Functional Traits and Grain Size in Species Assemblages of a Neotropical Dry Forest

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A Thesis Submitted to The Graduate School at the University of Missouri- St. Louis in partial fulfillment of the requirements for the degree
Master of Science in Biology

December 2011

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Abstract

A long-standing question in ecology is how so many tree species can coexist. New insight into assembly processes has been gained through functional traits that influence fitness. Such traits include the maximum height and diameter of a species as these describe the plant's ability to compete for light. Additionally, specific leaf area (SLA) describes the amount of leaf area for light capture per unit of biomass invested. SLA is one of the easiest traits to measure, but it depends on access to fresh leaf material which is not possible for samples from remote areas or historical collections e.g. herbaria. The study examined community assembly patterns based on species functional tratis in a species-rich tropical dry forest at the Madidi National Park (MNP) of Bolivia.

In my first chapter, a protocol to predict SLA for fresh leaves from dry leaves was developed. On the same leaf, area was repeatedly measured for fresh and dried leaves to generate four general mixed effects models, varying in their inclusion of the position in the crown where the leaf develops. The accuracy of the models was tested on leaves from an oak-hickory forest in USA. Both models performed well and are readily applicable to other datasets. A protocol for studies predicting SLA from dry leaves was developed.

In my second chapter, I investigated the distribution of trait values at plots of different sizes to understand processes that lead to different species assemblages. Deterministic (habitat filtering and competitive exclusion) and stochastic processes are potential drivers of species coexistence in assemblages. The importance of these non-exclusive processes in structuring assemblages at different scales remains unclear. I compared the trait dispersion of SLA (using models from chapter 1), maximum height, and maximum diameter of observed versus null species assemblages with metrics sensitive to deterministic processes. I found evidence for deterministic processes structuring species assemblages in the MNP. Competitive exclusion had greater importance at small grain sizes. Habitat filtering had greater importance at large grain sizes. Ecologically, the results indicate that stabilizing processes promote patterns of species diversity and co-existence in a species-rich tropical dry forest in Bolivia.

SPECIFIC LEAF AREA: A PREDICTIVE MODEL USING DRIED SAMPLES

INTRODUCTION

Plants allocate limited resources (e.g., carbon and nutrients) in the construction of leaves. Leaves in turn pay returns on this investment by harvesting energy from sunlight. Plants depend on this energy gained to maintain metabolic processes and build vegetative and reproductive organs (Wright et al. 2004). Biotic and environmental factors should provide strong selection to optimally allocate resources for light capture (Markesteijn 2010), a suboptimal allocation would lead to declining plant fitness. Leaf construction varies within and among individuals and species. This variation is driven in part by environment (e.g., variation in latitude, altitude, soil fertility, water availability, canopy height, light availability; Bongers and Popma 1990, Whitmore 1996, McDonald et al. 2003, Ackerly 2004, Sack et al. 2006), as well as phylogenetic background of the species. For instance, leaves exposed to direct sunlight are often small and thick with low surface to leaf mass and high photosynthetic capacity (Björkman 1981, Klich 2000, Rozendaal et al. 2006, Markesteijn 2010, Hulshof and Swenson 2010), while shade leaves are large and thin with low leaf mass to surface area (Evans and Poorter 2001, Rozendaal et al. 2006, Hulshof and Swenson 2010).

Variation in allocation strategies can be understood through the measure of morphological and physiological characteristics thought to influence plant performance, often denoted as functional traits (Grime 1979; Tilman 1988, Westoby et al. 2002, McGill et al. 2006). A series of leaf traits describing leaf allocation patterns and physiological function, known as the leaf economics spectrum, has shown tight coordination (Wright et al. 2004). These leaf traits include specific leaf area (or its inverse = leaf mass per area), photosynthetic capacity, nitrogen and phosphorous content, dark respiration rate, and lifespan (Wright et al. 2004). The leaf economics spectrum runs from quick to slow returns on investment of nutrients and dry mass (Wright et al. 2004). Species with high leaf nutrient concentrations, high photosynthetic and respiration rates, short leaf lifespan, and low dry mass per leaf area, are at the quick returns end of the spectrum, with the converse being true of species at the slow returns end of the spectrum (Coley et al. 1985, Choong et al. 1992, Ryser 1996, Reich et al. 1997, Reich 1998, Garnier *et al.* 2001, Wright et al. 2004).

Among the traits in the leaf economics spectrum, specific leaf area (SLA, ratio of fresh leaf area to dry mass) is one of the easiest to measure and can readily be determined for numerous species. SLA describes the amount of leaf area for light capture per unit of biomass invested. While the standard protocol for measuring SLA is simple, it requires access to recently collected sun-exposed leaves to determine fresh leaf area (Cornelissen et al. 2003). This recommendation limits the types of samples that can be used to measure SLA. For instance, previously collected and dried leaves, such as herbarium specimens, cannot be used. Additionally location within the crown of the plant can influence SLA values, with lower crown leaves typically being larger and having higher SLA values than upper crown leaves (Sack et al. 2006). However, information about collection location within a crown is seldom recorded for herbarium specimens.

Given the substantial ecological information that can be obtained by studying SLA and the limitation imposed by the protocol, the objectives here are to 1) develop models to predict SLA from dried samples that can extend the temporal, geographical, ecological and taxonomic scope of the technique allowing us to collect data from dried samples (e.g., stored in herbaria), 2) test the generality of these predictive models, and 3) propose the application of the models as a field standard method.

I hypothesized that 1) SLA values based on area measurements obtained from dried samples will be smaller than the respective SLA values based on area measurements obtained from fresh samples. This expectation follows from the fact that approximately 70% of a leaf's mass is water (Hopkins 1999), and therefore leaf area will be reduced after the leaf is dried. 2) As SLA is known to vary with environment (Bongers and Popma 1990, Whitmore 1996, Ackerly 2004, Sack et al. 2006, Rozendaal et al. 2006), then an accurate predictive model for SLA using dried samples should have as covariates information about the environment where the leaf developed (such as position of the leaf in the crown).

I developed four models to test the relationship between SLA from fresh and dried leaves, where leaf areas were measured on the same leaf. All sample leaves originated from a dry forest in northern Bolivia. I further tested the generality of the models with an independent dataset from trees in a Missouri, USA oak-hickory forest.

METHODS

Study site

The bulk of the present study was carried out in a dry forest in the Madidi National Park (MNP) in Northeastern Bolivia. The dry forest in the MNP is 1418 km^2 (Killeen et al. 2005) situated within the Tuichi river watershed, with an elevational gradient ranging from 600 to 1500 m. The region is characterized by having a single wet and a dry season per year, with three extremely dry months from June to August. It has a mean annual temperature of 20.5°C (Navarro 2002) and annual precipitation that varies between 1200 to 1400 mm (Müeller et al. 2002). The project "Floristic inventory of the Madidi region" established 16 1-ha plots in 2005. To examine questions of influence of the drying process on SLA, four of these plots were selected. These plots had high species richness and varied in floristic composition. They were also the most accessible logistically. The plots are located in Resina (14°20'0.5''S 68°34'20.6''W, 1034 m), Chirimayu (14°14'47.5''S 68°35'8.6''W, 850 m), Chaquimayu (14°15'8.7''S 68°31'9.1''W, 795 m), and Buena Hora (14°11'55.5''S 68°38'23.4''W, 1150 m).

Sampling methods

To make my predictive models broadly applicable, I sampled many species (n = 102), with 8 replicates within a species (dependent on availability) and 2 samples per individual. To capture the greatest amount of intra-crown plasticity (Rozendaal et al. 2006, Sack et al. 2006, Hulshof and Swenson 2010), from each individual, I collected one leaf from the top and one leaf from the bottom of each crown (i.e., sun and shade leaves within a given crown). Within each plot, all species that had tagged individuals with accessible crown leaves (via tree climbing) with minimal symptoms of pathogens or covered by epiphylls (lichens, fungi, liverworts) were sampled.

I harvested leaves that were fully expanded and mature with no obvious signs of senescence. Top crown leaves were collected from branches most exposed to sunlight, and bottom crown leaves were collected from lower crown shade branches. In each plot, one to eight individuals per species were sampled for a total of 541 individuals, 1082 leaves from 102 species across the four plots.

Petioles were included in leaf measures. In the case of compound leaf species, one leaflet was harvested and treated as a leaf, since a leaflet is functionally equivalent to a simple leaf (Bongers and Popma 1990, Kraft et al. 2008, Baroloto et al. 2010, Lebrija et al. 2010). To obtain fresh leaf areas, the top and bottom crown leaves were flatted together, if their size allowed, between Plexiglas sheets with a scale bar and photographs were taken. All leaves were then placed in envelopes and treated as if they were samples collected as

herbarium specimens. The leaves were pressed and dried with field stoves. Once the leaves were dried, a second photo was taken to obtain dry leaf area following the same procedures as when the leaves were fresh. Finally the leaves were placed in an oven for 24 h at 60°C and weighed to obtain dry mass. Dry mass measures were taken at the Institute of Ecology at the San Andrés University (La Paz, Bolivia). Leaf area was calculated from the digital photos of fresh and dried leaves with the program ImageJ (http://rsbweb.nih.gov/ij/). Additionally, I measured with calipers for both fresh and dried leaves, leaf thickness at the midpoint of the leaf between major veins. Two SLA values were obtained for each collected leaf, one using the fresh leaf area and dividing it by its dry mass (= SLA_{fresh}), and the second using the dried leaf area and dividing it by its dry mass (= SLA_{dry}).

Model fitting

SLA_{fresh} values in the dataset ranged from 0.005 m^2g^{-1} (*Calliandra chulumania* Barneby, Fabaceae) to 0.02 m^2g^{-1} (*Phyllostylon rhamnoides* (J. Poiss) Taub., Ulmaceae). My data cover most of the range of SLA_{fresh} values (0.0007 m^2g^{-1} to 0.07 m^2g^{-1}) sampled around the globe (GLOPNET; http://www.bio.mq.edu.au/~iwright/glopian.htm). For analyses, the data were log_{10} transformed. I ran standard major axis (SMA) regressions using the package 'smatr' in the R programming environment (R Development Core Team 2011, http://www.r-project.org/) to determine the correlation between fresh leaf area and dry leaf area, and between SLA_{fresh} of top crown leaves and SLA_{fresh} of bottom crown leaves.

