

# Functional trait variation and sampling strategies in species-rich plant communities

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## Summary

1. Despite considerable interest in the application of plant functional traits to questions of community assembly and ecosystem structure and function, there is no consensus on the appropriateness of sampling designs to obtain plot-level estimates in diverse plant communities.

2. We measured 10 plant functional traits describing leaf and stem morphology and ecophysiology for all trees in nine 1-ha plots in terra firme lowland tropical rain forests of French Guiana ( $N = 4709$ ).

3. We calculated, by simulation, the mean and variance in trait values for each plot and each trait expected under seven sampling methods and a range of sampling intensities. Simulated sampling methods included a variety of spatial designs, as well as the application of existing data base values to all individuals of a given species.

4. For each trait in each plot, we defined a performance index for each sampling design as the proportion of resampling events that resulted in observed means within 5% of the true plot mean, and observed variance within 20% of the true plot variance.

5. The relative performance of sampling designs was consistent for estimations of means and variances. Data base use had consistently poor performance for most traits across all plots, whereas sampling one individual per species per plot resulted in relatively high performance. We found few differences among different spatial sampling strategies; however, for a given strategy, increased intensity of sampling resulted in markedly improved accuracy in estimates of trait mean and variance.

6. We also calculated the financial cost of each sampling design based on data from our ‘every individual per plot’ strategy and estimated the sampling and botanical effort required. The relative performance of designs was strongly positively correlated with relative financial cost, suggesting that sampling investment returns are relatively constant.

7. Our results suggest that trait sampling for many objectives in species-rich plant communities may require the considerable effort of sampling at least one individual of each species in each plot, and that investment in complete sampling, though great, may be worthwhile for at least some traits.

**Key-words:** French Guiana, functional diversity, plant traits, specific leaf area, wood density, sampling design, tropical forest

## Introduction

Functional traits represent a foundation for defining plant resource use and life-history strategies (Grime 1979; Tilman 1988). Scaling up plant traits from individuals to communi-

ties provides a powerful approach to contemporary ecological questions (Wright *et al.* 2004; Swenson *et al.* 2006; Reich, Wright & Lusk 2007; Kleyer *et al.* 2008; Suding *et al.* 2008). The recent growth of large functional trait data bases has been fuelled by standardized protocols for the measurement of individual functional traits and intensive efforts to compile trait data (Cornelissen *et al.* 2003; Chave

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*et al.* 2009). Nonetheless, there remains no consensus for the most appropriate sampling design so that traits can be scaled from the individuals on whom measurements are made to the community or ecosystem levels at which inferences are drawn (Swenson *et al.* 2006, 2007, Reich, Wright & Lusk 2007; Kraft, Valencia & Ackerly 2008).

For many research questions, it is essential to be able to calculate community-level means accurately (Table 1). For example, understanding the links between climatic factors, vegetation types and ecosystem services requires a functional description of a large number of vegetation types across a climatic gradient (Wright *et al.* 2005; Cornwell *et al.* 2008; Malhado *et al.* 2009). An accurate estimation of the community-wide variance in functional traits is also often informative. For example, recent evaluations of the relative roles of environmental filtering vs. competition and niche differentiation in determining community structure have relied upon the breadth of functional traits among co-occurring species (Ackerly & Cornwell 2007; Kraft, Valencia & Ackerly 2008). Yet most recent studies rely on functional trait values compiled from the literature (Swenson *et al.* 2007). If community-wide trait variance is underestimated, the relative extent to which environmental filtering may structure these communities would be artificially inflated. In addition, within-species variability may be important to determine the breadth of species distributions and thus community assembly across environmental gradients, but such information is seldom available (Ackerly 2003).

Functional traits are also employed to parameterize a novel generation of dynamic global vegetation models (DGVMs) (Prentice *et al.* 2007). Recent advances in DGVMs develop vegetation descriptions from continuous trait landscapes based on functional trait measures rather than discrete categories of vegetation types (Hickler *et al.* 2006). As such, the accurate description of community-level trait variation will be essential to the future generations of integrated global change models.

