## LETTER

# How do traits vary across ecological scales? A case for trait-based ecology

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## Abstract

Despite the increasing importance of functional traits for the study of plant ecology, we do not know how variation in a given trait changes across ecological scales, which prevents us from assessing potential scale-dependent aspects of trait variation. To address this deficiency, we partitioned the variance in two key functional traits (leaf mass area and leaf dry matter content) across six nested ecological scales (site, plot, species, tree, strata and leaf) in lowland tropical rainforests. In both traits, the plot level shows virtually no variance despite high species turnover among plots and the size of within-species variation (leaf + strata + tree) is comparable with that of species level variation. The lack of variance at the plot level brings substantial support to the idea that trait-based environmental filtering plays a central role in plant community assembly. These results and the finding that the amount of within-species variation is comparable with interspecific variation support a shift of focus from species-based to trait-based ecology.

## Keywords

Ecological scaling, environmental filtering, leaf dry matter content, leaf functional traits, leaf mass area, plant community assembly, trait-based ecology, tropical rainforests, variance components.

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## INTRODUCTION

The amount of variation found in Nature has fascinated biologists since the days of the Victorian naturalists, continually stimulating novel research and shifts in paradigms as new facets of natural variation are recognized and their significance assessed. Characterizing natural variation and understanding its causes and consequences have been central to the conceptual development of ecology and evolution. For example, the study of genetic variation among individuals, populations and species is at the heart of the Modern Evolutionary Synthesis, and variation in life-history traits (or lack thereof) underlies the current debate between neutral and niche theories as explanations for patterns of community diversity. Even when the study of variation *per se* is not a primary goal, most fields of ecology and evolution are concerned to at least some extent with aspects of natural variation. Many disciplines are concerned especially with trait variation, which exists at all spatiotemporal and organizational scales: within a single organism (Pigliucci 2001), within a species (Valladares *et al.* 2000; Takahashi *et al.* 2005; McGill *et al.* 2006; Rozendaal *et al.* 2006), among species (Wright *et al.* 2001; Westoby *et al.*

2002) and among communities (Ackerly *et al.* 2002; Wright *et al.* 2004; Rozendaal *et al.* 2006; Ackerly & Cornwell 2007). Depending on the particular research questions, variation in traits typically is assessed over only a part of this spectrum of spatiotemporal variation. As a consequence, the study of trait variation is fragmented across disciplines. For example, there is considerable empirical and theoretical understanding of how variation between sun and shade leaves affects leaf function, but with less attention to how sun-shade plasticity of trees varies among species (Popma & Bongers 1988; Popma *et al.* 1992; Valladares *et al.* 2000; Takahashi *et al.* 2005; Rozendaal *et al.* 2006). Conversely, the extensive comparisons of interspecific variation that have advanced our understanding of the tradeoffs in traits defining foliar function (Wright *et al.* 2001; Westoby *et al.* 2002) purposely are restricted to sun leaves (Cornelissen *et al.* 2003). Similarly, studies of trait variation along environmental gradients focus on species mean and community-aggregated trait values (Ackerly *et al.* 2002; Wright *et al.* 2004; Rozendaal *et al.* 2006; Shipley *et al.* 2006b; Ackerly & Cornwell 2007; Cornwell & Ackerly 2009). While broad interspecific comparisons invariably focus on the mean with little or no attention to within-species variation among

co-occurring individuals (but see Bolnick *et al.* 2003), ecological genetic studies of plant plasticity often focus on only a few species to the exclusion of interspecific comparisons (but see Valladares *et al.* 2000). As a consequence of these fragmented and restricted approaches to the analysis of variation in traits, a general ecological understanding of trait variation across ecologically relevant spatial and temporal scales is lacking.

To our knowledge, no study has used a uniform sampling methodology to measure trait variation across the range of ecological scales spanning these diverse disciplinary perspectives. Identifying which scales have the most variation in traits will provide an important check on assumptions that underlie many existing theories. For example, under the environmental filtering paradigm, environmental conditions at a site are assumed to drive the optimal trait values, which then select for the appropriate species mixture (Keddy 1992; Diaz et al. 1998). Alternatively, the comparative paradigm views species as units of selection in an adaptive evolutionary history with tradeoffs among traits distinctly expressed across species, so that variation among species is large relative to that within species (Cornelissen et al. 2003). Bringing yet a different perspective, studies focusing on variation within species emphasize differences among individuals in a species resulting from genetic variation and/or phenotypic plasticity as the basis of differences in community composition among plots and sites. Such *a priori* expectations about the scaling of variation are rarely stated explicitly, and different disciplines work within paradigms rooted in different implicit assumptions. Identifying ecological scales that account for a large percentage of total variance can help resolve uncertainties and potential inconsistencies in these various disciplinary points of view. Identifying which scales account for most of the variation in traits can also can help focus research efforts on patterns and processes at spatiotemporal scales that are ecologically most important (McGill 2008).

In this study, we focus our efforts on the ecological scaling of variance in functional traits, i.e. traits that reflect adaptation to, and performance in, different environments (Violle *et al.* 2007). As functional traits can indicate how an individual relates and responds to its environment, their study offers a powerful approach to address ecological questions (McGill *et al.* 2006). This is especially true for highly diverse systems such as tropical forests where using species as the working unit often becomes overwhelming. Studies of functional traits can more efficiently provide ecologically informative insights into community composition and ecosystem function (Lavorel & Garnier 2002; McGill et al. 2006; Westoby & Wright 2006). For example, species abundance measurements might not be as informative as community trait composition if functionally equivalent species can substitute each other.