To examine different models to predict SLA_{fresh} from SLA_{dry}, I used the linear mixed effects (LME) function available in the R package 'lme4'. LME models present a statistical framework that allows simultaneous incorporation of fixed effects (SLA_{dry} and crown position) that I hypothesized *a prioiri* to influence SLA_{fresh}, as well as random effects (species and individuals) that may influence values of SLA but are not the focus of the current study. Another advantage of using LME models is that they allow for unbalanced datasets (e.g. different sample sizes of individuals within species).

Four models were used to predict SLA_{fresh}. The variables included in the models were discrete (position of the leaf in the crown, individuals, and species) and continuous (SLA_{dry}). The discrete variables were nested. SLA_{dry} was a fixed effect in all models. The crown leaf position was treated as a dummy variable (1 = top crown leaf and 0 = bottom crown leaf) that was either considered as a fixed or random effect depending on the model, and furthermore, considered as a fixed effect to generate a predictive model for SLA_{fresh} to be applied on dried samples where the position of collection within the crown is known. On the other hand, it was considered as a random effect to generate a model to predict SLA_{fresh} for those dried samples that do not have information about where in the crown they were collected. Species and individuals were considered as random factors in all models.

Models 1 and 2 were constructed to predict SLA_{fresh} from dried samples that do not require information about where in the crown the leaves were collected (Table 1). Model 1 assumes that to accurately estimate the parameters of the model, position of the leaf in the crown should be added as a random effects term because it influences the variability of leaf traits and as a consequence produces variation in SLA (Cornelissen et al. 2003, Rozendaal et al. 2006, Sack et al. 2006, Hulshof and Swenson 2010). This model has added species as a random effect because SLA_{fresh} values have a high interspecific variation (Hulshof and Swenson 2010). Model 2 applies the same scenario as model 1 but has added individuals as a random effect assuming that incorporating intra-species variation of SLA_{fresh} in the model will increase accuracy. Models 3 and 4 were built to predict SLA_{fresh} from dried samples that have information about where the leaves were collected in the crown (Table 1); position of

the leaf in the crown in both models was included as a fixed effect. Species was added as a random effects term in both models, and model 4 included individuals as a random effect.

Table 1. Candidate models. SLA_{fresh} = fresh leaf area/dry mass (m^2 g^{-1}), SLA_{dry} = dry leaf area/dry mass (m^2 g^{-1}), LP = leaf position in the crown, species = names of species, and individuals = number of individuals. The asterisk denotes that the variable was considered a random effects term in the model.

Model 1	$\log SLA_{fresh} = a + b\log SLA_{dry}$ + species* + LP*
Model 2	$\log SLA_{fresh} = a + b\log SLA_{dry}$ + species* + individuals* + LP*
Model 3	$\log SLA_{fresh} = a + b\log SLA_{dry} + c$LP + species*
Model 4	$\log SLA_{fresh} = a + b\log SLA_{dry} + c$LP + species*+ individuals*

Model selection

Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC) (Akaike 1974) were used for model selection. AIC is well suited for situations where the predictive capacity of the model is important, because AIC evaluates the likelihood of each model in the set, taking into account how well it fits the data, but also penalizing for adding model parameters (Burnham and Anderson 2002, Hilborn and Mangel 1997). BIC is a criterion for model selection that is based on the likelihood function. BIC also introduces a penalty for the number of parameters in the model, but this penalty term is larger in BIC than in AIC (Bhat and Kumar 2010). The model selection criterion in AIC and BIC is to find the lowest value (Hilborn and Mangel 1997, Bhat and Kumar 2010). In addition to AIC and BIC, I used analysis of variance (ANOVA) to compare fit between nested models (i.e., Models 1 versus 2, and Models 3 versus 4).

I estimated 95% confidence regions for the parameters in each of the models selected according to AIC and BIC by generating sampling distributions applying the Gibbs sampling algorithm of Markov Chain Monte Carlo (MCMC) methods (Manly 1997). I used package 'lme4' to run 1000 simulations, each having 1000 iterations. Only parameter estimates obtained in the 1000^{th} iteration of each simulation were kept as part of the sampling distributions (Manly 1997).

Model testing

My objective was to develop an accurate model to predict SLA_{fresh} from dried leaf samples. To determine if the models could be used to predict SLA_{fresh} from dried leaf samples for any plant species (not just for species from the Bolivian dry forests where I worked), I sampled plants from a temperate deciduous oak-hickory forest at Washington University in St. Louis' Tyson Research Center located in Eureka, MO (USA). I collected leaves from 5 individuals of *Quercus alba* L., *Fraxinus americana* L., *Celtis occidentalis* L., *Lonicera japonica* Thunb. Ex Murray, and *Juglans nigra* L. For each individual, I collected one leaf from the top (sun exposed) and one leaf from the bottom (shade) of the crown. Leaves were treated identically to the Bolivian leaves with all processing occurring at the University of Missouri-Saint Louis. Predicted SLA_{fresh} was obtained by applying the models constructed from the Bolivian samples. To determine the degree to which predicted SLA_{fresh} was determined by the models correlated with actual SLA_{fresh}, I performed standard major axis (SMA) regression of actual SLA_{fresh} onto predicted SLA_{fresh} by each model using the R package 'smatr'. I expected that if the predictive models generated were accurate the intercept of the regression would not deviate significantly from zero and the slope would not deviate significantly from one.

RESULTS

Variance in SLA_{fresh} values from the Bolivian dry forest was mainly explained by interspecific differences (50.6%) with smaller contribution from intraspecific differences (19.7%), and lastly 30% of variation was attributable to intra-individual differences. While intra-individual variation was high, it should be remembered that samples within an individual were selected to represent the most extreme values. When regressing SLA of top crown leaves onto SLA of bottom crown leaves, the slope (b = 0.9) was significantly different from one (P < 0.001), and the intercept (a = -0.2) was significantly different from zero (P < 0.001). Similarly, the slope (b = 1.12) of the regression of fresh leaf area onto dry leaf area was significantly different from one (P < 0.001), and the intercept (a = 6.25e-5) was significantly different from zero (P = 0.003) (Figure 1). Interestingly, ~14% of the leaves gained leaf area during the drying process. Additionally, I found that the variation of leaf thickness had no significant relation with SLA variation (data not shown).

Four candidate models were generated to predict SLA for dried leaves. Models 1 and 2 were built to predict SLA for dried samples that lack information about where in the crown they were collected (a situation typical for herbarium samples). The lower AIC and BIC values and highly significant ANOVA indicated that model 2 had more empirical support than the other candidate model (Table 2). Models 3 and 4 were built to predict SLA for dried leaf samples that have information about where in the crown the leaves where collected. The AIC and BIC values and the highly significant ANOVA indicated that model 4 had higher empirical support than model 3 (Table 2). I conclude that the predictive models (2 and 4) that include species and individuals as random effects were more accurate models to predict SLA.

I compared the two selected models (2 and 4) to gauge the importance of information on crown position when it is available, using AIC, BIC and ANOVA. Model 4 performed significantly better (P <0.001; model 4 AIC -1470.1, BIC -1440.2) than model 2 (AIC -1460.2, BIC -1430.3). These results suggest that a more accurate prediction of SLA for samples from MNP is obtained from dried leaf samples when it is known where in the crown the samples were collected.

Table 2. Estimated parameters (as denoted in Table 1) of the candidate models developed for 109 species from Madidi National Park, Bolvia. Including Akaike information criterion (AIC), Bayesian information criterion (BIC), and the *P*-value obtained in the ANOVA.

Models	*a*	*b*	*c*	AIC	BIC	ANOVA (*P*-value)
Model 1	-0.19	0.87		-3111.3	-3086.4	
Model 2	-0.17	0.88		-3269.1	-3239.2	<2.2e-16
Model 3	-0.20	0.87	0.04	-3120.6	-3095.7	
Model 4	-0.18	0.88	0.04	-3279.2	-3249.3	<2.2e-16

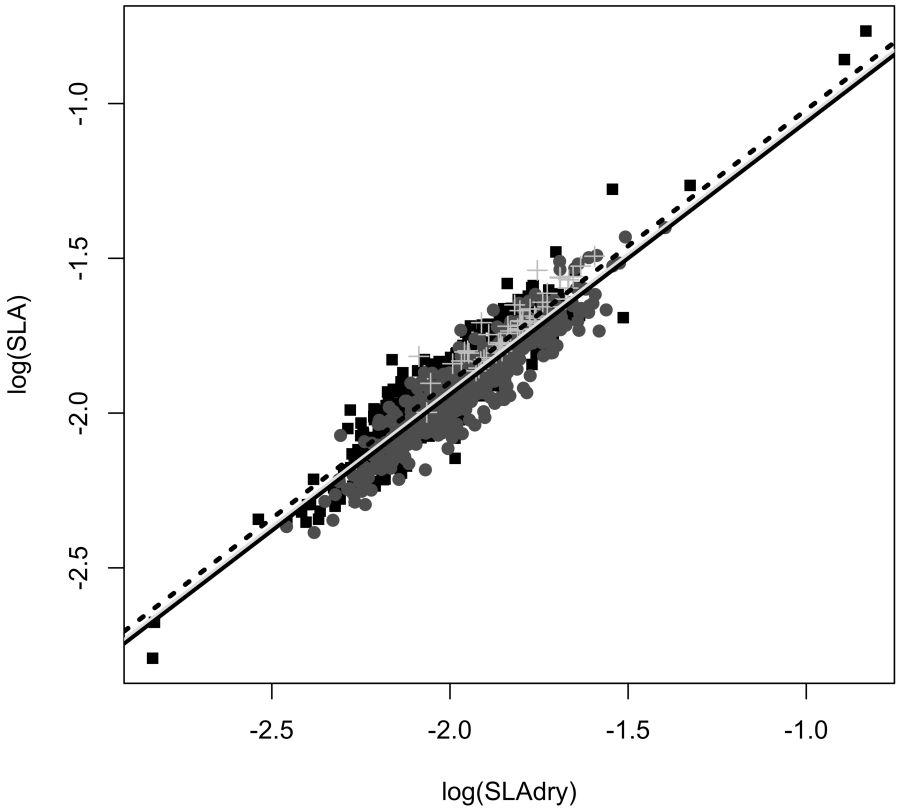

Figure 1. The regression between SLA_{dry} and SLA_{fresh} for 109 species from Madidi National Park, Bolivia and 5 species from Tyson Research Center, Eureka, MO, USA. For the Bolivian data, bottom crown leaves are denoted by dark grey circles and top crown leaves are denoted by black squares. The solid light grey line corresponds to model 2, which does not require position of the leaf in the crown and the black lines correspond to model 4, which requires the knowledge of position of the leaf in the crown. The solid black line corresponds to bottom crown leaves, and the dashed black line corresponds to top crown leaves. The USA data are denoted by the light grey crosses.