Here, we emphasize that there currently exists no consensus for the most appropriate sampling design of plant traits in the field. This is largely because the exact sampling design depends on the research question. However, the fast pace of development of plant trait meta-analyses also suggests that trait acquisition in the field is a factor limiting the growth of plant trait data bases. To assess which sampling design would in principle be optimal for many of the research questions mentioned above, we use an original and intensive trait data set to contrast various sampling strategies. We measured traits for every individual tree in nine 1-ha plots in tropical lowland rainforest ( $N = 4709$ ). Each plant was sampled for 10 functional traits related to wood and leaf morphology and ecophysiology. Here, we contrast the trait means and variances obtained with a full sampling strategy with those of other sampling designs used in the recent literature, which we obtain by simulation. We assess the differences in community-level estimates of functional trait means and variances among design types and sampling intensities. We then contrast the relative costs of these designs and discuss the appropriateness of different sampling designs and intensities for different questions and systems.

## Materials and methods

### FIELD SAMPLING

Field sampling was conducted in nine 1-ha plots representing a gradient of precipitation and geological substrates of lowland tropical forests in French Guiana. Sampling was conducted under the auspices of the BRIDGE project, which aims to test alternative theories of community assembly (for complete details, see <http://www.ecofog.gf/Bridge/index.html>). In each plot, all trees > 10 cm diameter at breast height (d.b.h.) were mapped and measured for height and d.b.h. During inventories in 2007 and 2008, each tree in each plot was climbed to obtain a branch for leaf samples and herbarium vouchers. Samples were collected by professional tree climbers instructed to retrieve the

**Table 1.** The relative importance of accurate measures of community mean and variance of functional trait values to major ecological questions using a trait-based approach in ecology

Question	Importance of estimating community trait mean	Importance of estimating community trait variance	References
What are the roles of niche vs. neutral processes in community assembly?	Important to understand mechanisms	Vital to define functional trait breadth	Ackerly & Cornwell (2007), Kraft, Valencia & Ackerly (2008)
To what extent do environmental filters structure communities across landscapes?	Essential link to environmental measures at the community/plot scale	Important for detecting differences among plots	Cavender-Bares, Kitajima & Bazzaz (2004b), Cavender-Bares <i>et al.</i> (2004a), Ter Steege <i>et al.</i> (2006)
How does community composition effect environmental change?	Essential link to environmental measures at the community/plot scale	Important for detecting differences among plots	Cornwell <i>et al.</i> (2008)
How does community composition respond to environmental change?	Essential link to environmental measures at the community/plot scale	Important for detecting differences among plots	Suding <i>et al.</i> (2005), Gross & Cardinale (2007)
How does land use change alter ecosystem functioning?	Important to parameterize DGVMs	Important to parameterize DGVMs	Hickler <i>et al.</i> (2006), Prentice <i>et al.</i> (2007)

DGVMs, dynamic global vegetation models.

branch receiving the most light in the crown of each tree. Herbarium vouchers were made for every single stem to verify botanical determinations at the Cayenne herbarium (CAY), with consultation of taxonomic specialists when necessary. Overall, 4709 stems representing 728 species were sampled.

For each tree, three recently-expanded leaves were collected from the next-to-last shoot along the sampled branch. For compound leaves, we considered a leaflet as the laminar unit. Laminar surface area was measured with WinFolia software (Regent Instruments, Toronto, Canada) from digital scans made in the field using a portable scanner (Canon LiDE 60, Canon USA, Lake Success, NY, USA). Leaf thickness was measured for each lamina as the mean of three measurements using a Mitutoyo digital micrometer (Mitutoyo Instruments, Singapore). Leaf toughness was estimated as the average of three punch tests for each lamina using a Chatillon E-DFE-2 digital penetrometer (Ametek, Largo, FL, USA). Leaf total chlorophyll content was estimated using three values per lamina from a Minolta SPAD 502DL meter (Spectrum Technologies, Plainfield, IL, USA) with subsequent calculations using calibrations for French Guianan tropical trees (S. Coste, unpublished). Laminar dry mass was measured following drying to constant mass at 50 °C (around 72 h), and specific leaf area (SLA) was calculated for each lamina as the ratio of leaf surface area to leaf mass. We used the mean foliar trait values for each individual tree for all analyses in this study.

A 2-g subsample of each leaf sample was ground to fine powder using a grinding mill (Retsch MM200; Retsch GmbH & Co., Hahn, Germany). Leaf chemical analyses were conducted on a 3-mg subsample of the resulting powder by the Stable Isotope Facility of the University of California at Davis, including C and N content (%) and stable carbon and nitrogen isotope composition (‰).