To begin to address the lack of knowledge on ecological scaling of variance, we present a standardized and integrative study designed to assess how variation in two key foliar traits – leaf mass area (LMA) and leaf dry matter content (LDMC) (Reich *et al.* 1999; Wright *et al.* 2004; Shipley *et al.* 2006a) – is distributed across six hierarchically structured ecological scales – leaf, canopy strata (sun vs. shade), tree, species, plot (400 m$^2$) and site (4–8 plots per site) – in the lowland tropical forests of Panama.

## MATERIAL AND METHODS

### Traits and scales

We assessed variation in LMA and LDMC across six hierarchical ecological scales: 1 – among leaves within a canopy stratum; 2 – between sun and shade strata within a tree; 3 – among trees within a species; 4 – among species within a plot; 5 – among plots within a site; 6 – among sites. These six scales contain a mixture of taxonomic (species) and spatial factors both with explicit environmental gradients (strata and site) and with no immediately obvious environmental gradient (leaf, tree and plot). Plots within our three sites were established systematically 60–80 m apart, staying within the same habitat and subject only to local topographic variation, whereas the three sites themselves were purposely arrayed on a steep rainfall gradient across the Isthmus of Panama. This nested design was chosen to help compare pre-existing disciplinary perspectives on variation at these scales and to contain a logically nested spatial structure. These are the scales most commonly studied by ecologists and the two traits are among the most important in defining foliar function in plants (Reich *et al.* 1999; Weiher *et al.* 1999; Wright *et al.* 2004; Shipley *et al.* 2006a). Both LMA and LDMC are key traits in the leaf economic spectrum and have the advantage of well-established sampling protocols with low error variance (Garnier *et al.* 2001; Cornelissen *et al.* 2003). LMA, or the foliar mass per unit area (g m$^{-2}$), is part of the leaf economic spectrum and closely correlated to photosynthetic capacity, nitrogen content per mass and leaf lifespan (Wright *et al.* 2004). LDMC, or the ratio of a leaf's dry mass to its water-saturated mass (g g$^{-1}$), reflects the fundamental tradeoff in investing resources in structural tissues vs. liquid-phase processes and therefore has been argued to be the root variable governing correlations among the traits in the leaf economic spectrum (Shipley *et al.* 2006a).

### Data collection

To capture trait variation among sites, we sampled three old-growth lowland tropical forests located along the

precipitation gradient across the Isthmus of Panama: Parque Nacional Metropolitano (PNM: *c.* 1800 mm year$^{-1}$), Barro Colorado Island (BCI: *c.* 2600 mm year$^{-1}$), and Parque Nacional San Lorenzo (PNSL: *c.* 3000 mm year$^{-1}$) (Smithsonian Tropical Research Institute 2007). We chose the sites to assess the relative importance of climate on trait variability; site moisture balance and other indices of water availability are climate measures known to have a large influence on leaf traits (Reich *et al.* 1999; Wright *et al.* 2001, 2004, 2005). To measure variation among plots within a site, at each site we sampled 20 m × 20 m plots located 60–80 m apart from center to center: four in the 1 ha PNM permanent plot (due to the smaller size of site), eight in the 50 ha BCI permanent plot and eight in the 6 ha PNSL permanent plot. The plots were located in a systematic fashion along transects drawn through the center of the sites for BCI and PNSL and at the north, south, east and west sides of the canopy cranes at PNM and PNSL. The systematic establishment of plots at fixed intervals within a site means that no *a priori* selection was made to minimize or maximize plot heterogeneity, successional status or level of disturbance. Because each plot level measurement tallies all coexisting tree species, in this study, we use the plot level to represent a forest community in the sense of a set of species growing together and interacting in a locality. We acknowledge that different spatial scales can and have been used to measure plant communities and that our operational definition of community is one of many possible. To assess variation among tree species within a plot and among individuals within a species, we sampled six leaves from all trees of all species located in the plots for individuals with a diameter at breast height (dbh) greater than 10 cm. To capture the variation between strata within a tree and among leaves within a stratum, we collected three newly matured leaves from each of the sun and shade strata in each tree. To control for temporal variation in traits that occurs between seasons and years, all data were collected during the 2007 rainy season (September to December). Ontogenetic variance, which is associated to decline in photosynthetic capacity with leaf age, is another source of temporal variation that merits future study; due to logistic constraints we were not able to include this component in this study. Instead, we collected only the youngest fully expanded leaves to control for trait variation due to leaf development and aging. Samples were collected using a canopy crane in the four PNM plots and in four of the eight PNSL plots and using a shotgun in the eight BCI plots and in the remaining four PNSL plots. We sampled a total of three sites, 20 plots, 119 species, 322 trees and 1910 leaves (see comment in Appendix S1 for additional information on sample size). Measurements of the two leaf traits generally follow Cornelissen et al. (2003). The differences between our protocol and that suggested in this handbook are: (1) that

we sampled all trees with a dbh > 10cm of all species present in the plot instead of only the most healthy trees from the most abundant species; (2) we sampled from both the sun and shade strata, instead of only sun leaves; and (3) the leaf rehydration procedure was different. Extra precautions were taken to make sure all leaves were fully rehydrated before measuring the fresh weight. Because trees likely are in different hydration state in the field, it is crucial to get water-saturated LMA and LDMC values to have standardized trait values that are comparable among trees, plots and sites. To minimize water loss, the cut ends of harvested branches were immediately put in water-filled and sealed test tubes and then placed in an air-tight plastic bag. At the end of the field day, the base of each branch was re-cut under water and left immersed overnight to rehydrate in an air-tight plastic bag. Rehydration took place in the dark at ambient temperature for 12–20 h. The following day, three of the youngest, fully developed leaves were selected randomly on each branch.