To provide an accurate predictive model, I estimated the sampling distribution of the parameters in the models to determine the confidence region. I obtained a sample from the Bayesian posterior distribution of the parameter estimates (*a* and *b*) for both selected models using MCMC methods. For Model 2, a high number of points were concentrated near the mean point (*a* = -0.17, *b* = 0.88) (Table 3). For Model 4, a high number of points were concentrated near the mean point (*a* = -0.19, *b* = 0.88). The bivariate distribution of the 10000 parameter estimates for Models 2 and 4 were positively correlated, the covariance of the parameters was also positive (Table 3) indicating that *a* increases with increasing *b*.

Table 3. Mean, variance and covariance values of the samples generated from the Bayesian posterior distribution of the parameters (*a* and *b*) for models 2 and 4 using MCMC methods for 109 species from Madidi National Park, Bolivia.

Models	Mean of *a*	Variance of *a*	Mean of *b*	Variance of *b*	Covariance of *a* and *b*
Model 2	-0.17	0.006	0.88	0.00015	0.0003
Model 4	-0.19	0.006	0.88	0.00015	0.0003

The third objective of this study was to propose the application of these predictive models as a field standard. To accomplish this, I applied the models obtained from data gathered in Bolivia to data collected in a temperate deciduous oak-hickory forest at Washington University at St. Louis' Tyson Research Center in Eureka, Missouri, USA, and determined the degree to which predicted SLA_{fresh} correlated with actual SLA_{fresh}. The range of SLA_{fresh} for samples collected in the USA were within the range of SLA values for samples collected in Bolivia (Figure 1).

When regressing the actual SLA_{fresh} onto predicted SLA_{fresh}, both predictive model regressions (from Models 2 and 4) had slopes not significantly different from one, intercepts not significantly different from zero, and $R^2 \geq 0.80$ (Table 4, Figure 2). Additionally, I regressed SLA_{fresh} top crown leaves onto SLA_{fresh} bottom crown leaves from the experiment, and I found that they were not significantly different from a slope of one and an intercept of zero (slope = 1.08, $P = 0.7$; intercept = 0.09, $P = 0.8$). From these results, I concluded that model 2 predicts SLA_{fresh} for dried leaf samples more accurately than model 4.

Table 4. Results from the standard major axis (SMA) regression of actual SLA_{fresh} onto predicted SLA_{fresh} for 5 species from Tyson Research Center, Eureka, MO, USA. The 95% confidence intervals are given in parenthesis (CI_{high}—CI_{Low}).

Model	Intercept	Slope	R^2
2	0.20 (-0.02—0.42)	1.08 (0.96—1.22)	0.84
4	0.23 (-0.02—0.49)	1.11 (0.97—1.26)	0.80

DISCUSSION

SLA is an easy to measure functional trait that provides insight into leaf allocation and function. The main drawback of the existing SLA protocol is the requirement for measures of fresh leaf area, limiting the types of samples that can be used. In this study, using samples from a Bolivian dry forest, I generated two models to predict SLA from dried leaf samples. One model requires information about where in the crown the leaf was collected, while the other model does not require knowledge of leaf position. The accuracy of the models as a global standard were tested on data collected in a very different forest, a temperate deciduous oak-hickory forest near St. Louis, MO, USA. While both models performed well, the simpler model — not requiring information about crown position (model 2) — provided the best prediction of SLA. I believe that I have established models that should be readily applicable to other datasets. I have also established a protocol that is easy to follow for studies that would like to make equations specific to their study species.

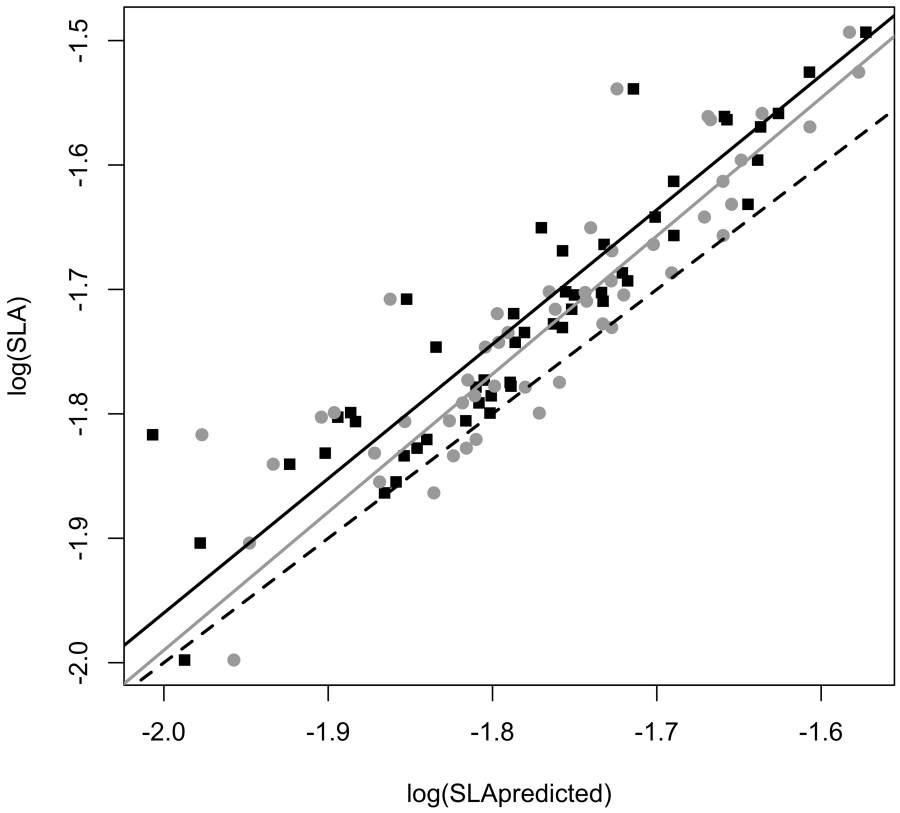

Figure 2. The correlation of actual SLA_{fresh} versus predicted SLA_{fresh} for 5 species from Tyson Research Center, Eureka, MO, USA. Grey circles are values predicted with the model that requires position of the leaf in the crown (Model 4), and black squares are values predicted with the model that does not require position of the leaf in the crown (Model 2). The dashed line is a 1:1 relation between actual $log(SLA_{fresh})$ and predicted $log(SLA_{fresh})$. The solid grey line is the regression line of the relation between actual log(SLA) and predicted $log(SLA_{fresh})$ which was predicted with the model that requires position of the leaf in the crown (Model 4) and the solid black line is the regression between actual $log(SLA_{fresh})$ and predicted $log(SLA_{fresh})$ which was predicted with the model that does not require position of the leaf in the crown (Model 2).

Amendments to the SLA protocol for dry leaf samples

While the standard protocol for SLA (Cornelissen et al. 2003) provides a useful tool for researchers, I propose the following modifications. These modifications make the protocol more accessible for researchers working with dried leaf samples (e.g., stored in herbaria or remote field conditions) to obtain SLA data that they can compare to other studies. First, in the section *What and how to collect?* I suggest it is possible to use dried leaf samples when fresh leaf samples are not available. From dried samples, mature fully expanded leaves with no herbivore or pathogen damage should be selected avoiding folded leaves. The targeted leaf should be removed including its petiole and measured following

the current protocol. Second, in the section *Measuring* I suggest measures of area of the dried leaf be taken as explained in the measurement of fresh leaf area in the Cornelissen et al (2003) protocol. After, the leaf should then be placed in an oven at 60 °C for 24 h and weighed. SLA_{dry} can be obtained by dividing dry leaf area by its dry mass. This value should be used in the following equation to obtain predicted SLA,

$$Log_{10}SLA = -0.17 + 0.88(Log_{10}SLA_{dry})$$

In cases where leaves can be collected in the field but fresh leaf area cannot be obtained, the crown position where the samples were collected should be noted. The samples should be processed as mentioned above, and SLA should be predicted from the following equation,

$$Log_{10}SLA = -0.18 + 0.88(Log_{10}SLA_{dry}) + 0.04(position\ of\ the\ leaf\ in\ the\ crown)$$

If the leaf was collected from a branch at the top of the crown a value of one should be used, and if the leaf was collected from a branch at the bottom of the crown, a value of zero should be used.

Effects of drying on leaf area

Because leaves contain a large amount of water, I hypothesized that SLA_{dry} would be smaller than SLA. In the dataset, I found that fresh leaf area was significantly greater than dry leaf area, which was not surprising. While the predicted tendency was found across the entire dataset, 14% of the collected samples gained rather than lost leaf area during the drying process. One possible explanation is that leaves that gained area were thicker and that when pressed while drying they added area. Interestingly, no relationship between change in leaf area with drying and leaf thickness was found. Furthermore, leaf area increased in simple and compound leaf species, and from leaves collected from the bottom and top of the crown. However, I did find that the image quality from those samples that increased in leaf area as they dried was lower. The program ImageJ, which I have used to measure leaf area, uses a threshold tool to select the object that is going to be measured. The threshold process can be difficult if the image does not have enough contrast (e.g., if the leaves are pale, if they have shadows, or if the resolution is low and then edges are hard to identify) (Davidson 2011). In these cases, the area of the object to be measured can be under or overestimated. A way to identify problematic leaves is to measure leaf area several times to obtain a mean and standard deviation for identification and validation.

