategies in species-rich plant
 503 communities. *Functional Ecology*, **24**, 208-216.

504 Bernard-Verdier, M., Navas, M.L., Vellend, M., Violle, C., Fayolle, A. & Garnier, E.
 505 (2012) Community assembly along a soil depth gradient: contrasting patterns of
 506 plant trait convergence and divergence in a Mediterranean rangeland. *Journal of*
 507 *Ecology*, **100**, 1422-1433.

508 Carmona, C.P., Azcárate, F.M., de Bello, F., Ollero, H.S., Lepš, J., Peco, B. (2012)
 509 Taxonomical and functional diversity turnover in Mediterranean grasslands:
 510 interactions between grazing, habitat type and rainfall. *Journal of Applied Ecology*,
 511 **49**, 1084-1093.

512 Cornelissen, J.H.C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D.E,
 513 Reich, P.B., Steege, H., Morgan, H.D., M.G.A. van der Heijden, Pausas, J.G. &
 514 Poorter, H. (2003) A handbook of protocols for standardised and easy
 515 measurement of plant functional traits worldwide. *Australian Journal of Botany*,
 516 **51**, 335-380.

517 Cornwell, W.K. & Ackerly, D.D. (2009) Community assembly and shifts in plant trait
 518 distributions across an environmental gradient in coastal California. *Ecological*
 519 *Monographs*, **79**, 109-126.

520 Díaz, S. & Cabido, M. (2001) Vive la différence: plant functional diversity matters to
 521 ecosystem processes. *Trends in Ecology & Evolution*, **16**, 646-655.

522 Díaz, S., Lavorel, S., Bello, F. de, Quétier, F., Grigulis, K. & Robson, T.M. (2007)
 523 Incorporating plant functional diversity effects in ecosystem service assessments.
 524 *Proceedings of the National Academy of Sciences*, **104**, 20684-20689.

525 Grime, J.P. (1998) Benefits of plant diversity to ecosystems: immediate, filter and
 526 founder effects. *Journal of Ecology*, **86**, 902-910.

527 Gross, N., Börger, L., Soriano-Morales, S.I., Le Bagousse-Pinguet, Y., Quero, J.L.,
 528 García-Gómez, M., Valencia-Gómez, E., Maestre, F.T. (2013) Uncovering
 529 multiscale effects of aridity and biotic interactions on the functional structure of
 530 Mediterranean shrublands. *Journal of Ecology*, **101**, 637-649

531 Hulshof, C.M. & Swenson, N.G. (2010) Variation in leaf functional trait values within
 532 and across individuals and species: an example from Costa Rican dry forest.
 533 *Functional Ecology*, **24**, 217-223.

534 Jost, L. (2007) Partitioning diversity into independent Alpha and Beta components.
 535 *Ecology*, **88**, 2427-2439.

536 Kichenin, E., Wardle, D.A., Peltzer, D.A., Morse, C.W. & Freschet, G.T. (2013),
537 Contrasting effects of plant inter- and intraspecific variation on community-level
538 trait measures along an environmental gradient. *Functional Ecology*, **27**, 1254-
539 1261.

540 Laughlin, D.C., Joshi, C., van Bodegom, P.M., Bastow, Z.A. & Fulé, P.Z. (2012) A
541 predictive model of community assembly that incorporates intraspecific trait
542 variation. *Ecology Letters*, **15**, 1291-1299

543 Lavorel, S. & Garnier, E. (2002) Predicting changes in community composition and
544 ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional*
545 *Ecology*, **16**, 545-556.

546 Lavorel, S., Grigulis, K., McIntyre, S., Williams, N.S.G., Garden, D., Dorrough, J.,
547 Berman, S., Quétier, F., Thébault, A. & Bonis, A. (2008) Assessing functional
548 diversity in the field - methodology matters!. *Functional Ecology*, **22**, 134-147.

549 Le Bagousse-Pinguet Y., de Bello F., Vandewalle M., Lepš J. & Sykes M. (2014)
550 Species richness of limestone grasslands increases with trait overlap: evidence
551 from within- and between-species functional diversity partitioning. *Journal of*
552 *Ecology*, **102**, 466-474.

553 Lepš, J., de Bello, F., Šmilauer, P. and Doležal, J. (2011) Community trait response to
554 environment: disentangling species turnover vs intraspecific trait variability
555 effects. *Ecography*, **34**, 856-863.

556 Mason, N.W.H. & de Bello, F. (2013) Functional diversity: a tool for answering
557 challenging ecological questions. *Journal of Vegetation Science*, **24**, 777-780.