## Data analysis

Using a restricted maximum likelihood (REML) method in the 'lme' function of R (version 2.6.1) and data normalized by $\log_{10}$ transformations, we fitted a general linear model to the variance across six scales nested one into another (i.e. nested ANOVA with random effects) in this increasing order: leaf, strata, tree, species, plot and site. A variance component analysis was performed on this model using the 'varcomp' function of R (R Development Core Team 2007). The code used in R to calculate the variance partitioning of the traits across the six nested ecological scales for the full model on LMA was:

```
varcomp.LMA<-varcomp(lme(log(LMA)~1,
random=~1|Site/Plot/Species/Tree/
Strata, data = d, na.action = na.omit),1)
```

The same model applies to LDMC, only replacing LMA by LDMC in the R code. The 'ape' and 'nlme' libraries are necessary to use the 'varcomp' and 'lme' commands. These analyses in R were cross-checked with Matlab code using a traditional Type I sum-of-squares (Gower 1963). As the results from Type I sum of squares and REML estimates were very similar, we report only the results from R/REML which is known to be unbiased (see Box 1 and Appendix S1 for further explanations of the nested ANOVA analyses and details on the codes used). While Bartlett's and Fininger's tests suggest that the assumption of homogenous variance among the members of a group are not met, we note that homoscedasticity matters in estimating *P*-values only, not for the calculation of the actual statistics – here the variance component (Zar 1996).

In general terms, in a nested ANOVA the variance components represent the variances around the means.

**Box 1** Explanation of the analyses and interpretation of nested ANOVAS with random effects

To clarify the analysis, Table 1 presents the ANOVA table for a Type I sum of squares analysis (Gotelli and Ellison 2004) in a simplified (3 levels, balanced) version of the actual (6 levels, unbalanced) analysis performed.

**Table 1** Nested ANOVA table for a Type I sum of squares analysis with 3 levels and a balanced design

| Source | Sum squares, SS | degrees of freedom, d.f. | Mean squares, MS | Expected MS, E(MS) |
|---|---|---|---|---|
| Among leaves within strata (includes measurement error) | $SS_L = \sum_{t=1}^{T}\sum_{s=1}^{S}\sum_{l=1}^{L}\left(Y_{lst}-\overline{Y_{st}}\right)^2$ | $d.f._L = T*S*(L-1)$ | $MS_L = \frac{SS_L}{d.f._L}$ | $E(MS)_L = \sigma_L^2$ |
| Between strata within trees | $SS_S = L\sum_{s=1}^{S}\left(\overline{Y_{st}}-\overline{Y_t}\right)^2$ | $d.f._S = T*(S-1)$ | $MS_S = \frac{SS_S}{d.f._S}$ | $E(MS_S) = \sigma_L^2 + L\sigma_S^2$ |
| Among trees within total | $SS_T = LS\sum_{t=1}^{T}\left(\overline{Y_t}-\overline{Y}\right)^2$ | $d.f._T = T-1$ | $MS_T = \frac{SS_T}{d.f._T}$ | $E(MS_T) = \sigma_L^2 + L\sigma_S^2 + LS\sigma_T^2$ |
| Total | $SS_{Total} = \sum_{l=1}^{L}\sum_{s=1}^{S}\sum_{t=1}^{T}\left(Y_{lst}-\overline{Y}\right)^2$ $= SS_L + SS_S + SS_T$ | $d.f._{Total} = T*S*L-1$ | $MS_{Total} = \frac{SS_{Total}}{d.f._{Total}}$ | $E(MS_{Total}) = \sigma_{Total}^2$ |

Here, a trait $Y_{lst}$ is measured on $l = 1\ldots L$ leaves per stratum, $s = 1\ldots S$ strata per tree and $t = 1\ldots T$ trees. Mean values are taken at each level so:

$$\overline{Y_{st}} = \frac{1}{L}\sum_{l=1}^{L}Y_{lst} \tag{1}$$

is the mean value of strata $s$ of tree $t$, averaged over all the leaves within the strata of that tree (strata–tree). Similarly,

$$\overline{Y_t} = \frac{1}{LS}\sum_{s=1}^{S}\sum_{l=1}^{L}Y_{lst} \tag{2}$$

is the mean value of tree $t$, averaged across all leaves of both strata of that tree. Finally,

$$\overline{Y} = \frac{1}{LST}\sum_{t=1}^{T}\sum_{s=1}^{S}\sum_{t=1}^{T}Y_{lst} \tag{3}$$

is the grand mean, i.e. the mean value of all leaves of all strata of all trees.