Trunk xylem density ( $\rho_{xt}$ ) was calculated as the ratio of mass of samples dried for 72 h at 103 °C after removal of bark, to their fresh volume calculated by displacement of liquid volume. Wood samples were collected from boles using a 7 mm core to *c.* 3 cm depth at 1 m above ground level. Table 2 summarizes the traits measured and the range of values represented in the data set.

#### SIMULATING SAMPLING STRATEGIES

We investigated the effectiveness of four taxon-free strategies (requiring no botanical determinations; Lavorel *et al.* 2008) in addition to three taxon-explicit strategies that varied in the amount of field sam-

**Table 2.** Functional traits measured in the study, the sample size measured, and the range of values observed. Sample sizes are reduced for some traits because of sample condition or because the trait was not measured for all plots

Attribute (Abbreviation)	Unit	<i>N</i>	Range in data set
Laminar thickness (Thickness)	µm	4623	93.4–1036
Laminar toughness (Toughness)	N	4605	0.22–13.1
Laminar surface area (Surface)	cm <sup>2</sup>	4604	10.2–151.2
Laminar total chlorophyll (Chlorophyll content)	µg mm <sup>-2</sup>	4663	210–2164
Specific leaf area (SLA)	cm <sup>2</sup> g <sup>-1</sup>	4591	3.1–121.1
Foliar N <sub>m</sub> (Leaf N)	µg g <sup>-1</sup>	2063	0.6–5.5
Foliar C : N ratio (C : N ratio)	g g <sup>-1</sup>	2063	10.0–52.3
Foliar <sup>15</sup> N composition (Leaf <sup>15</sup> N)	‰	2063	-4.0 to +8.7
Foliar <sup>13</sup> C composition (Leaf <sup>13</sup> C)	‰	2063	-36.5 to -26.2
Trunk xylem density (Bole density)	g cm <sup>-3</sup>	2789	0.24–0.98

**Table 3.** A summary of sampling designs compared in this study. Entries are sorted by financial cost relative to complete sampling of all individuals in the field. For a detailed explanation of costs, see Appendix S1

Strategy	Sampling intensity*	Botanical intensity†	Cost (€)	Relative cost (%)
CLUMP	1	0	1228	6.7
GRID	1	0	1228	6.7
RANDOM	1	0	1228	6.7
SIZE	1	0	1228	6.7
CLUMP	2	0	1336	7.3
GRID	2	0	1336	7.3
RANDOM	2	0	1336	7.3
SIZE	2	0	1336	7.3
CLUMP	5	0	2180	12.0
GRID	5	0	2180	12.0
RANDOM	5	0	2180	12.0
SIZE	5	0	2180	12.0
CLUMP	10	0	4280	23.5
GRID	10	0	4280	23.5
RANDOM	10	0	5453	29.9
SIZE	10	0	3760	20.6
CLUMP	20	0	5927	32.5
GRID	20	0	7027	38.5
RANDOM	20	0	7027	38.5
SIZE	20	0	6533	35.8
DATABASE	0	100	500	2.7
COMMON_SP	~15	0	6617	36.3
ONE_PER_SP	~33	100	8830	48.4
BRIDGE	100	100	18 240	100.0

\*Percentage of stems requiring sampling under this strategy.

†Percentage of stems requiring herbarium determinations of botanical identifications so that the strategy can be effected.

pling required (Table 3). Some of these strategies were identified based on recent published studies. The basis of comparison was the BRIDGE strategy, in which every individual in each plot was sampled. In RANDOM, individual trees were randomly selected from the plot. Although we have not seen this strategy used in the literature, it provides a basis for comparison with other taxon-free sampling designs. In CLUMP, nine random locations within the plot were selected, and trees were chosen from those locations. A variant of CLUMP is used by the RAINFOR project (e.g. Patiño *et al.* 2009). The SIZE strategy controls for potential effects of canopy structure or ontogeny on trait values (Poorter, Bongers & Bongers 2006). Here, trees were divided into size-classes based on d.b.h., with breaks that divided the community into three equal parts. Sampling of random individuals was stratified among size-classes. In GRID, an evenly spaced grid of locations was generated, and the individuals nearest the vertices of the grid were selected. In subsequent re-samples, the position of the grid in the plot was randomly shifted. Each of these four taxon-free sampling strategies was performed at five levels of intensity: 1%, 2%, 5%, 10% and 20% of the individuals in each plot; sampling beyond this intensity is unrealistic, as costs quickly approach those of complete sampling (see Appendix S1, Supporting Information).