Leaf crown position

My second prediction was that an accurate predictive model of SLA for dried samples should have covariates that describe the environment where the leaf developed, such as position of the leaf in the crown. However, in the results from sampling species from the temperate deciduous oak-hickory forest, I found that model 2, which was generated to predict SLA without information on position of the leaf in the crown, was more accurate in predicting SLA than model 4, which includes leaf position in the crown as a covariate. This means that leaves for these species from this forest did not differ in SLA for top and bottom crown leaves. This finding was surprising since bottom and top crown SLA were significantly different in the dataset from Bolivia, and also Hulshof and Swenson (2010) found similar results in data collected in a dry forest in Costa Rica. A possible explanation for these differences could be that temperate deciduous forests in USA are less stratified in their irradiance within the crowns of trees. Additionally, it should be noted that even for the

Bolivian species model 4 did not lead to large shifts in the model parameters, suggesting it had a significant but weak influence on SLA. When available, I suggest that both models should be tested on data for other ecosystems and that it is likely model 4 will be more accurate in ecosystems where the canopy has greater light stratification.

A second striking finding regarding crown position in the Bolivian forest was that ~42% of the trees had bottom crown leaves (shade leaves) with lower SLA than top crown leaves (sun leaves). This was further supported by a positive coefficient for crown position in the models. However, top and bottom crown leaves from the experiment developed in USA were found to be not significantly different (P = 0.7). These findings do not support the general trend found in other studies in which sun leaves were reported to have lower SLA than shade leaves (Rozendaal et al. 2006, Sack et al. 2006, Hulshof and Swenson 2010). However, other studies have reported species with shade leaves that have lower SLA than sun leaves (Talbert and Holch 1957, Niinemets and Kull 1994, Carr 2000, Richardson et al. 2000). Based on these different results reported I recommend when possible that intracrown SLA differences should be evaluated.

Top crown leaves are difficult to collect in tropical forest

Considering that upper crown sun leaves are difficult to collect in most tropical forests due to their tall stature, standardized methods such as the SLA protocol proposed by Cornelissen et al. (2003) may be difficult to apply. In most situations, it will be easier to collect leaves from bottom crown branches exposed to the sun. Sack et al. (2006) have reported that the variation of SLA between top crown sun leaves and bottom crown sun leaves is minimal and that more variation is explained by differences in irradiance. They have found that bottom crown internal leaves (shade leaves) differ strongly from bottom crown external leaves (sun leaves). Considering these results and the height of tropical forest trees, I suggest that bottom crown external leaves (sun leaves) may be easier to collect and bottom crown exterior versus interior leaves likely represent the extremes in SLA through the crown of the tree. However, it would be useful to explicitly test this expectation.

In this study, I developed two models that can be used to predict SLA from dried leaf samples. Both models can readily be applied to dry tropical forests and deciduous temperate forests. I feel confident that they should be widely applicable across different study systems. I recommend however that they be validated when possible in other systems before applying them, (e.g., the importance of crown position for prediction may differ). A nice application of these models is that they allow data collected from herbarium samples or from sampled collected in remote locations to be compared to other studies around the world. For instance, herbarium samples could be used to ask questions about shifts in SLA over time as a result of climate change. Or herbarium samples can be used to evaluate the amount of intraspecific variation across large geographic ranges. These predictive models extend the temporal, geographical, ecological, and taxonomic scope of SLA studies.

REFERENCES

Ackerly, D. 2004. Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. Ecological Monographs 74:25–44.

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19: 716–723.

Björkman, O. 1981. Responses to different quantum flux densities, pp. 57–107 in Physiological Plant Ecology I. Responses to the Physical Environment. O.L. Lange, P.S. Nobel, C.B. Osmond & H. Ziegler (eds.). Springer, Heidelberg/New York.

Bongers, F. and J. Popma. 1990. Leaf characteristics of the tropical rain forest flora of los Tuxtlas, Mexico. Botanical Gazette 151: 354–365.

Burnham, K. P. and D. R. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. 2nd ed. Springer-Verlag, New York.

Carr, D. J. 2000. On the supposed changes in stomatal frequency and size with height of leaf insertion. Annals of Botany 86: 911–912.

Cornelissen, J.H.C., S. Lavorel, E. Garnier, S.Diaz, N.Buchmann, D.E. Gurvich, P.B. Reich, H. ter Steege, H.D. Morgan, M.G.A. van der Heijden, J.B. Reich, J.G. Pausas, and H. Pooter. 2003. A Handbook of protocols for standardized and easy measurement plant functional traits Worldwide. Australian Journal of Botany 51: 335–380.

Evans, J.R. and Poorter, H. 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant, Cell & Environment 24: 755–767.

Garnier, E., B. Shipley, C. Roumet, and G. Laurent. 2001. A standardized protocol for the determination of specific leaf area and leaf dry matter content. Functional ecology 15: 688–695.

Hilborn, R. and M. Mangel. 1997. The ecological detective: confronting models with data. Princeton University press. Pp. 152–160.

Hulshof, C. and N. Swenson. 2010. Variation in leaf functional trait values within and across individuals and species: an example from a Costa Rican dry forest. Functional ecology 24: 217–223.

Klich, M.G. 2000. Leaf variations in Elaegnus angustifolia related to environmental heterogeneity. Environmental and experimental botany 44:171–183.

Kraft, N.J.B., R. Valencia, and D. Ackerly. 2008. Functional traits and niche-based tree community assembly in an Amazonian forest. Science 322: 580–582.

Manly, B. 1997. Randomization, bootstrap and Monte Carlo methods in Biology. 2nd ed. Chapman and Hall press. Pp. 330–337.

Markesteijn, L. 2010. Drought tolerance of tropical tree species; Functional Traits, Trade-offs and Species Distribution. PhD thesis, Wageningen University, the Netherlands. 204 pp.

McDonald, P.G., C.R. Fonseca, J.M. Overton, and M. Westoby. 2003. Leaf-size divergence along rainfall and soil-nutrient gradients: is the method size reduction common among clades?. Functional Ecology 17: 50–57.

Niinemets, Ü. and K. Kull. 1994. Leaf weight per area and leaf size of 85 Estonian woody species in relation to shade tolerance and light availability. Forest Ecology and Management 70: 1–10.

R Development Core Team. 2009. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reich, P., M. Walters, and D. Ellsworth. 1997. From tropics to tundra: Global convergence in plant functioning. Proceedings of the national academy of sciences 9: 13730–13734.

Richardson, A. D., G. P. Berlyn, P. M. S. Ashton, R. Thadani, and I. R. Cameron. 2000. Foliar plasticity of hybrid spruce in relation to crown position and stand age. Canadian Journal of Botany 78: 305–317.

Rozendaal, D.M.A., V.H. Hurtado, and L. Poorter. 2006. Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. Functional Ecology 20: 207–216.

Ryser, P. 1996. The importance of tissue density for growth and life span of leaves and roots: a comparison of five ecologically contrasting grasses. Functional ecology 10:717–723.

Talbert, C. M., and A. E. Holch. 1957. A study of the lobbing of sun and shade leaves. Ecology 38: 655–658.

Weiher, E., A. van der Werf, K.Thompson, M. Roderick, E. Garnier, and O. Eriksson. 1999. Challenging Theophrastus: A common core list of plant traits for functional ecology. Journal of vegetation science 10:609–620.

Whitmore, T.C. 1996. A review of some aspects of tropical rain forest seedling ecology with suggestions for further enquiry, pp. 3–39 in: The Ecology of Tropical Forest Tree Seedlings (M.D. Swaine ed.) MAB UNESCO Series Vol. 17. Parthenon, Paris.

Wright, I.J., P.B. Reich, M. Westoby, D.D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J.H.C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P.K. Groom, J. Gulias, K. Hikosaka, B.B. Lamont, T. Lee, W. Lee, C. Lusk, J.J. Midgley, M. Navas, Ü. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V.I. Pyankov, C. Roumet, S.C. Thomas, M.G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature 428: 821–827.

GRAIN SIZE AND FUNCTIONAL TRAITS IN SPECIES ASSEMBLAGES OF A NEOTROPICAL DRY FOREST

INTRODUCTION

A long standing question in ecology is how and why so many species coexist. Explanations have entailed deterministic and stochastic processes. Deterministic processes are "rules" or "filters" thought to structure species assemblages according to traits that determine fitness differences among species across environments (Diamond 1975, Weiher and Keddy 1999, Cornwell et al. 2006). On the other hand, stochastic processes may structure species assemblages independent of such traits (Hubbell 2001). Uncertainty remains regarding the importance of these two kinds of process, and how they interact to structure species assemblages defined at different grain sizes in the landscape (Weiher and Keddy 1995, Weiher and Keddy 1999, Cavender-Bares et al. 2006, Cavender-Bares et al. 2009, Kraft and Ackerly 2010).

Recent progress in our understanding of species assemblages has occurred through a focus on functional traits (Shipley 2010) where species assemblages are examined in terms of morphological, physiological or reproductive traits that are thought to influence fitness; combinations of these traits define species' ecological strategies (Grime 1979; Tilman 1988, Westoby et al. 2002, McGill et al. 2006). Optimal strategies may shift with changes in environmental settings. Two kinds of deterministic processes have been considered in this trait-based approach, namely the habitat filtering model and the inter-specific competition model. Both invoke inter-specific differences in ecological strategies (Kraft et al. 2008), but they predict distinct effects on the distribution of functional traits of co-occurring species (Cornwell et al. 2006, Cavender-Bares et al. 2006).

In the habitat filtering model, it is assumed that only species having functional traits within a particular range of values can tolerate the environmental conditions that occur in a given site (the assumption being that environmental conditions are relatively homogeneous within the site). In other words the environment filters the regional pool of species, limiting the composition of an assemblage to a subset of species that possess functional traits conferring high fitness in that environment. Consequently, it is expected that functional traits values for species co-occurring in any given assemblage range within a limited trait space relative to the trait space defined by all species in the regional pool (van der Valk 1982, Keddy 1992, Weiher *et al.* 1998, Weiher and Keddy 1999, Cornwell *et al.* 2006, Cornwell and Ackerly 2006).

In the inter-specific competition model, species with similar functional trait values are assumed to compete strongly and, therefore, frequently exclude each other from a given species assemblage. Such competitive exclusion is thought to happen when there is asymmetrical competition (i.e., there is a competitively superior species, MacArthur and Levins 1967), but also under scenarios involving unstable co-occurrence, presence of natural enemies, and priority effects (Chase and Leibold 2003). Despite the variety of scenarios for competitive exclusion allowed by this model, a constant element is that competitive exclusion is particularly likely between species with similar functional trait values. Therefore, it is expected that functional trait values for co-occurring species in any given assemblage be evenly dispersed in the trait space (Cornwell and Ackerly 2009, Kraft and Ackerly 2010).