As is always the case in ANOVA, we equate the Mean Square (MS) calculated from observed data, MS = SS/d.f., to the expected MS or E(MS) and use this equation to calculate estimators for variances $\sigma_?^2$. The top row of Table 1 is used to estimate the variance among leaves within one strata-tree, $\sigma_L^2$, as: $\sigma_L^2 = MS_L = SS_L/d.f._L$. With $\sigma_L^2$ estimated and $L = 3$ known, we can then quickly get an estimator for the variance at the strata level, $\sigma_S^2$, by $MS_S = E(MS_S) = \sigma_L^2 + L\sigma_S^2$ or solving for $\sigma_S^2$, gives $\sigma_S^2 = (MS_S - \sigma_L^2)/L$. We can make sense of the formula $E(MS_S) = \sigma_L^2 + L\sigma_S^2$ by noting that by definition

$$E(MS_S) = MS_S = \frac{L\sum_{s=1}^{S}\left(\overline{Y_{st}}-\overline{Y_t}\right)^2}{T*(S-1)} = \sigma_L^2 + L\sigma_S^2 \tag{4}$$

If we divide both sides by $L$ we get:

$$\frac{\sum_{s=1}^{S}\left(\overline{Y_{st}}-\overline{Y_t}\right)^2}{T*(S-1)} = \sigma_L^2/L + \sigma_S^2 \tag{5}$$

Thus, the variance of strata-level observed values around tree mean value (left hand side) is the sum of the variance between strata ($\sigma_S^2$) plus the variance due to using a strata-level value that is a mean across leaves ($\sigma_L^2/L$) because the $var(\overline{Y}) = var(Y)/N$. This calculation of variances at a given level $\sigma_?^2$ using $E(MS_?)$ and estimated variances $s_?^2$ at lower levels can continue up the hierarchy. Variance components (the percentage of the total variance found at a level) can then be calculated by the simple formula, say for strata, of $vc_S = s_S^2/(s_L^2 + s_S^2 + s_T^2) = s_S^2/s_{Total}^2$. Note that as in any nested ANOVA, the bottom variance $\sigma_L^2$ captures both variance between leaves and variance due to measurement error. The actual details are slightly more complicated to deal with six levels and the unbalanced design (Gower 1963).

**Table 2** Variance partitioning of the full nested linear models on Leaf Mass Area (LMA) and Leaf Dry Matter Content (LDMC) across six nested ecological scales. All data were log$_{10}$ transformed prior to analysis. n = 1910 leaves. Square brackets represent the 95% confidence intervals, which were calculated by bootstrapping (500 runs with 1910 randomly sampled data points with replacement)

| | % variance of trait [95% C.I.] | |
|---|---|---|
| Ecological scale | log LMA | log LDMC |
| Leaf and error | 10 [7–9]* | 15 [9–15]* |
| Strata | 16 [16–21] | 16 [12–26] |
| Tree | 22 [19–28] | 17 [12–30] |
| Species | 21 [16–26] | 35 [25–43] |
| Plot | 0 [0–0] | 0 [0–0] |
| Site | 30 [27–32] | 16 [13–19] |

*See comment 1 in Appendix S1.

So, for any given level (e.g. the species), we first calculate each group mean (e.g. the mean value of each species), then we calculate the variance of these group means around the group mean of the higher level to which they belong (e.g. the variance of the species means around the mean of their plot).

Trait differences between plots may be due to differences in environment or differences in species composition, with the latter known to be high in tropical forests due to high species diversity. To quantify species turnover, we calculated the mean Sorenson ($L$) and Chao-Sorensen-Raw Abundance-Based ($L_{abd}$) index values between plots for each site and between sites (Chao *et al.* 2005). Because of the high species turnover between plots, we also built an alternative model that left out the species level to verify that variation in foliar traits at the species level was not confounded with variation at the plot level (See Appendix S1). Species could have been modeled as either crossed or nested factors, but as shown below this variance component is closer to nested than crossed due to the very high turnover in species composition in tropical forests. In addition, an analysis with the factors crossed instead of nested gave similar results as shown in Table S2.

### RESULTS

The partitioning of variance in LMA and LDMC reveals fairly balanced distributions of variance across five of the six ecological scales. However, the plot scale stands out as containing none of the total variance in either foliar trait (Table 2, Fig. 1 and Figure S1). The results for the two traits are similar, except for a minor difference in the scale at which most variation occurs: sites account for 30% of total variance for LMA, but species for 35% of total variance in LDMC.

The $L$ and $L_{abd}$ similarity indices measuring species turnover across the study sites indicate that the precipitation gradient across which we sampled is strong enough to have major effects on species composition: between PNM and BCI $L = 0.02$ and $L_{abd} = 0.03$; between BCI and PNSL $L = 0.26$ and $L_{abd} = 0.22$ and between PNM and PNSL $L = 0.00$ and $L_{abd} = 0.00$. The average similarity indices between plots in a site are: for PNM $L = 0.33$ and $L_{abd} = 0.40$; for BCI $L = 0.13$ and $L_{abd} = 0.21$ and for PNSL $L = 0.23$ and $L_{abd} = 0.36$. In other words, at each site only a small percentage of species co-occur between neighbouring plots. The average species richness is 46 ($\pm$ 29) per site and 12 ($\pm$ 4) per plot. The average density of trees with a dbh > 10cm is 109 ($\pm$ 55) per site and 16 ($\pm$ 6) per plot.

To check that the analysis was successfully differentiating plot level variation from species level variation, which could have been confounded due to some albeit small overlap in species composition among plots in a site, we ran the same variance partitioning analysis with the species scale removed. This alternative model results in essentially the same pattern of variance distribution found in the full model, except that in the alternative model the variance component at the tree level increased to include the variance that was originally attributed to species in the full model (Table S1). The results from the analyses with the species level crossed (Table S2) show that crossing or nesting the species level does not affect the overall pattern of variance partitioning.