558 Mason, N.W.H., de Bello, F., Doležal, J. and Lepš, J. (2011) Niche overlap reveals the
559 effects of competition, disturbance and contrasting assembly processes in
560 experimental grassland communities. *Journal of Ecology*, **99**, 788-796.

561 Mason, N.W.H., de Bello, F., Mouillot, D., Pavoine, S. & Dray, S. 2013. A guide for
562 using functional diversity indices to reveal changes in assembly processes along
563 ecological gradients. *Journal of Vegetation Science*, **24**, 794-806.

564 Mason, N.W.H., Richardson, S.J., Peltzer, D.A., Wardle, D.A., De Bello, F. & Allen,
565 R.B. (2012) Changes in co-existence mechanisms along a long-term soil
566 chronosequence revealed by functional trait diversity. *Journal of Ecology*, **100**,
567 678-689.

568 McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006) Rebuilding community
569 ecology from functional traits. *Trends in Ecology & Evolution*, **21**, 178-185.

570 Messier, J., McGill, B.J. & Lechowicz, M.J. (2010) How do traits vary across
571 ecological scales? A case for trait-based ecology. *Ecology Letters*, **13**, 838-848.

572 Mouchet, M.A., Villéger, S., Mason, N.W.H. & Mouillot, D. (2010) Functional
573 diversity measures: an overview of their redundancy and their ability to
574 discriminate community assembly rules. *Functional Ecology*, **24**, 867-876.

575 Pakeman, R.J., Lennon, J.J. & Brooker, R.W. 2011. Trait assembly in plant assemblages
576 and its modulation by productivity and disturbance. *Oecologia*, **167**, 209-218.

577 R Core Team (2013). *R: A language and environment for statistical computing*. R
578 Foundation for Statistical Computing, Vienna, Austria.

579 Rao C.R. (1982) Diversity and dissimilarity coefficients-a unified approach. *Theoretical*
580 *Population Biology*, **21**, 24-43.

581 Ricotta, C. & Moretti, M. (2011) CWM and Rao's quadratic diversity: a unified
582 framework for functional ecology. *Oecologia*, **167**, 181-188.

583 Schielzeth, H. (2010) Simple means to improve the interpretability of regression
584 coefficients. *Methods in Ecology and Evolution*, **1**, 103-113.

585 Shipley, B., Vile, D. & Garnier, E. (2006) From plant traits to plant communities: a
586 statistical mechanistic approach to biodiversity. *Science*, **314**, 812-814.

587 Spasojevic, M.J. & Suding, K.N. (2012) Inferring community assembly mechanisms
588 from functional diversity patterns: the importance of multiple assembly
589 processes. *Journal of Ecology*, **100**, 652-661.

590 Violle C., Enquist B.J., McGill B.J., Jiang L., Albert C.H., Hulshof C., Jung V. &
591 Messier J. (2012) The return of the variance: intraspecific variability in community
592 ecology. *Trends in Ecology and Evolution*, **27**, 244-252.

593 Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A. & Wright, I.J (2002) Plant
594 ecological strategies: Some leading dimensions of variation between species.
595 *Annual Review of Ecology and Systematics*, **33**, 125-159.

596

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Appendix S1. Soil water content along the topographical gradient

Appendix S2. Community weighted mean (CWM) and functional diversity (FD) along the topographical gradient.

Appendix S3. Boxplots representing the error of the different intensities for the LOC strategy.

Appendix S4. Boxplots representing the reductions in error attained using the example of EFF strategy proposed in the main text (2 individuals per species and quadrat if the cover of the species in the quadrat is <25% and 4 individuals otherwise).

608

609 **Table 1.** Partitioning of variance of the three studied traits across the considered
 610 levels.

Level	% variance of trait (bootstrap 95% C.I.)		
	Height	log LA	SLA
Individuals within species and error	15.64 (12.77-15.98)	12.79 (12.68-15.89)	22.38 (18.37-23.56)
Species within quadrats	49.09 (48.46-52.02)	70.70 (70.59-74.26)	59.13 (58.02-65.27)
Quadrats	35.26 (33.37-37.20)	16.50 (11.19-17.48)	18.49 (16.47-20.83)

611

Table 2. Results (mean \pm standard error) of the 1,000 repeated measures ANCOVA models performed to analyze the proportion of the variance (% VE) in error explained by the considered factors.