An alternative to the deterministic models above is the stochastic, neutral or null model in which the spatial distribution of species is not limited by environmental conditions. Instead species are ecologically equivalent so that competitive exclusion can occur but the winner is not determined based on similarity in functional trait values (Hubbell 1997,

Hubbell 2001). The neutral model relies on the assumption that dispersal and demographic processes have prominent roles in structuring species assemblages (Hubbell 2001). It predicts that the distribution of functional trait values in a species assemblage is not different from random draws of trait values from the regional species pool.

The relevance of the different models is thought to be contingent on the spatial grain size used to define species assemblages (Swenson et al. 2006, Cavender-Bares et al. 2006, Kraft and Ackerly 2010). In particular, one hypothesis (hereafter referred to as *hypothesis 1*) suggests that at large grain sizes (called "habitat scale") environmental filters govern the organization of species assemblages, while biotic interactions such as competitive exclusion have the greatest impact at small grain sizes (called "neighborhood scale") (Hardin 1966, Roughgarden 1983, Tilman 1994, Weiher and Keddy 1995, 1999, Webb et al. 2002, Swenson et al. 2007, Cavender-Bares et al. 2009, Kraft and Ackerly 2010).

I propose an alternative working hypothesis (hereafter referred to as *hypothesis 2*) according to which the relevance of the habitat filtering model depends on the relationship between grain size and spatial heterogeneity of the environmental conditions within the area ascribed to species assemblages. I take for granted that the spatial heterogeneity increases with area (Williamson 1987, Bell et al. 1993, Storch et al. 2002, Bridges et al. 2007). Under this assumption, hypothesis 2 suggests that environmental filters are particularly important in the organization of species assemblages defined at small grains, because species assemblages at these sizes would occur within a fairly homogeneous environment that filters the regional pool of species in a consistent fashion. On the other hand, species assemblages defined at large grains would encompass higher environmental heterogeneity (microtopography, soil nutrients, sun exposure, slope, water availability) and thus may not be structured by any consistent effect of habitat filtering. As in the inter-specific competition model above, I also expect biotic interactions to most strongly affect species assemblages defined at small grains, because in such assemblages individuals may competitively interact more readily than at larger grains. In short, according to hypothesis 2 and in contrast to hypothesis 1, the importance of both, the habitat filtering model and the inter-specific competition model, will increase as the grain size used to define species assemblages decreases.

A recent study, partially consistent with both hypotheses, of Amazonian tree species assemblages defined plot grain sizes from 25 to 10000 m^2 (0.0025 to 1 ha) (Kraft and Ackerly 2010). It showed evidence of habitat filtering across plots of all sizes, but the evidence of competitive exclusion was restricted to relatively small plots, ranging from 25 to 400 m^2 (0.0025 to 0.04 ha). However, power to detect the pattern predicted by the model emphasizing competitive exclusion decreased as plot size increased. An earlier study in the same Amazonian forest defined tree species assemblages using 400 m^2 (0.04 ha) plots, and found strong evidence of habitat filtering and competitive exclusion (Kraft et al. 2008). These results indicate that topographic habitats (ridgetops and valley bottoms) support species assemblages with divergent strategies. Beyond these findings, all derived from a 25 ha plot in a lowland Amazonian forest of Ecuador, there seems to be little work on how the models of habitat filtering and competitive exclusion may differentially apply to species assemblages at various grain sizes.

Here I test predictions from the two hypotheses above by examining the distribution of functional traits across tree species assemblages defined according to non-contiguous plots (24.1 ha in total) scattered across a tropical dry forest in the Bolivian Andes.

METHODS

Study site

The Madidi National Park (MNP) comprises an area of 18,957 km^2 in northwestern Bolivia (SERNAP 2011). The park includes 1,442 km^2 of dry forest within an elevational gradient of 600—1500 m; approximately 700 km^2 is pristine forest. This park is thought to hold one of the largest and best conserved areas of dry forest in the Neotropics (Kessler and Helme 1999). The closest meteorological station is located 50 km away, in Apolo at 1430 m of elevation, with a mean annual temperature of 20.5 °C (Navarro 2002). The mean annual precipitation ranges from 1200 to 1400 mm and there are 3.5 dry months per year (Müeller et al. 2002).

The dry forest at MNP lies along the watershed of the Tuichi River and its tributaries, the Machariapo and Resina Rivers (Cayola et al. 2007). It is surrounded by Amazonian forest at lower elevations and humid Andean forests at higher elevations. Both evergreen and deciduous species can be found in the dry forest, leading to a mix of deciduous and semi-deciduous dry forests. At least 1119 vascular plants occur in the area (Cayola et al. 2010).

I tested predictions derived from each hypothesis using data on 16 permanent plots (1 ha) and 81 non-permanent plots (0.1 ha) established by the "Floristic Inventory of the Madidi region" project from 2003 to 2005. Individuals with ≥10 cm of diameter at breast height (dbh; 130 cm above the ground) were censused in permanent plots and individuals with ≥2.5 cm of dbh were censused in non-permanent plots. For every individual found in permanent and non-permanent plots, height and dbh were recorded. Species occurrence in each plot was documented with herbarium vouchers deposited in the National Herbarium of Bolivia and in the Missouri Botanical Garden (MBG).

Species assemblages

I defined species assemblages using two grain sizes; large and small grain sizes were defined separately for permanent and non-permanent plots. The larger grain sizes were the sizes of the entire plot: 1 ha for permanent plots and 0.1 ha for non-permanent plots. The smaller grain sizes were defined by the minimum sizes of adjacent and non-overlapping square quadrats within the plots defined during plot set up: 20 x 20 m (0.04 ha) quadrats within permanent plots and 10 x 10 m (0.01 ha) quadrats within non-permanent plots. To avoid non-independence among species assemblages defined by small grain sizes within a single plot (i.e., non-independence among 25 0.04 ha quadrats within a single 1 ha permanent plot, and among the 10 0.01 ha quadrats within a single 0.1 ha non-permanent plot), I randomly selected only one quadrat within each plot, and tested the predictions of interest using only data on the species assemblages defined by these randomly selected quadrats.

Trait sampling

Three functional traits were considered in the analysis: specific leaf area (SLA, m^2g^{-1}), maximum height (H_{max}, m), and maximum diameter (D_{max}, m). These traits were chosen because they are components of important plant ecological strategies (Westoby et al. 2002, Ackerly 2004). H_{max} and D_{max} represent the competitive ability to capture light (Falster and Westoby 2005, Maharjan et al. 2011) and growth strategy (Anten and Hirosel 1999). SLA represents strategies of carbon investment and gain (Wright et al. 2004, Cornwell and Ackerly 2010).

At the time of plot set up, height and dbh were obtained for all individuals in the plot. Maximum sizes (H_{max} and D_{max}) were obtained following the method suggested by King et al. (2006). The method is based on the abundance of species. To estimate the maximum

size of common species (>500 individuals), the largest three values were averaged. For less common species (100–500 individuals), the two largest values were averaged, and for rare species (<100 individuals) the largest observed value were used.

SLA values were obtained from herbarium specimens collected in the dry forest of the MNP and deposited at MBG. To obtain SLA for freshly collected samples from dry samples, I applied the predictive model developed by Torrez et al. (Chapter 1, this thesis). A mature expanded leaf without herbivory was excised (including its petiole) from an herbarium specimen. For species with compound leaves, multiple leaflets per specimen were excised (Hulshof and Swenson 2010). An image was captured for each leaf/leaflet to obtain dry leaf area using the program ImageJ (http://imagej.nih.gov/ij/). Leaves were placed in an oven at 60°C for 24 h and then weighed to obtain dry mass. Dry leaf area per dry mass entered as a predictor variable in the model developed by Torrez et al. (Chapter 1) to obtain fresh leaf area per dry leaf mass. For species with compound leaves, I obtained SLA values for every leaflet and then calculated a mean SLA value for each specimen. Mean SLA values were obtained from at least 10 specimens for each species (Hulshof and Swenson 2010). However for a few rare species (120 species), mean SLA values were obtained using fewer specimens. In those cases, I excised more than one leaf per herbarium specimen.

D_{max} and H_{max} values were obtained for all the species (n = 463), however SLA values were obtained only for those species that had available specimens at the MBG (n = 319). Trait values were $\log_{10}$ transformed for analysis.

Species pools

I generated two types of regional species pools. One type, hereafter referred to as *entire species pool*, was generated separately for permanent and non-permanent plots, and included all species occurring in permanent and non-permanent plots, respectively. A second type of species pool was defined to detect a pattern consistent with inter-specific competition in a background of habitat filtering (Kraft and Ackerly 2010). I referred to this second type of species pool as *species pool in a background of habitat filtering*. It was generated separately for each species assemblage, and included only species that a) were in the *entire species pool* and b) had trait values within the range of trait values observed in a given species assemblage. For example, if SLA values range from 0.005–0.05 m^2g^{-1} in the *entire species pool*, and from 0.005–0.01 m^2g^{-1} in a given species assemblage A, then the *species pool in a background of habitat filtering* for the species assemblage A is composed of species in the *entire species pool* that have SLA values within 0.005–0.01 m^2g^{-1}.

Detecting non-random patterns of trait dispersion

For each observed species assemblage, trait dispersion patterns were compared to null expectations estimated based on 999 random species assemblages. Each of these random assemblages had species richness equal to the species richness in the respective observed species assemblage and was created by a null model that drew species from the regional species pool irrespective of species trait values. In three versions of this null model, the probability of including any species from the species pool in a particular random assemblage was determined by presence-absence, abundance, and frequency of occurrence across plots (Kraft et al. 2008). Species from observed and null species assemblages were matched to their respective SLA, H_{max}, and D_{max} values to calculate five metrics sensitive to deterministic patterns of trait dispersion. I compared these metrics between observed and null species assemblages to detect non-random patterns of trait dispersion. Following Kraft et al. (2008), species with missing SLA values were included in species pools and null models, but excluded from the calculation of SLA dispersion metrics.