We also examined three taxon-explicit sampling strategies that inherently vary in their required sampling intensity. The COMMON\_SP strategy is based on a taxonomic abundance weighting that has been employed in temperate herbaceous plant communities (e.g. Garnier *et al.* 2007). The 30 most common species in the entire data

set were sampled for up to three individuals in each plot, obviating the botanical determination and sampling of rare species. We chose the cut-off of 30 species in our system because it represented a natural break in the species-abundance curves, below which species were represented by fewer than an average of three individuals per plot. The ONE\_PER\_SP strategy samples a single individual from each species in each plot and thus requires a full taxonomic inventory prior to sampling. Trait values for all individuals of each species in the plot were set equal to those of the sampled conspecific. A variant of this method was used by Kraft, Valencia & Ackerly (2008). Finally, in the DATABASE strategy, trait values were assigned to individuals based on shared taxonomic status (Swenson *et al.* 2006; Ter Steege *et al.* 2006). Our application of this method set the trait values of every individual equal to their species mean, using a data base of all individuals and species sampled in the BRIDGE project (4709 and 683 respectively). This implementation of the DATABASE strategy probably overestimates its accuracy, both because the trait values come from a regional data base including only the plots being studied, rather than from other regions, and because species–trait combinations for which no data were available were left as missing data (rather than replacement with genus means).

The benefits of each sampling strategy were assessed by simulation. The mean and variance of each normalized trait value as measured by the BRIDGE strategy was assumed to be the most accurate possible representation of the true plot-level mean and variance. The values obtained by every other strategy were compared with the BRIDGE values in the following way.

We simulated performing each strategy 1000 times at the five prescribed intensities (1%, 2%, 5%, 10% and 20%), in each of the nine plots in the BRIDGE network. For each re-sampling event, we calculated the per cent difference of the trait values thus obtained to the plot-level means and variances. Resampled means within  $\pm 5\%$  of the true plot-level mean, and resampled variances within  $\pm 20\%$  of the true plot-level variance were considered successes, and those outside these envelopes, failures. We chose the threshold values to provide the greatest discrimination among the sampling designs we simulated; the larger threshold for trait variance reflects the inherent variation in these plant functional traits (Wright *et al.* 2004; Patiño *et al.* 2009). The number of successes and failures for each combination of plot, strategy, intensity and trait was tabulated.

The per cent variance explained (PVE) in the frequency of success was partitioned in two pairs of ANCOVAs. The first two focused on taxon-free strategies and on leaf traits, which were measured in all nine plots. In them, plot, strategy, intensity and trait predicted the accuracy of the estimates for mean and variance respectively. The second pair focused on a single plot in which all traits were sampled, and included all strategies. It was structured similar to the first pair but without a plot term. In all four models, third- and fourth-order interactions were nonsignificant, and were dropped from the model. *P*-values and significance tests provide little guidance in these situations, due to their dependence on sample size. Hence, we use the sums of squares to compute the variance explained by each independent variable and interactions. All analyses were performed in R 2.9, and the code used to conduct the analyses is available in Appendix S2.

## Results

Overall, the estimation of plot-level trait values did not vary substantially among plots. Differences among plots explained less than 1% of the variance in the estimates of means, and 5.2% of the variance in the estimates of variances. Further-

more, strategies and intensities had similar performance among plots. In other words, there were no strong plot by strategy interactions (PVE < 0.05% for mean and variance), nor plot by intensity interactions (PVE < 1.6%). Additionally, the four taxon-free sampling strategies included in these analyses (CLUMP, GRID, RANDOM and SIZE) provided equally accurate estimates of the mean and variance in trait values (PVE < 0.11%). For more detailed analyses investigating all traits, we chose a single plot where all traits had been measured on most individuals, the Acarouany plot (Fig. 1).