### DISCUSSION

The patterns in the variance components of these two key foliar traits are rich in information in many respects, and some of the findings from these results are quite surprising or contrary to conventional wisdom. There are three main elements that we would like to emphasize: (1) trait variance is fairly evenly distributed across all scales (except the plot level), (2) the total amount of within-species variation is roughly equal to the amount of interspecific variation, and (3) the plot level is responsible for only a minute percentage of the total variance.

First, the relatively uniform distribution of the variance among five of the six ecological scales – i.e. leaf, tree, strata, species and site – suggests that processes at all five scales are equally important in determining trait values. The main ecological and evolutionary processes driving variation at different scales are sketched in Fig. 2. These results indicate that all components of variation ought to be taken into account in any one study, although in practice this is often not feasible. Most studies discount variation occurring at levels other than the focal study level. Yet in some cases, we can expect that the variation occurring at different levels will affect the results obtained and the conclusions drawn. For
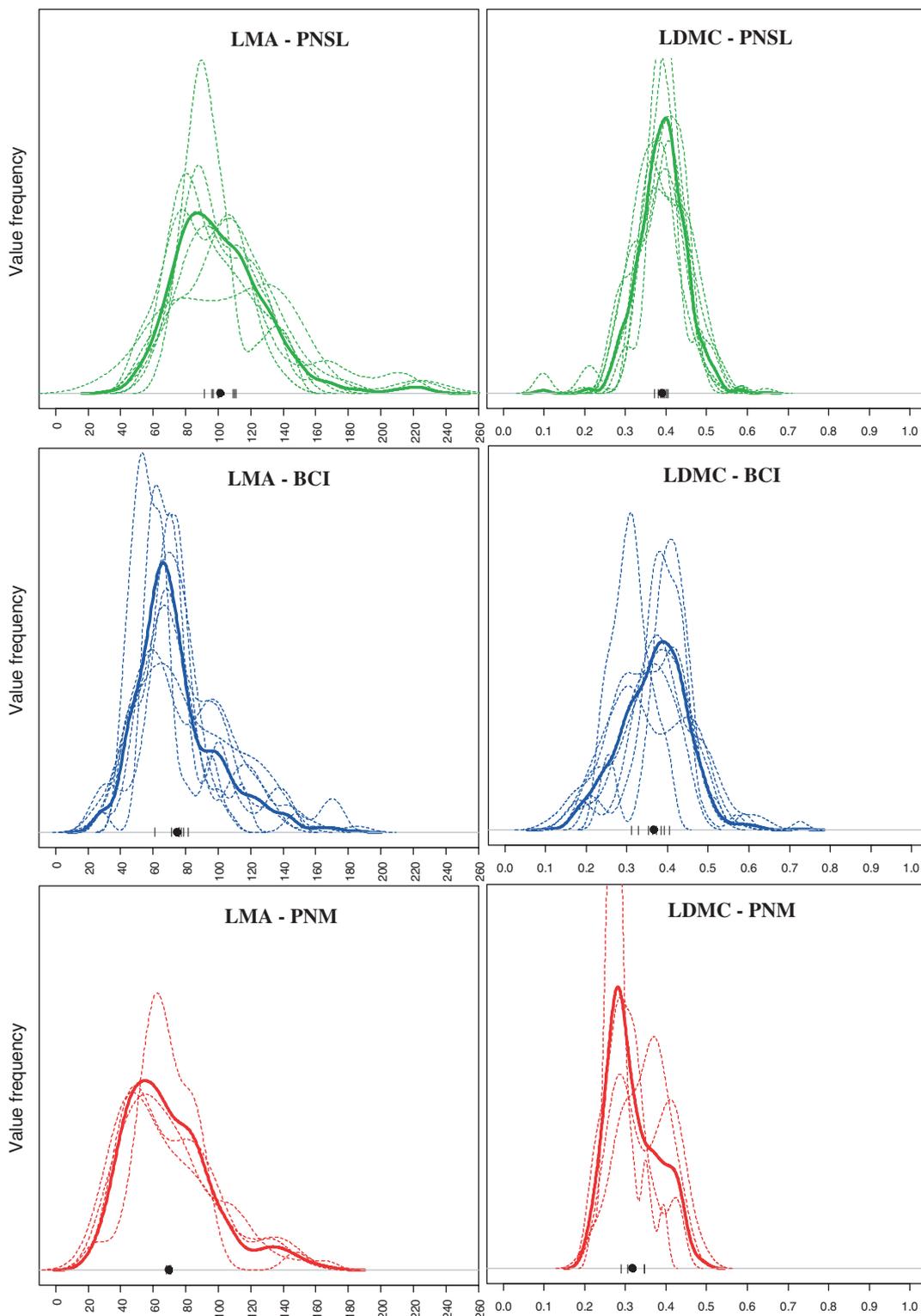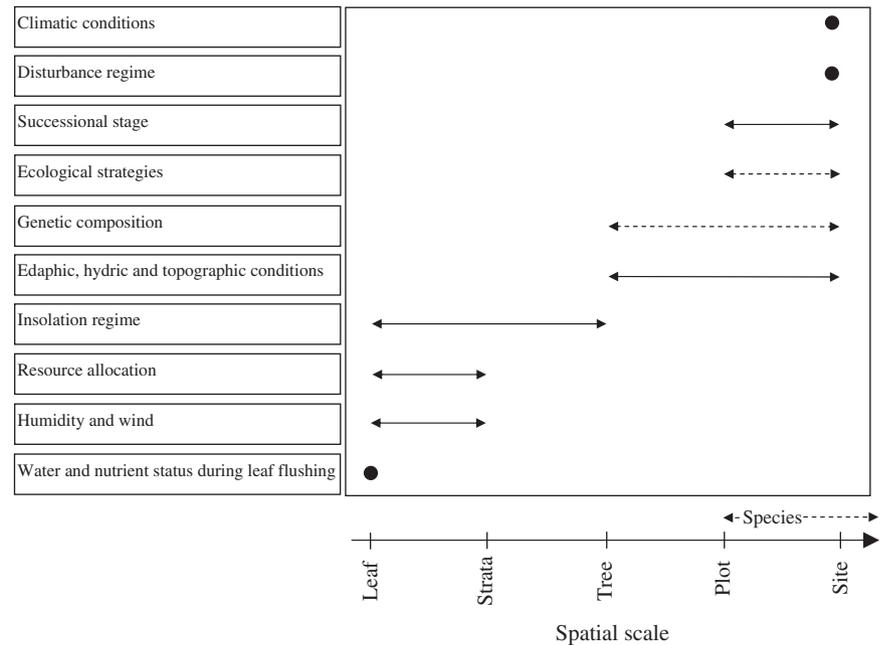
**Figure 1** Frequency distributions of LMA and LDMC value of individual leaves for each site. The solid lines represent the sites. Dashed lines represent the individual plots in each site. The site mean values are shown by a bullet point (•) on the abscissa and the plot mean values by a tick mark (|). These graphs show that plots within a site have not only similar means trait values, but also similar trait value frequency distribution, i.e. the overall trait distributions is conserved at the plot level.