I used four metrics to detect patterns of competitive exclusion (even dispersion of trait values) in species assemblages. First, I used *kurtosis* to measure the peakedness of trait values in species assemblages. This metric represents one aspect of how trait values are spread in trait space. If competitive exclusion structures the observed species assemblages, then kurtosis in the distribution of trait values in the observed species assemblages would be smaller than that of the respective random assemblages (Stubbs and Wilson 2004, Kraft et al. 2008, Cornwell and Ackerly 2008, Kraft and Ackerly 2010). Second, I measured the distance in trait space from each species to its nearest neighbor (NN) in the assemblage. I used the standard deviation of the NN values (SDNN) as a second metric to detect patterns of even dispersion of trait values expected according to the model emphasizing competitive exclusion. If competitive exclusion structures the observed species assemblage, then SDNN in the observed species assemblages would be smaller than that in the respective random assemblages (Ricklefs and Travis 1980, Stubbs and Wilson 2004, Kraft et al. 2008, Kraft & Ackerly 2010).

I used two other metrics to detect patterns of even spacing predicted by the model of competitive exclusion against a background of habitat filtering. One of them was obtained by dividing SDNN by the range of trait values present in the species assemblage (henceforth SDNNr) (Stubbs and Wilson 2004, Kraft and Ackerly 2009, Kraft and Ackerly 2010). To obtain the last metric, I calculated all neighbor distances (ND) as the difference between adjacent species in the assemblage, and then quantified the standard deviation of the ND divided by the range of trait values in the species assemblage (henceforth SDNDr) (Ingram and Shurin 2009, Kraft & Ackerly 2010). If competitive exclusion structures observed species assemblages within a background of habitat filtering, then SDNNr and SDNDr in the observed species assemblages would be smaller than that in the respective random assemblages (Stubbs and Wilson 2004, Ingram and Shurin 2009, Kraft and Ackerly 2009, Kraft and Ackerly 2010).

I used the trait range (TR) of a species assemblage as a metric to detect patterns of restricted trait dispersion predicted by the model of habitat filtering. This metric is the difference between the maximum and minimum trait values present in the species assemblage. If habitat filtering structures species assemblages, then the range of trait values in observed species assemblages would be smaller than that in the respective random assemblages (Stubbs and Wilson 2004, Cornwell et al. 2006, Ingram and Shurin 2009, Kraft and Ackerly 2009, Kraft and Ackerly 2010).

I used two levels of analysis to determine if values of the five metrics of trait dispersion (kurtosis, SDNN, SDNNr, SDNDr and TR) for the observed species assemblages deviated from those in the respective random assemblages as expected from deterministic models emphasizing competitive exclusion and habitat filtering. The first is the analysis at the level of single species assemblages. To determine if single species assemblages deviated significantly from random species assemblages, I examined whether the value of each of the five metrics of trait dispersion fell below the fifth percentile of the distribution of the metrics for the respective 999 random species assemblages. The results of this level of analysis were summarized as the percentage of species assemblages at each grain size that differed (relative to the fifth percentile, equivalent to a one-tailed test with alpha = 0.05) from random expectation. The second level of analysis focused on the difference in metrics of trait dispersion between each species assemblage and the central tendency of the respective 999 random species assemblages, aggregated across plots at each grain size. In particular, I used Wilcoxon signed rank tests to determine if the values of the metrics of trait

dispersion for the observed species assemblages deviated from those in the respective random assemblages.

Testing predictions from the two hypotheses

Hypothesis 1 predicts that the effect of habitat filtering on the structure of species assemblages is higher at a larger than at smaller grain sizes and that the effect of competitive exclusion is higher at smaller than at larger grain sizes. In contrast, hypothesis 2 predicts that both habitat filtering and competitive exclusion affect the structure of species assemblages more heavily at small than at large grain sizes. I used two criteria to examine support for these predictions. First, to support a given prediction, I determined if there was a nonrandom pattern of trait dispersion, consistent with the prediction, at the grain size where the hypothesis predicts a deterministic process (habitat filtering or competitive exclusion) has greater importance. Methods in the previous section describe how non-random patterns of trait dispersion were detected. Second, if the first criterion was met, then I examined if the deviation from a random pattern of trait dispersion was higher at the grain size at which the hypothesis predicts the strongest effect of the deterministic process. For this purpose, I used a Wilcoxon signed rank tests to compare small and large grain size in terms of standardized effect size of trait metrics sensitive to deterministic patterns of trait dispersion (see above description of these metrics). Standardized effect sizes are the difference between the observed metric and the average value of the metric for null species assemblages, divided by the standard deviation of the metric for the null species assemblages.

RESULTS

Trait based species assemblage structure

Results from the null model weighted by frequency of occurrence were more conservative than results obtained from the null model weighted by abundance or presence-absence (Appendix, Table 1A, 1B, 1C). Thus I report only results obtained from the null weighted by frequency of occurrence.

Evidence of deterministic processes was found at small and large grains at 1 ha and 0.1 ha plots (Table 1, Figure 1) for the three functional traits analyzed. The range of SLA and H_{max} values were significantly smaller than the null expectation at large grain size of 0.1 ha plots (Table1, Figure 1A), suggesting habitat filtering structured these species assemblages. Evidence of competitive exclusion was found through analysis of kurtosis for SLA at small and large grain sizes of 0.1 ha plots, and for D_{max} at the large grain size of 0.1 ha plots (Table 1, Figure 1B). Patterns of even spacing of trait values for H_{max} were found through analysis of SDNN at large grains of 0.1 and 1 ha plots (Table 1, Fig 1 C). Similar patterns were found for SLA with the analysis of SDNN at both grain sizes of 0.1 ha plots, suggesting that competitive exclusion structured those species assemblages.

In a background of habitat filtering, evidence of competitive exclusion was found through the analysis of SDNDr with SLA at small grain sizes of 0.1 ha plot (Table 1, Figure 1E). No evidence of competitive exclusion was found with the analysis of SDNNr (Table 1, Figure 1D).

When observed species assemblages were considered individually to detect deterministic processes through the analysis of range, kurtosis, SDNN, and SDNNr, a few species assemblages had significant *P*-values. Similarly, only a few observed metric values fell below the 5% extreme of the null distribution (Table 1). However, through the analysis of SDNDr for SLA more than 50% of the individual species assemblages had significant *P*-values (Table 1). Although many individual species assemblages were indistinguishable from

null species assemblages, grain size-wide tests were statistically significant, which is evidence against a random assembly of co-occurring species.

Effect of nonrandom processes at different grain sizes

I found that range for SLA and H_{max} met the first criterion to test the prediction of habitat filtering from hypothesis 1 (Table 1). The effect of habitat filtering at large grain sizes was not significantly greater than at small grains for SLA (Table 2, Figure 2 and 3). Thus, it did not meet the second criterion. However, the effect of habitat filtering at large grain was significantly greater than at small grain sizes for H_{max}, which met the second criterion. I conclude that I found support for the prediction that habitat filtering has greater importance in structuring species assemblages at large grains.

Additionally, I found that SDNDr, SDNN, and kurtosis for SLA met the first criterion to test the prediction of competitive exclusion from the two hypotheses (Table 1). Through the analysis of SDNDr, I found that the effect of competitive exclusion at small grains was significantly greater than at large grains (Table 2, Figure 4), which met the second criterion. However, through the analyses of SDNN and kurtosis, I found that the effect of competitive exclusion at small grains was not significantly greater than at large grains (Table 2, Figure 4), which did not meet the second criterion. Therefore, I found support for the prediction that competitive exclusion has greater importance in structuring species assemblage at small grain sizes.

Table 1. *P*-values of Wilcoxon signed rank test are reported for small and large grain sizes of 1 and 0.1 ha plots for three functional traits. Numbers in parenthesis are percentage of individual assemblages that had observed metric values below the fifth percentile of the null distribution of metric values. The asterisk denotes grain sizes that met the first criterion.

Trait	Grain size (ha)	Habitat filtering	Competitive exclusion			
		Range	Kurtosis	SDNN	SDNNr	SDNDr
SLA						
	1	0.3 (0)	0.2 (0)	0.2 (6.3)	0.5 (6.3)	1 (6.3)
	0.04	0.4 (0)	0.9 (0)	0.4 (0)	1 (0)	0.2 (19)
	0.1	**0.01** (5)*	**<0.001** (8.6)	**0.002** (6.2)	1 (0)	1 (11.1)
	0.01	0.06 (6.2)	**<0.001** (5)*	**<0.001** (4)*	1 (6.2)	**< 0.001** (56.8)*
D_{max}						
	1	0.9 (12.5)	0.3 (18.8)	0.4 (6.3)	0.43 (6.3)	0.13(12.5)
	0.04	0.97 (0)	0.59(12.5)	0.91 (0)	0.84(0)	0.59(0)
	0.1	0.45 (3.7)	**0.03** (3.7)	0.84 (6.2)	1 (4.9)	1 (1.2)
	0.01	0.16 (6.2)	0.32 (2.5)	0.07 (3.7)	0.9 (2.5)	0.7 (1.2)
H_{max}						
	1	0.11 (25)	0.08 (0)	**0.008** (18.8)	0.25 (6.3)	0.74 (0)
	0.04	0.47 (6.3)	0.81 (0)	0.97 (6.3)	1 (0)	0.94 (0)
	0.1	**<0.001** (7.4)*	0.47 (4.9)	**0.03 (11.1)**	0.59 (8.6)	0.96 (7.4)
	0.01	0.16 (9.9)	0.11 (11.1)	0.14 (11.1)	0.2 (2.5)	0.5 (1.2)