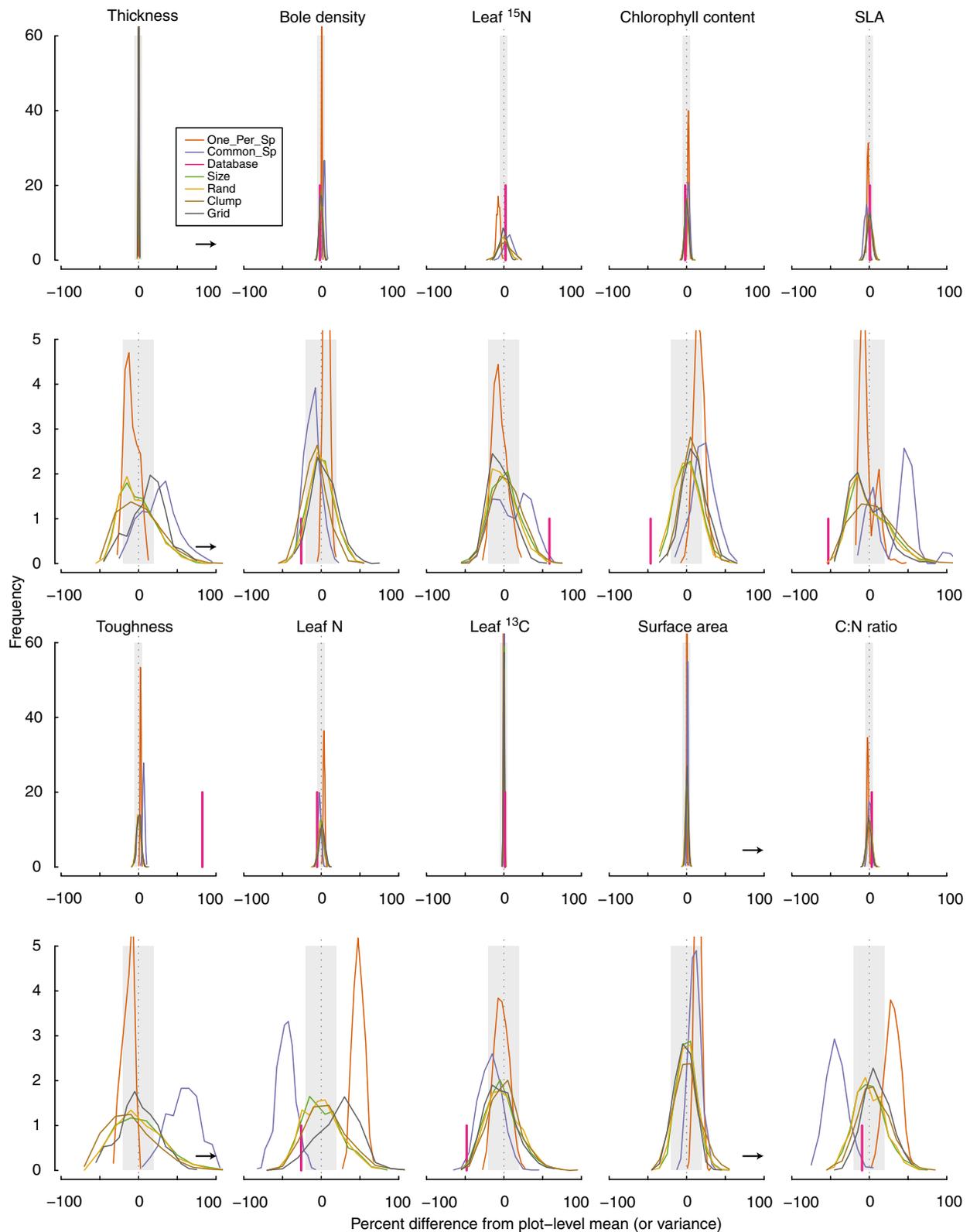
In Acarouany, the accuracy of predictions of mean and variance was significantly and positively correlated ( $P < 0.0001$ ,  $R^2 = 0.22$ ). The sampling strategy explained a substantial portion of the variance in estimation accuracy (PVE<sub>MEAN</sub> = 4.1, PVE<sub>VARIANCE</sub> = 8.3; Table 4). This difference is due to the generally good performance of the ONE\_PER\_SP strategy, and the erratic results of the DATABASE strategy (Fig. 2). The performance of DATABASE also generated a trait by strategy interaction, as DATABASE estimated the values of some traits well and others poorly (PVE<sub>MEAN</sub> = 20.4, PVE<sub>VARIANCE</sub> = 34.1). Some traits, such as leaf dry mass, were consistently inaccurately predicted, relative to other traits. In fact, variation among traits was the single most important predictor of estimation accuracy for mean trait values. Even increased intensity did not improve the estimation accuracy of these obstinate traits, generating a minor trait by intensity interaction (PVE<sub>MEAN</sub> = 5.5, PVE<sub>VARIANCE</sub> = 5.7). Intensity on its own explained substantial variance (PVE<sub>MEAN</sub> = 29.3, PVE<sub>VARIANCE</sub> = 22.0). However, increasing intensity improved the accuracy of estimation by the same magnitude across all strategies, such that there was no interaction between strategy and intensity (PVE < 0.6).