**Figure 2** Processes affecting trait variation and their scale of effect. The abscissa represents the spatial scale and the ordinate lists the processes. The scale of species is drawn using a dashed line and overlaps the plot and site levels. The processes affecting a spatial scale via its effects on species are also drawn using a dashed line.

example, in the study by Shipley *et al.* (2006b), we can wonder if the assembly rule found to determine local species composition would be so deterministic had the amount of variation within a species been considered. It is critical to decide which components of variation should be taken into account considering the ecological question addressed. A deeper understanding of the pattern of variation across scales of different traits and in different ecosystems appears to be a *sine qua non* requirement to making informed decisions. More studies such as this one but in more diverse ecosystems and with different traits need to be carried out.

Second, the approximately equal distribution of variance in traits within and among species may well be contrary to prevailing assumptions. For LMA, the fraction of total variance within species (the sum of all the variation occurring at levels below species, i.e. leaf + error, strata and tree levels) is 48%, much greater than the strictly interspecific fraction of 21%. For LDMC, the within-species fraction of total variance also is 48% compared with the strictly interspecific fraction of 35% (Figs 1 and 2 and Figure S1). The relative abundance of intra- and interspecific variation remains for both traits when analyses are performed on sun and shade leaves separately (Table S3). High levels of plasticity in these traits have been described in the past (Valladares *et al.* 2000) and indeed are often controlled for in comparisons among species (Weiher *et al.* 1999; Cornelissen *et al.* 2003; Wright *et al.* 2004), but this study, the first designed to compare variation across a wide range of scales, clearly shows that within-species variation is a significant, not minor, component of the overall variance in these traits.

These results suggest that for these two key foliar traits many species can be functionally redundant in tropical forests. The finding that the amount of within-species variation is similar to interspecific variation does not mean that species are indistinguishable from one another based on these traits, but that their overlap is generally larger than the difference between their means. Yet, if these traits show more variation within than among species, this raises the question of when (for which questions and in what contexts) it is desirable to work with the mean functional trait values of different species (Table 2, Fig. 3). It is becoming clear that the amount of variation around the mean trait value of a species can be as important as the mean itself, so both the mean and standard deviation of trait values should be reported and interpreted. Ecologists increasingly appreciate that within-species variation can have consequences for community dynamics and structure (Bolnick *et al.* 2003; Clark *et al.* 2004; Clark 2005).

As a corollary to the importance of considering variance in a trait, it is important to carefully characterize the nature of the mean values of traits when reporting or collating data. For example, while it is legitimate and often necessary to control for variation in a trait such as LMA by measuring only sun leaves, it would be preferable to refer to these measurements as $LMA_{max}$ or $LMA_{sun}$ to avoid confusion with the mean $LMA_{tree}$ determined across all canopy strata, because the information held in these two traits measurements and the conclusions that can be drawn from them are quite different.