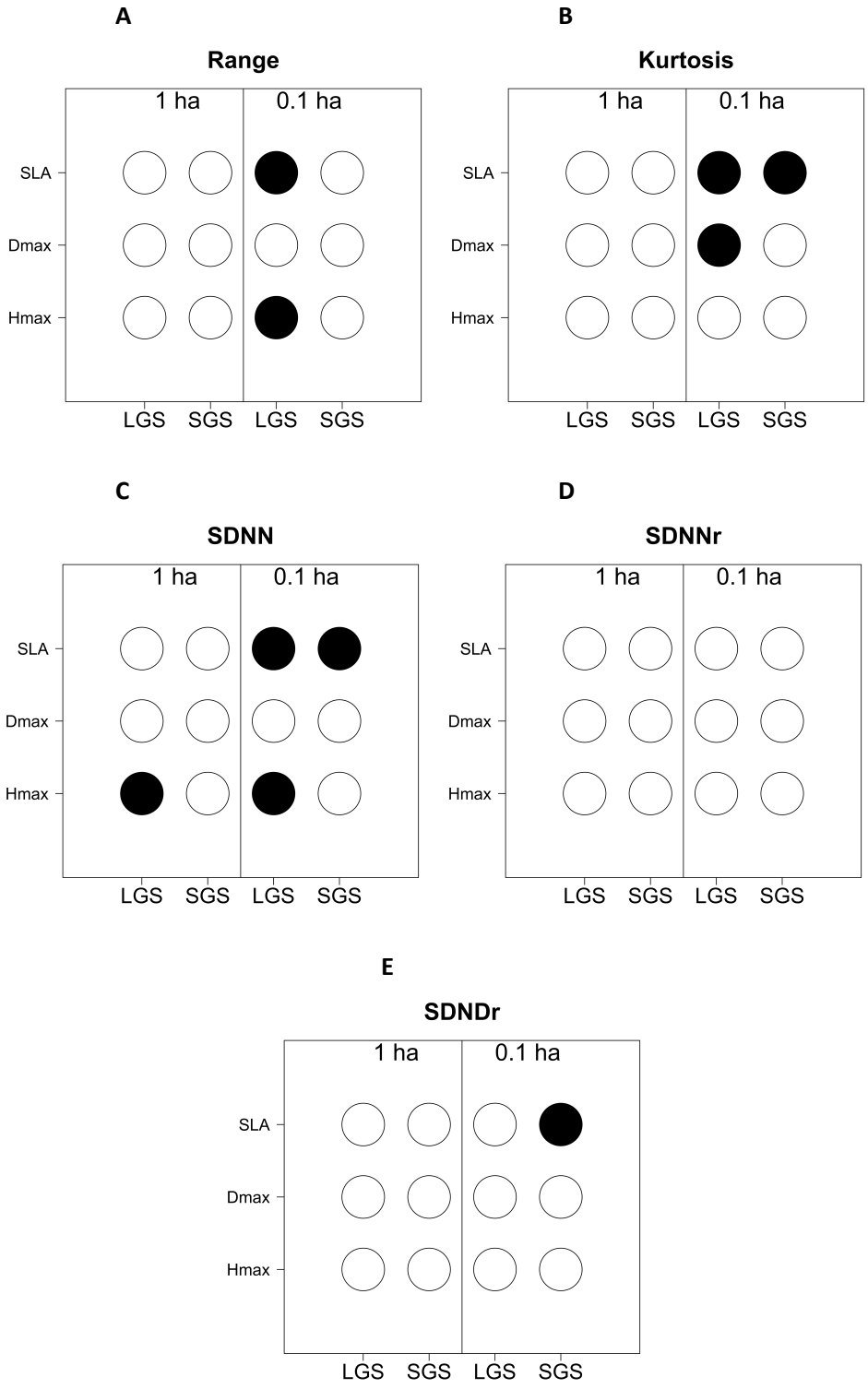

Figure 1. Summary of trait test at small grain sizes (SGS) and large grain sizes (LGS) at 1 and 0.1 ha plots. Black filled circles indicate that Wilcoxon signed rank test had *p*-values <0.05. SLA is specific leaf area, D_{max} is maximum diameter and H_{max} is maximum height. Large and small grain sizes of 1 and 0.1 ha plots are on the x axis, the first two columns on the left of each graph belong to 1 ha plot, and the second two are large and small grain sizes.

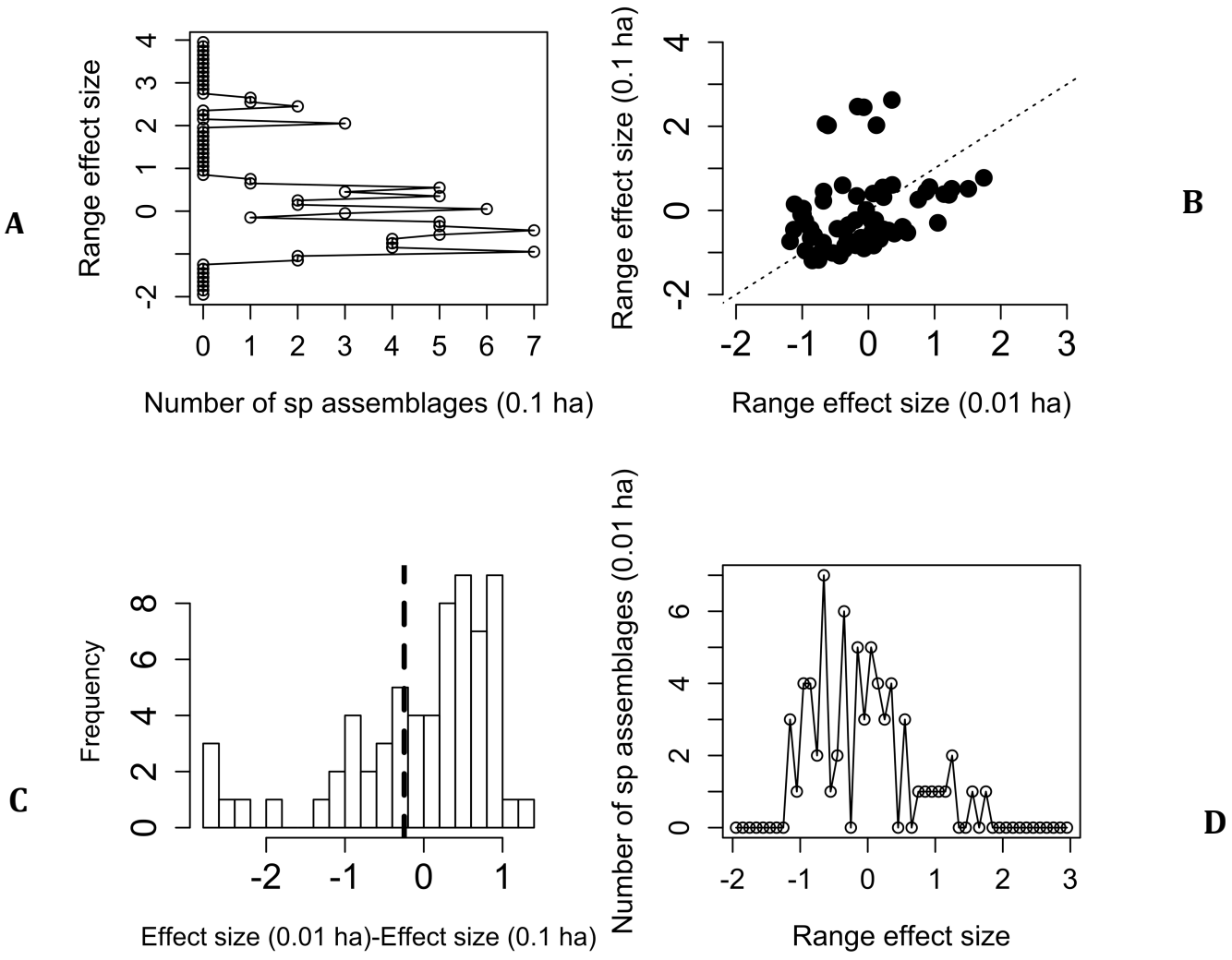

Figure 2. Range effect size values of small (0.01 ha) and large (0.1 ha) grain sizes of SLA at 0.1 ha. **A.** Effect size distribution of values in relation to the number of species assemblages of large grain size at 0.1 ha. **B.** Correlation between range effect size values of small and large grain at 0.1 ha. Dashed line indicates a 1:1 relation between range effect size values of small and large grain at 0.1 ha. **C.** Frequency distribution of the difference between range effect size values of small grains and range effect size values of large grains. Dashed line indicates the mean of the difference between range effect size values of small grains and range effect size values of large grains. **D.** Effect size distribution of values in relation to the number of species assemblages of small grain size at 0.1 ha.