Source	d.f.	CWM		FD	
		SS	% VE	SS	% VE
Quadrats	39	74052 \pm 172	13.50 \pm 0.02	7423 \pm 22	9.69 \pm 0.02
Trait	2	55605 \pm 107	10.14 \pm 0.01	7045 \pm 31	9.17 \pm 0.02
Strategy	2	91931 \pm 130	16.79 \pm 0.02	15675 \pm 44	20.49 \pm 0.04
Intensity	1	2661 \pm 16	0.48 \pm 0	518 \pm 6	0.67 \pm 0.01
Trait:Strategy	4	31049 \pm 108	5.65 \pm 0.01	4744 \pm 26	6.16 \pm 0.02
Trait:Intensity	2	427 \pm 7	0.08 \pm 0	54 \pm 2	0.07 \pm 0
Strategy:Intensity	2	287 \pm 9	0.05 \pm 0	90 \pm 3	0.12 \pm 0
Residuals	2467	292783 \pm 765	53.31 \pm 0.03	41048 \pm 103	53.63 \pm 0.04

Table 3. Parameters of the regressions describing the variation in functional trait structure indicators (CWM and FD) across the studied gradient. For each trait and indicator, we show the parameters of the regression model with the lowest AIC value, as well as its significance level (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; NS: $p > 0.05$). Additionally, for each of the sampling strategies (GLO, MAX and LOC; see main text for a full description of the strategies), and a subset of sampling intensities, we show the results (average parameter \pm sd) of 1,000 resampling events. The cases in which the 95% confidence interval of the simulated parameters did not include the parameter attained with the most intensive strategy (10 individuals per species and quadrat) are shown in bold type. However, for the MAX strategy with 10 individuals, results in bold type indicate that the regression parameter for MAX10 is not within the 95% confidence interval of the parameter attained with the most intensive strategy.

CWM					FD		
HEIGHT		β_0	β_1	β_2 (x1000)	β_0	β_1 (x1000)	β_2 (x1000)
<i>INTENSIVE</i>		14.19***	0.41***	-9.36 ^{NS}	1.21***	4.64***	-0.34**
Strategy	Intensity						
GLO	5	13.67±0.57	0.20±0.02	-6.97±1.61	1.24±0.04	2.08±0.65	-0.34±0.07
	10	13.65±0.40	0.20±0.01	-6.84±1.11	1.23±0.03	2.12±0.45	-0.34±0.05
	20	13.65±0.24	0.20±0.01	-6.86±0.64	1.23±0.01	2.22±0.28	-0.34±0.03
MAX	5	13.40±0.35	0.29±0.01	-11.04±0.66	1.33±0.03	4.06±0.51	-0.37±0.04
	9	14.38±0.10	0.29±0.01	-11.04±0.23	1.33±0.01	4.11±0.18	-0.38±0.01
	10	14.39	0.29	-11.04	1.33	4.12	-0.38
LOC	1	14.19±0.42	0.41±0.02	-9.36±2.47	1.20±0.03	4.13±0.75	-0.32±0.09
	2	14.20±0.26	0.41±0.01	-9.34±1.69	1.20±0.02	4.40±0.60	-0.33±0.07
	3	14.20±0.20	0.41±0.00	-9.38±1.22	1.20±0.02	4.50±0.47	-0.33±0.05

LEAF AREA							
		β_0	β_1 (x1000)	β_2	β_0	β_1 (x1000)	β_2
<i>INTENSIVE</i>		4.84***	22.32**	-	1.09***	-0.03 ^{NS}	-
Strategy	Intensity						
GLO	5	4.83±0.08	-0.01±2.56	-	1.10±0.01	-1.08±0.32	-
	10	4.83±0.05	-0.06±1.71	-	1.10±0.01	-1.12±0.22	-
	20	4.83±0.03	0.03±1.20	-	1.10±0.01	-1.11±0.16	-
MAX	5	4.77±0.04	9.82±1.49	-	1.12±0.01	-1.01±0.19	-
	9	4.77±0.01	9.84±0.55	-	1.12±0.00	-1.02±0.08	-
	10	4.77	9.83	-	1.12	-1.03	-
LOC	1	4.84±0.03	22.21±3.49	-	1.10±0.01	0.09±0.35	-
	2	4.84±0.02	22.27±2.10	-	1.10±0.01	0.02±0.23	-
	3	4.84±0.02	22.29±1.59	-	1.09±0.00	-0.01±0.18	-