Our third and most important finding is that the plot level accounts for essentially none of the total variance in
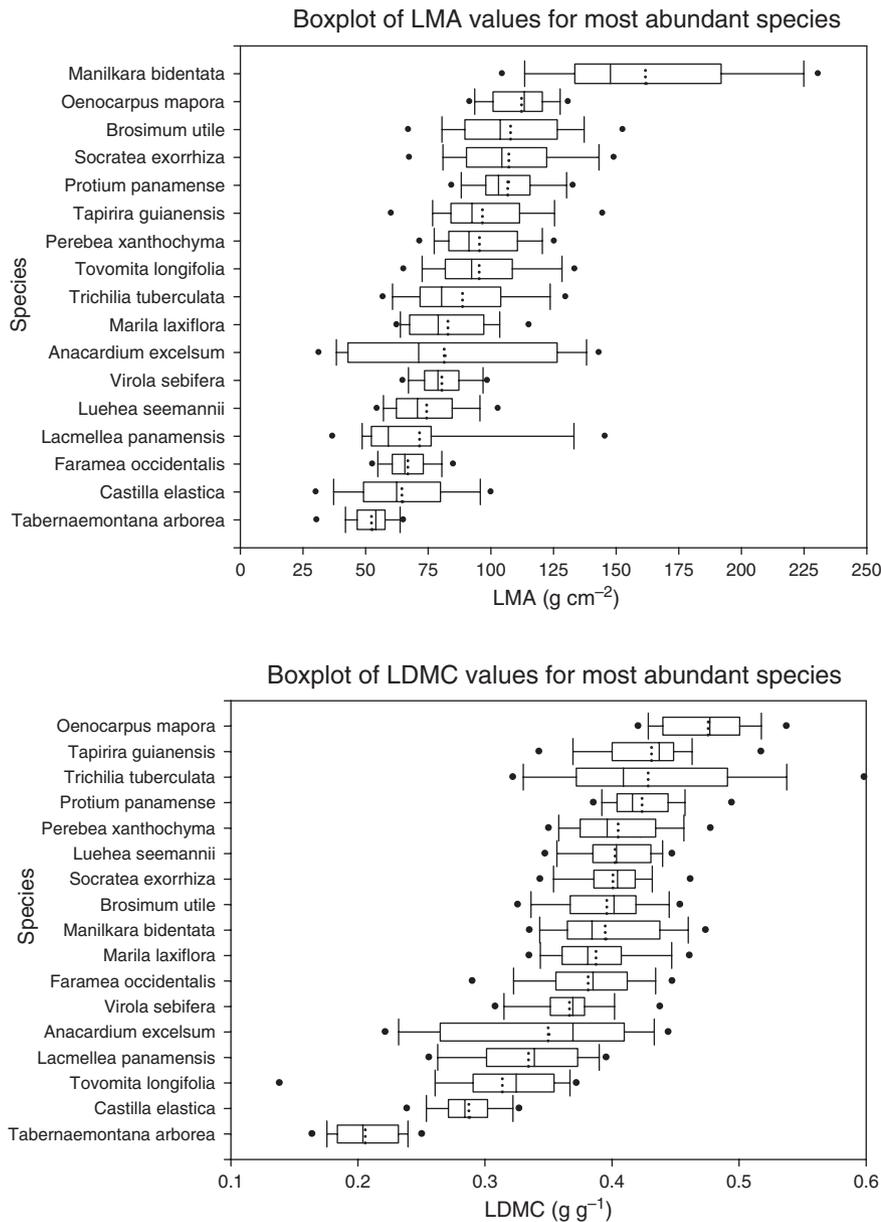
### Boxplot of LMA values for most abundant species



### Boxplot of LDMC values for most abundant species



**Figure 3** Box plot of LMA and LDMC for the 17 most common species in our dataset. Only species for which five trees or more were sampled are illustrated. Dotted line is the species' mean trait value. While the sample size is not uniform amongst species, there is no correlation between species' sample size and variance (LMA: $P = 0.18$, d.f. = 15; LDMC: $P = 0.42$, d.f. = 15), nor is there a correlation between mean species value and variance (LMA: $P = 0.93$, d.f. = 15; LDMC: $P = 0.69$, d.f. = 15).

LDMC and LMA. We have ruled out the possibility that this result could be an artefact of imperfect nesting of species within plots by considering alternative partitioning models. The first one with species removed (Table S1) also finds no variance at the plot level and the second one that crosses the species level with the other scales gives equivalent results as the analysis with the levels nested (Table S2). Figure 1 illustrates that the plots at a site not only share a common mean trait value but also a similar distribution of trait values. The negligible amount of variation in these individual traits between neighbouring plots despite their very different species composition suggests a filter operating on the overall distribution of trait values within a site. If true, then the presence of an individual tree within a local community is controlled via a filter operating on functional traits rather than on species identity *per se*. This filter on functional trait values will secondarily act as a coarse filter on species assemblage because, as shown by our results, each species has a substantial but finite degree of variability in its expression of trait values. Given this high functional redundancy among species (fig. 15, Clark *et al.* 2004), many different combinations of species and individuals can produce the overall distribution of trait values at a given site. If, for example, a given stand shows a deficiency in the conserved trait distribution, a species that dispersed into that stand may or may not 'fit' into the assembling

community – according to the degree that it can plastically shift its trait values – and match the deficient part of the conserved trait distribution at the site. Conversely, many different species might be able to 'fit' into the community either because the central tendency of a species for a given trait corresponds to the required trait value, or because the level of trait variability of the species is high enough to allow it to take that trait value.

This suggested explanation for the observed scaling of LMA and LDMC follows the multiple alternative designs hypothesis proposed by Marks & Lechowicz (2006), whereby many different combinations of traits can produce equivalent fitness in a given set of environmental conditions. Alternatively, the observed pattern might arise in species-specific adaptations to heterogeneous microenvironments that lead to different optimal trait values within any one habitat. It would be surprising, however, that the influence of local heterogeneity would be so consistent across plots and sites. A third mechanism that might lead to this pattern is a traditional view of environmental filters, which supposes that abiotic filtering at a locality favours a single optimum, but that biotic interactions create spread around that central tendency. It seems unlikely, however, that the balance of abiotic and biotic environmental regulation would be so uniform across plots and sites. Whatever the underlying mechanisms affecting species assembly in plant communities may be, it is clear in our data that the distribution of trait values can be conserved at the plot level.