## Discussion

Choosing the most appropriate design for sampling plant functional traits requires consideration of both the relative benefits of a design addressing a given ecological question, and the relative costs of implementing alternative strategies. We address each of these in turn below.

### TRAIT MEANS AND VARIANCE

We assessed the benefits of sampling strategies by the accuracy of their estimations of trait means and variance in sampled communities. Our simulations provide sobering news for the application of existing trait data to many important ecological questions. The DATABASE strategy frequently provided inaccurate estimations of community-wide mean trait values, and almost never gave accurate estimations of trait variance. Among the other taxon-explicit strategies, the ONE\_PER\_SP species design (e.g. Kraft, Valencia & Ackerly 2008) performed relatively well. The most likely explanation for this result invokes substantial intraspecific trait variation due to either regional genetic variation, environmental plasticity or both (Lavorel *et al.* 2008). This result is concordant



**Fig. 1.** Examples of sampling design performance at the Acarouany plot. Shown are frequency distributions of the per cent difference between the observed trait values (mean and variance) and those estimated by six different sampling strategies. The shaded vertical bars represent the designated threshold for success:  $\pm 5\%$  for plot-level means and  $\pm 20\%$  for plot-level variance (see text for details). Each curve represents the distribution of trait values obtained from 1000 simulations. The DATABASE strategy yields one estimate per trait, and is represented by a small dark vertical bar (when deviations exceeded  $\pm 100\%$  an arrow indicates the direction of the deviation). For clarity, taxon-free strategies are shown only at their highest sampling intensity (20% of stems).

**Table 4.** The success of predicting the mean and variance of trait values varied among strategies, intensities and traits, as well as their interactions. Data are from the Acarouany plot, as depicted in Fig. 1

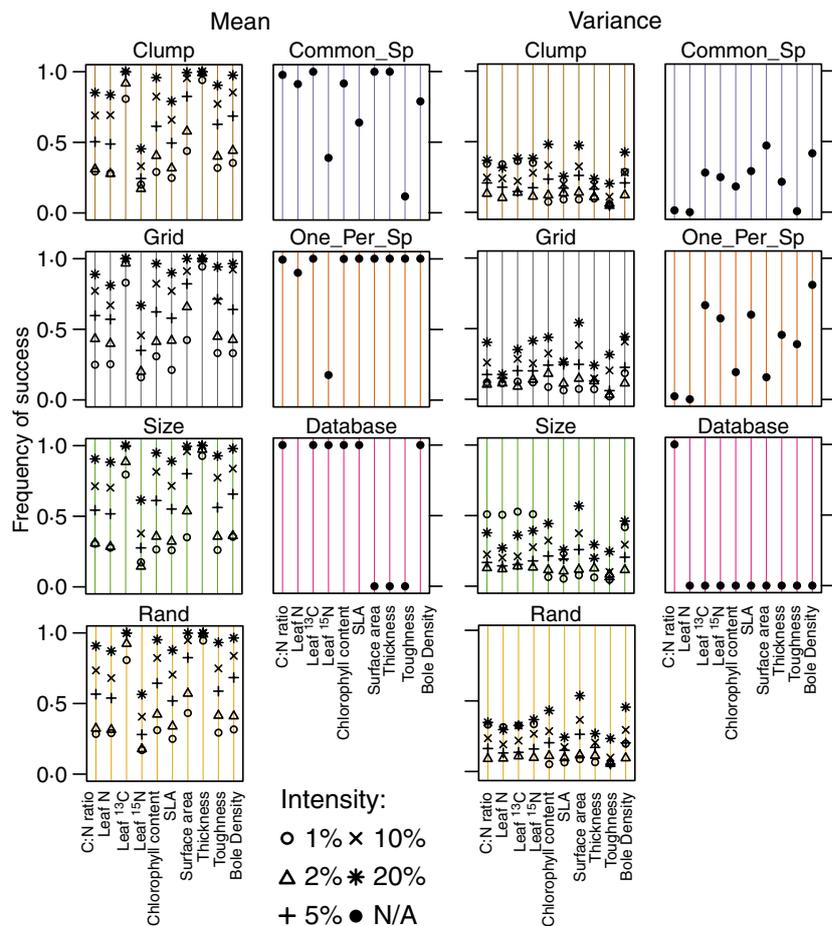
Predictor	d.f.	Mean		Variance	
		Sum of squares	Per cent variance explained	Sum of squares	Per cent variance explained
Trait	9	6.919	34.66	0.658	12.27
Strategy	6	0.816	4.09	0.443	8.26
Intensity	1	5.845	29.27	1.179	21.97
Trait by strategy	54	4.072	20.39	1.831	34.12
Trait by intensity	9	1.088	5.45	0.307	5.71
Strategy by intensity	3	0.005	0.07	0.029	0.54
Residuals	147	0.008	6.07	0.919	17.13