SLA							
		β_0	β_1 (x1000)	β_2 (x1000)	β_0	β_1 (x1000)	β_2 (x1000)
<i>INTENSIVE</i>		29.55***	0.32***	-21.65**	1.09***	0.29 ^{NS}	-0.22***
Strategy	Intensity						
GLO	5	28.54±0.97	0.08±0.03	-14.46±3.07	1.09±0.02	-0.26±0.30	-0.16±0.04
	10	28.52±0.62	0.08±0.02	-14.58±2.10	1.09±0.01	-0.28±0.20	-0.16±0.03
	20	28.55±0.41	0.08±0.01	-14.63±1.22	1.08±0.01	-0.27±0.13	-0.16±0.02
MAX	5	30.70±0.37	0.12±0.01	-30.77±1.05	1.17±0.02	-0.20±0.12	-0.35±0.04
	9	30.68±0.16	0.12±0.01	-30.75±0.44	1.17±0.01	-0.23±0.09	-0.35±0.01
	10	30.68	0.12	-30.75	1.17	-0.24	-0.35
LOC	1	29.52±0.51	0.32±0.03	-21.54±2.62	1.11±0.03	0.58±0.33	-0.25±0.08
	2	29.55±0.34	0.32±0.02	-21.69±1.76	1.10±0.02	0.44±0.20	-0.23±0.05
	3	29.56±0.25	0.32±0.01	-21.64±1.33	1.10±0.01	0.38±0.15	-0.23±0.03