It is interesting to examine the implications of our findings for neutral theory (Hubbell 2001). The presence of a strong filter determining trait composition in a plot supports niche theory by showing that that deterministic, adaptive processes drive community assembly. However, the fact that this filter acts on traits irrespective of species also brings support to a central aspect of the neutral theory, which argues that species identity per se does not matter. This suggests that the resolution of the debate between niche and neutral theory will not happen until we move beyond the species-centered perspective.

The idea that species assemblage of a local community should be determined by some sort of environmental filters acting on traits has been suggested previously (Janzen 1985; Keddy 1992; Weiher & Keddy 1995; Diaz et al. 1998; Lavorel & Garnier 2002) and in a few cases demonstrated (Shipley et al. 2006b; Cornwell & Ackerly 2009), but our results are unique in: (1) showing that species identity alone is not the critical factor in the filtering process, (2) showing that the entire distribution of community trait values is conserved, not just the community mean trait value, (3) explicitly comparing variation among plots within a site to variation among sites and among species to benchmark the relative strength of the processes driving trait variation, and (4) showing at precisely which scale (plot) that the critical

filter is occurring. Perhaps the most surprising aspect of these results is that environmental filtering can be so strong on the overall distribution of a trait, yet influence species composition so little because the relative amount of within-species trait variation is large. These are remarkable results, but we should consider how consistent they are likely to be, given changes in the sampling design: (1) other traits, (2) other life forms and (3) other environments.

With regard to traits, the fact that the pattern of variance partitioning across scales is largely consistent between LMA and LDMC supports the proposition that both traits reflect fundamental processes that underlie positioning along the leaf economic spectrum (Shipley et al. 2006a). In this dataset measured at the leaf level, we found the correlation coefficient between LMA and LDMC to be 0.34. Although the variance partitioning across scales is broadly similar between the two traits, LMA has a higher site level variance than LDMC (30% vs. 16% respectively) and lower species level variance (21% vs 35%, respectively). This suggests that LMA is more strongly influenced by water availability and other environmental variables whereas LDMC is relatively more fixed by the evolutionary history of a species, which is consistent with earlier analyses of the functional and evolutionary relationships among traits in the leaf economic spectrum (Shipley et al. 2006a). Given this difference even between two highly integrative traits that represent key elements of production capacity, we should not expect a similar analysis performed on traits less central to plant function to show variance components indicating trait-based filtering leading to community assembly. With regard to life form, it is likely that the variance partitioning will depend on the range of life forms and growth forms sampled. This study looked specifically at trees and we can expect a study including a larger spectrum of growth forms such as for example shrubs, epiphytes, lianas or ferns to produce different variance partitioning patterns. Note also that the results are influenced by the sampling intensity at the different level of the hierarchy. Increasing the number of leaves sampled within a branch would either leave the leaf level variance unchanged or increase it (thereby strengthening our conclusions on the importance of intraspecific variation). Finally, in terms of the generality of these results across disparate environments, habitats and biomes, we recognize that the strength of the environmental factors acting at different spatial scales vary across different biomes. In this study, the environmental gradient in Panama and the high levels of alpha- and beta-diversity in the tropics may underpin our results. Whether this is the cause or consequence of the observed variance component pattern is unclear, but it is clear that additional, well-designed studies clarifying the links between species richness, variance component patterns and the underlying evolutionary and ecological processes driving trait variation will be a key to

understanding community assembly and dynamics. This study is the first step in that direction and it will be interesting to see whether future studies working on different traits and in different biomes give comparable results.

If the results of this hierarchical scaling of variance in foliar function prove to hold more generally, the existence of a leaf trait filter on community assembly will encourage some exciting lines of inquiry. The suggestion that the distribution of traits associated with the leaf economic spectrum is regulated in a site invites us to identify other major ecological strategy dimensions and to verify whether these axes of variation are subject to comparable environmental filtering, thereby further controlling community assembly. Given the evenness of the variance partitioning patterns for both LMA and LDMC, it is clear that ecologists working with traits at the species level could benefit from the wider perspective provided by considering within-species variation. More generally this study demonstrates that the ecological scaling of variance is highly valuable and informative for many disciplines – the structure of variance across ecological scales should be a concern regardless of the traits and problems under investigation. While the mean values of traits probably matter ecologically, they only reveal their full significance when set in the context of their variance. Most importantly, our findings remind us that ecologists interested in community assembly have much to gain by shifting their focus from species to traits (Lavorel & Garnier 2002; McGill et al. 2006).

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## SUPPORTING INFORMATION

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

**Appendix S1** Supplementary material.
**Figure S1** Stacked bar chart of LMA and LDMC variance partitioning across six nested ecological scales.
**Table S1** Variance partitioning of an alternative nested linear model of LMA and LDMC across five ecological scales (Leaf, Strata, Tree, Plot and Site), leaving out the species scale.
**Table S2** Variance partitioning of an alternative nested linear model of LMA and LDMC across six ecological scales, with the species level crossed.
**Table S3** Variance partitioning of alternative nested linear models of LMA and LDMC on the sun and shade leaves separately.

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