with recent Amazon-wide estimates for tree xylem density, where local environmental factors explained up to 22% of variability in sapwood density (Patiño *et al.* 2009).

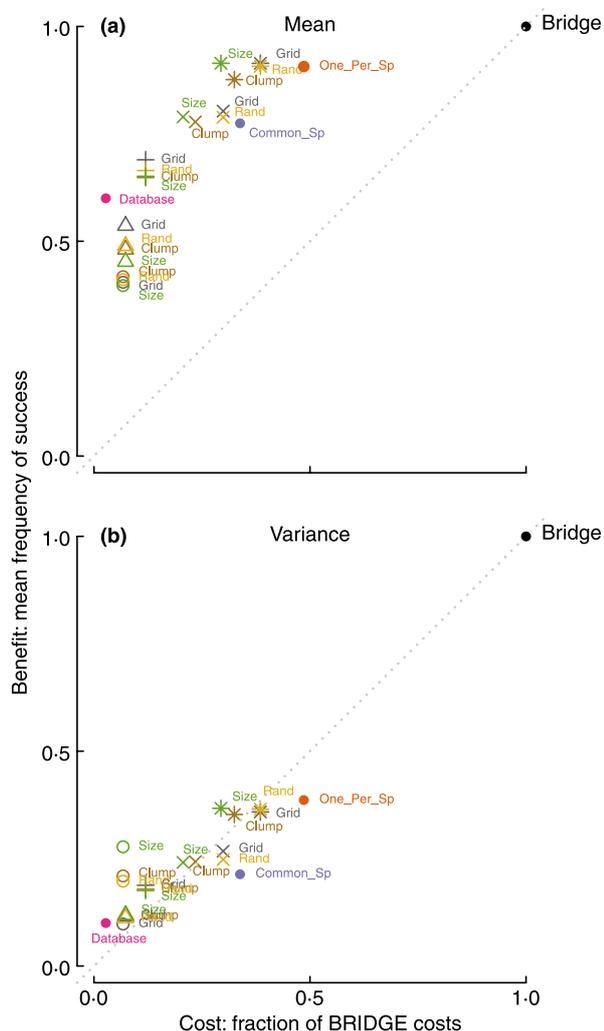
In contrast, the COMMON\_SPP design did not perform very well, suggesting that rare species in our system represent rare phenotypes that influence both community functional diversity (trait variance) and mean trait values (Fig. 3). In other plant communities with fewer rare species or with rare

species that do not exhibit rare phenotypes, abundance-based taxon sampling may be more appropriate. For example, the application of a 10% prevalence rule to taxon-explicit sampling across a network of plots in European prairies may capture 80% of the local abundance (Garnier *et al.* 2007) and resulted in accurate estimates of functional diversity (Lavorel *et al.* 2008), whereas our similar COMMON\_SPP strategy results in the sampling of only about 40% of the stems in an average plot and provided rather poor estimates of community trait means and variance (Figs 2 and 3).

Rare species limit the appropriateness of the DATABASE strategy in plant communities exhibiting high beta-diversity (species turnover among communities at a landscape scale), because many locally rare species may not have been previously measured. Our simulations may actually overestimate the typical performance of the DATABASE strategy, because we used a data base that contained all species in the plots we studied. When applying data bases among projects, the problem of rare species may be strongly limiting. A potential solution that has been used in several studies is to apply to rare species the mean trait values of species in the data base with shared nearest ancestors (Baker *et al.* 2004; Ter Steege *et al.* 2006). However, the implicit assumption to this approach is that trait values are conserved among closely-related species. This assumption has not received much support in the few



**Fig. 2.** Interaction plot showing the effects of sampling strategy and sampling intensity on the frequency of successfully estimating the plot-level mean and variance for each trait. The results presented are from a single plot, Acarouany, which was selected because all traits were measured for most individuals there. Similar results were obtained in the other eight plots.