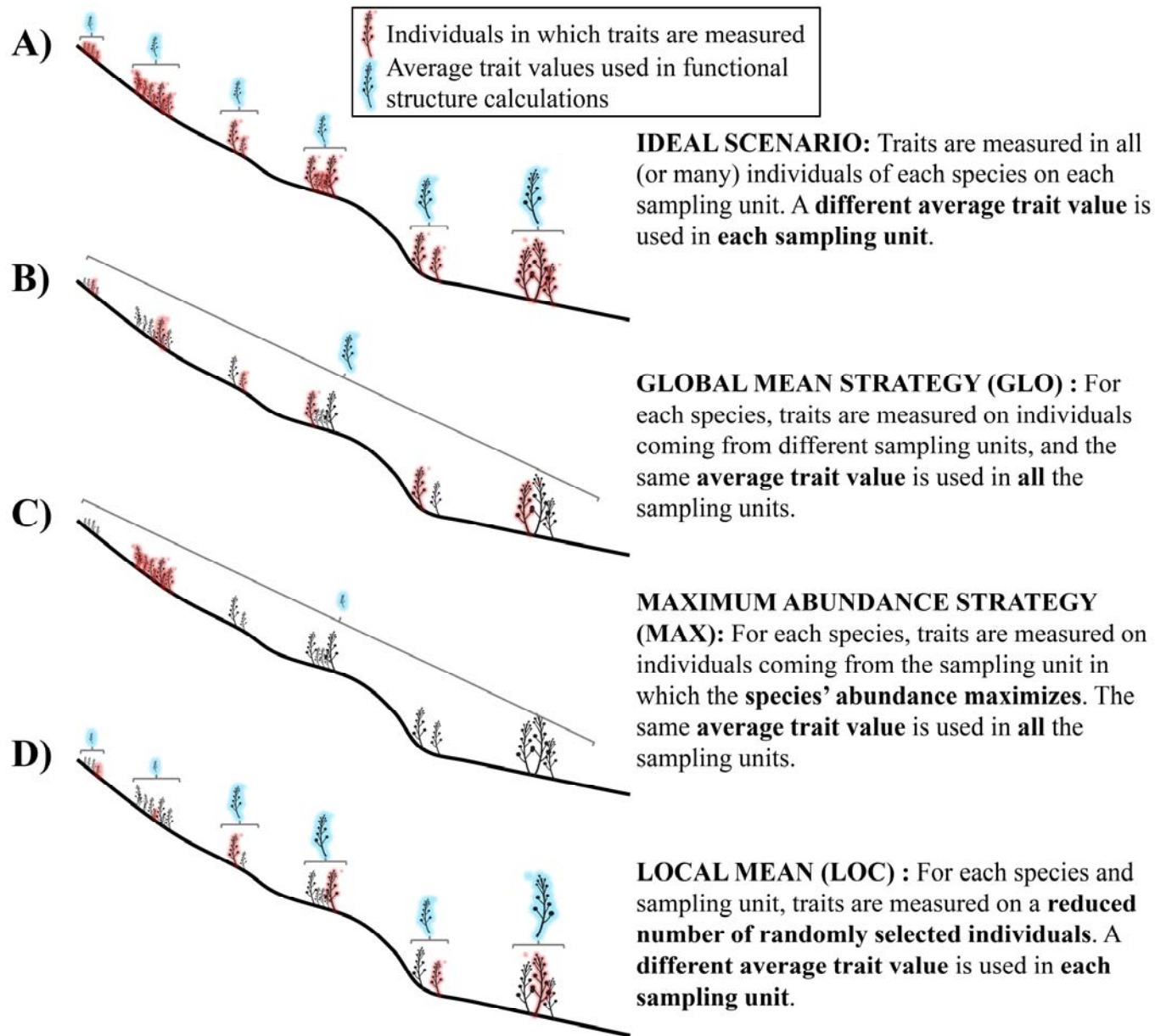


Fig. 1. (Colour online, B&W in print) Illustration of the different sampling strategies considered in this paper. For any given species, in an ideal scenario (A), the traits of all (or many) individuals are measured in all the plots in which the species is present, and the averages of the individuals of each plot are used to calculate the functional trait structure indices (CWM_I and FD_I ; see main text). However, this strategy implies a great effort that is not feasible in most occasions. In this paper we compare three alternative strategies: the global mean strategy (GLO; B); the maximum abundance strategy (MAX; C); and the local mean strategy (LOC; D).

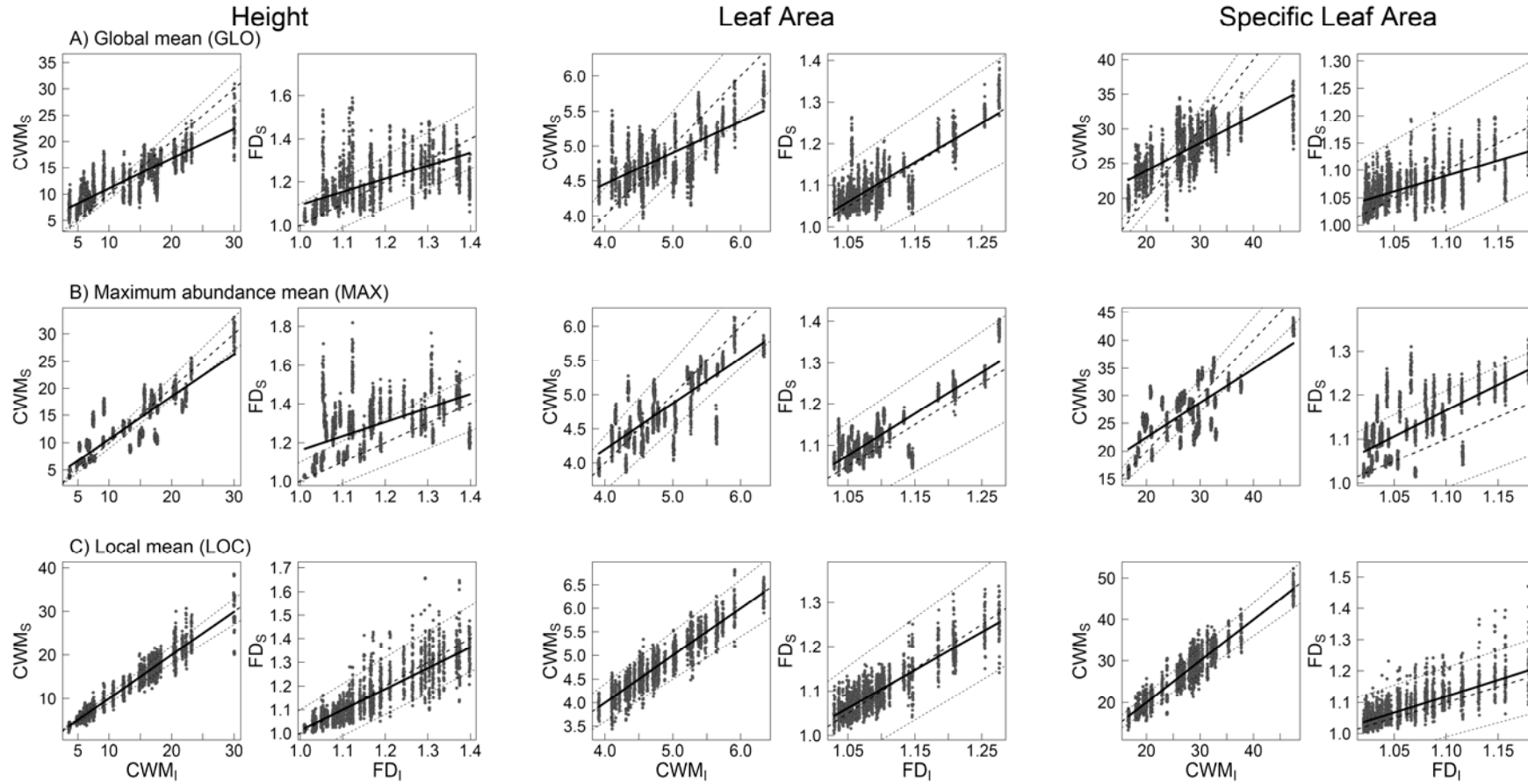


Fig. 2. Relationship between the indicators of functional trait structure attained using the most intensive strategy (10 individuals per species and quadrat; CWM_I and FD_I) and the indicators of functional trait structure obtained using the three studied sampling strategies (CWM_S and FD_S). For each strategy, we show the results of 50 simulations for one of the simulated sampling intensities -5 individuals for species for GLO (255 individuals across the whole gradient) and MAX (255) and one individual per species and quadrat for LOC (257). The total number of individuals (in parentheses) is similar for the three strategies. Continuous black lines represent a linear regression fitted for each case, which considered all the simulations (1,000). Dotted lines indicate a $\pm 10\%$ level of accuracy, and the dashed line indicates a 1:1 relationship. No systematic departures from this line indicate no bias in the estimations of the corresponding functional trait structure parameter.

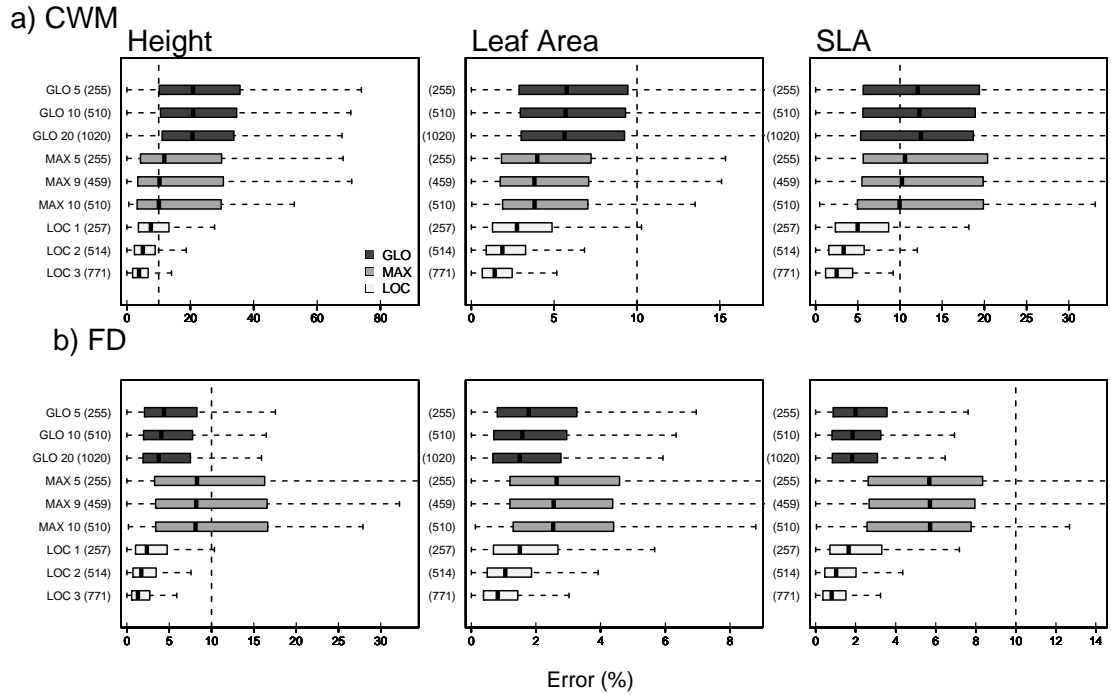


Fig. 3. Boxplots representing the error (expressed in percentage) of the different sampling strategies and intensities. For each trait and indicator of functional trait structure (CWM and FD), we calculated the ratio between the CWM and FD values obtained following the different sampling strategies and the CWM_I and FD_I of each quadrat. Error is the absolute difference between 1 and this ratio. Values close to 0 indicate a small error (i.e. simulated values are very close to the ones attained using the most intensive strategy of 10 individuals per species and quadrat). The dashed line represents a 10% level of error. The total number of individuals sampled across the gradient is indicated in parenthesis.