**Fig. 3.** Cost–benefit analysis of sampling strategies and intensities in terms of success in estimating the (a) mean and (b) variance of trait distributions. Costs, in euros, are scaled to those of a BRIDGE mission (18 240€ per plot). Increasing sampling intensity leads to more cost-effective missions. Even so, to accurately estimate the variance in trait values, complete sampling is necessary.

plant groups where it has been tested for multiple traits (Cavender-Bares *et al.* 2004a).

We found surprisingly slight differences in performance among the taxon-free (field-based sampling) strategies, whereas more intense sampling yielded consistently more accurate results. In fact, intensity explained 65.3% of the variance in estimates of variance among all plots for the four taxon-free strategies. This suggests that investments in complicated spatial sampling designs may yield a lower return, in terms of estimation accuracy, than investments in more intense sampling.

#### A COST–BENEFIT ANALYSIS

The most appropriate sampling design also depends on the resources available, in terms of finance, personnel and access to equipment. As an initial investigation of these potential

costs, we estimated the financial costs associated with each sampling strategy in this study. Details of these calculations are provided in Appendix S1. The cost for completely sampling a plot with the BRIDGE strategy is 18 240€. Other strategies cost substantially less due to reductions in field effort as well as reduced laboratory and herbarium costs associated with processing fewer samples (Table 2).

Figure 3 illustrates the relative costs of each sampling strategy vs. the benefits they provide, in terms of the frequency with which they were found to provide accurate estimates of trait mean or variance. The dotted line on these panels represents a cost–benefit ratio equivalent to that of the BRIDGE (complete sampling) strategy.

With regard to estimating mean trait values, strategies alternative to BRIDGE were consistently cost-effective. On the other hand, strategies alternative to BRIDGE clearly failed to accurately estimate the variance of trait values. This indicates that in situations where accurate estimation of plot-level variance is desired, complete censuses are essential.

ONE\_PER\_SP is a relatively expensive strategy, in that it requires a complete botanical census as well as climbing, sampling and analysing *c.* 200 trees per hectare. Of all the sampling strategy alternatives to BRIDGE, however, ONE\_PER\_SP yielded the most accurate estimations of both trait means and variances. ONE\_PER\_SP may represent a viable sampling strategy in situations where the costs of sample acquisition are reduced, for example, in some herbaceous plant communities, or communities of juvenile trees. In contrast, the cost of the DATABASE strategy is minimal, requiring, at a maximum, the verification of certain botanical identifications. However, the estimates it yields are rarely accurate.

At low sampling intensities, the taxon-free strategies perform similarly, but as sampling intensity increases, SIZE has better performance. The costs associated with SIZE in forest systems are marginally lower than the other sampling strategies, because more sampling can be made from the ground, which reduces the need for expensive professional climbers. Our results thus recommend size-stratified sampling in situations where taxon-free methods are preferred (e.g. identifications are too expensive or beyond project scope). Overall, the relative performance of designs was strongly positively correlated with relative financial cost, suggesting that sampling investment returns are relatively constant in this system.

#### Conclusions

Our results indicate that trait sampling for many objectives in species-rich plant communities requires considerable effort to obtain reliable estimates of the mean and variance of trait values. Sampling at least one individual of each species in each local community seems like a reasonable compromise in the tropical forests we studied. Such an approach would provide a reliable estimate of mean trait values when attempting to respond to questions comparing a large number of local communities (Ter Steege *et al.* 2006; Cornwell *et al.* 2008). However, to address questions of functional diversity and

community assembly at the local scale, for which an accurate estimate of the variance of trait values in a local community becomes vital (Cavender-Bares *et al.* 2004a; Kraft, Valencia & Ackerly 2008), even the one individual of each species approach may be insufficient. We suggest that, in these studies, the investment in complete sampling may be worthwhile for at least some traits. Of course, the extent to which we can extrapolate to other plant systems, such as temperate forests or grasslands, remains speculative. Nonetheless, our results underline the need to match research questions with not only budgetary and practical constraints but also with particular properties of the study system (e.g. frequency of rare species).

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## Supporting information

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

**Appendix S1.** Calculations of the costs of different sampling designs  
**Appendix S2.** Code in the R-language for simulation of sampling designs.

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