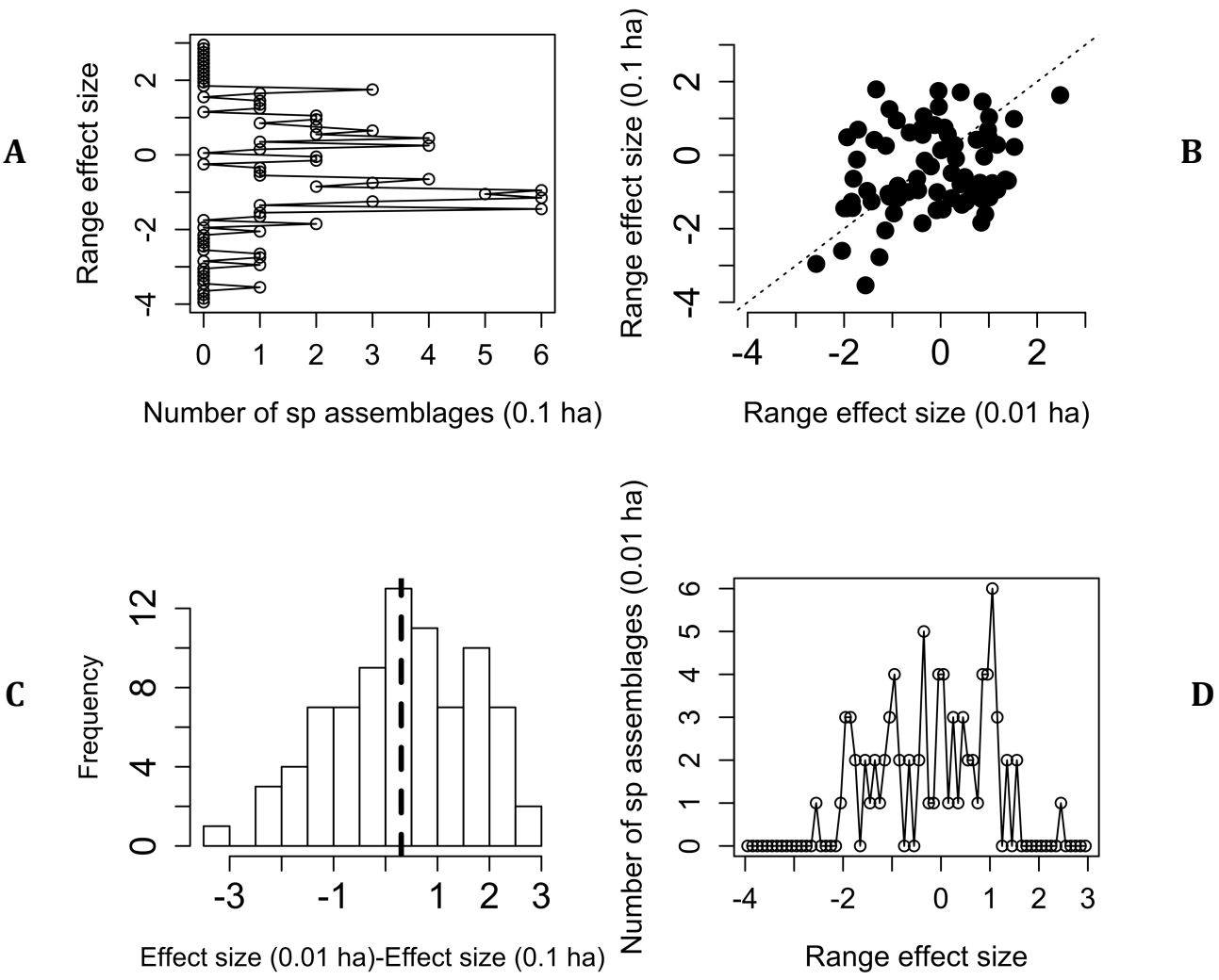

Figure 3. Range effect size values of small (0.01 ha) and large (0.1 ha) grain sizes of H_{max} at 0.1 ha. **A.** Range effect size distribution of values in relation to the number of species assemblages of large grain size at 0.1 ha. **B.** Correlation between range effect size values of small and large grain at 0.1 ha. Dashed line indicates a 1:1 relation between Range effect size values of small and large grain at 0.1 ha. **C.** Frequency distribution of the difference between range effect size values of small grains and range effect size values of large grains. Dashed line indicates the mean of the difference between range effect size values of small grains and range effect size values of large grains. **D.** Range effect size distribution of values in relation to the number of species assemblages of small grain size at 0.1 ha

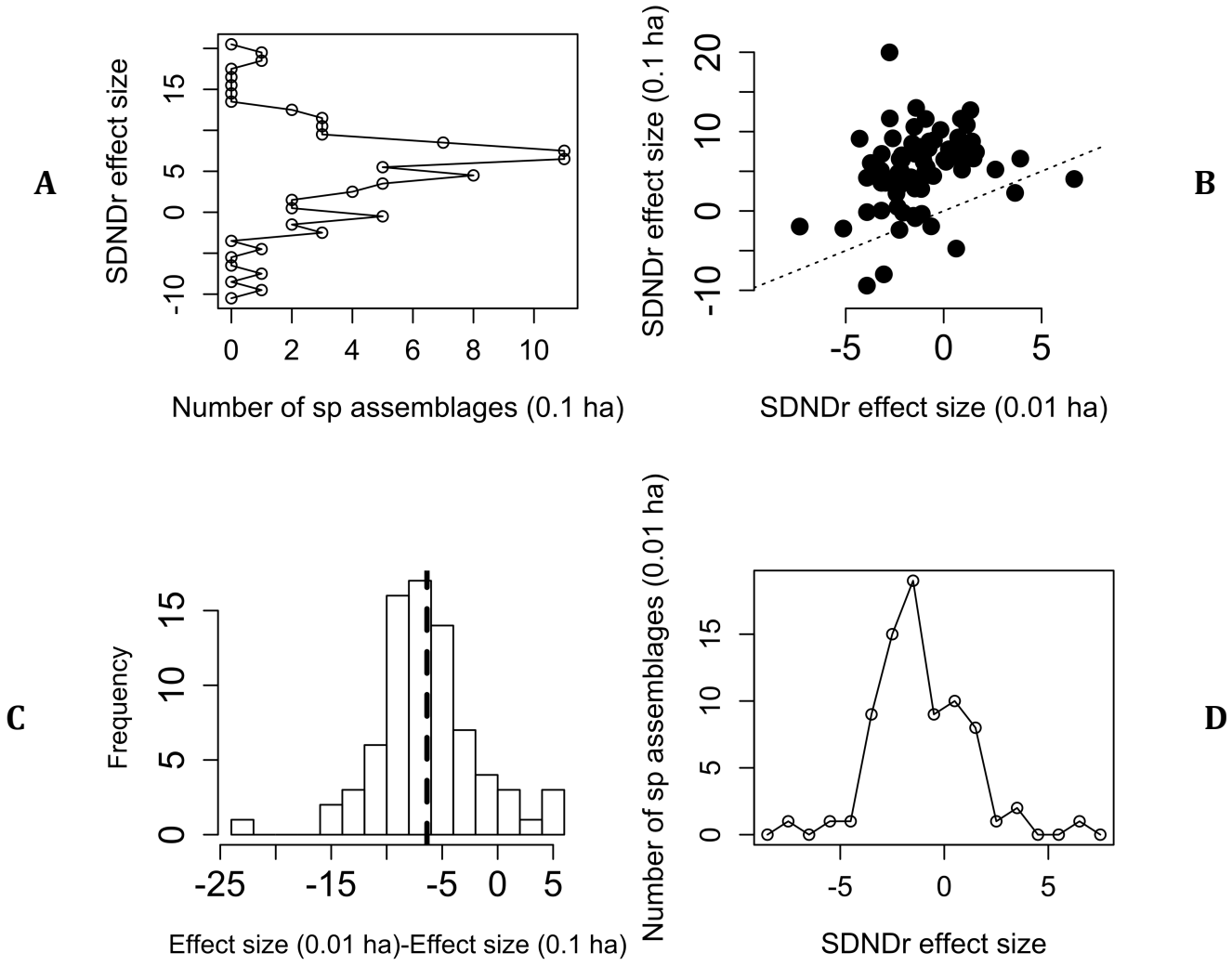

Figure 4. SDNDr effect size values of small (0.01 ha) and large (0.1 ha) grain sizes of SLA at 0.1 ha. **A.** SDNDr effect size distribution of values in relation to the number of species assemblages of large grain size at 0.1 ha. **B.** Correlation between range effect size values of small and large grain at 0.1 ha. Dashed line indicates a 1:1 relation between SDNDr effect size values of small and large grain at 0.1 ha. **C.** Frequency distribution of the difference between SDNDr effect size values of small grains and SDNDr effect size values of large grains. Dashed line indicates the mean of the difference between SDNDr effect size values of small grains and SDNDr effect size values of large grains. **D.** SDNDr effect size distribution of values in relation to the number of species assemblages of small grain size at 0.1 ha.

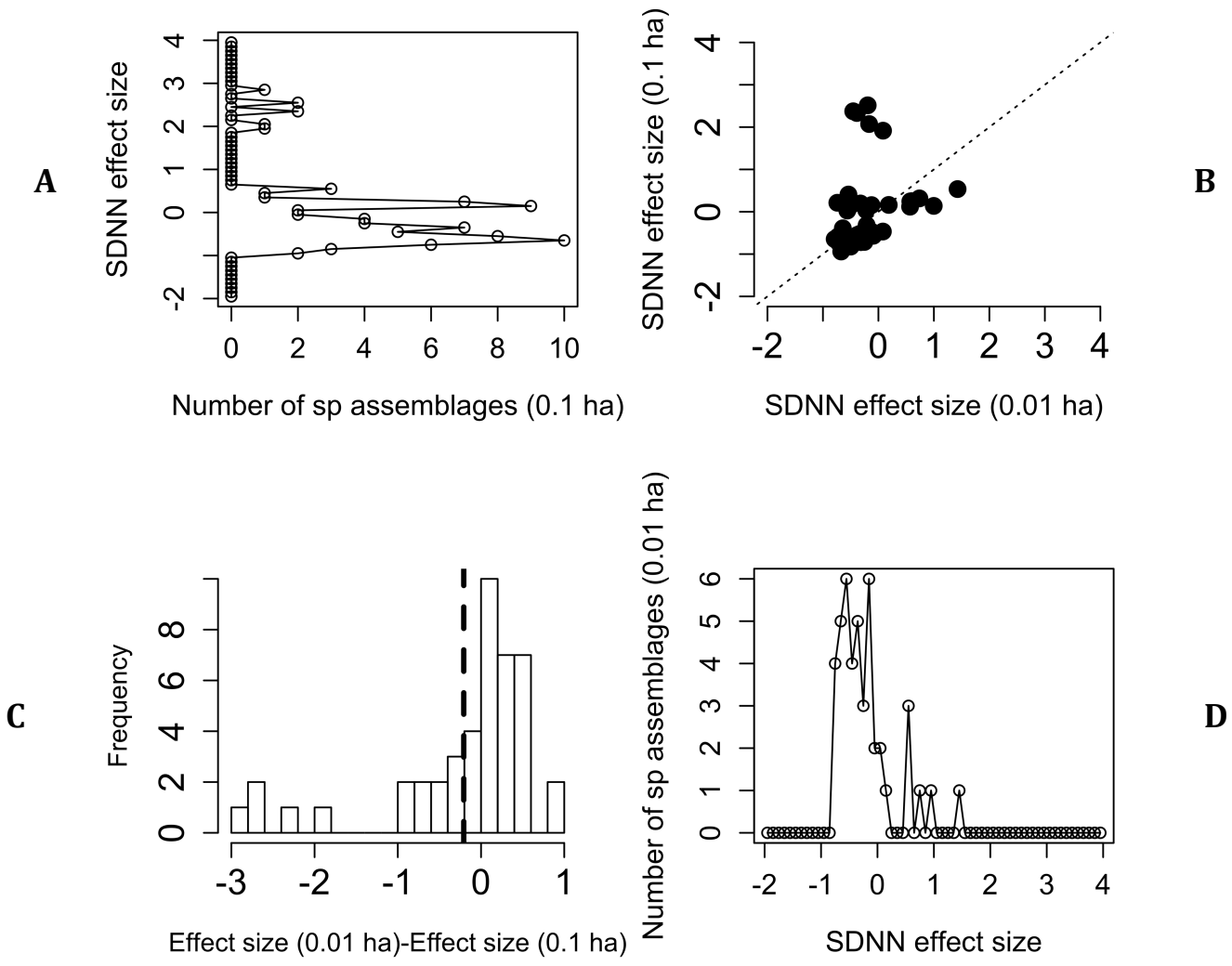

Figure 5. SDNN effect size values of small (0.01 ha) and large (0.1 ha) grain sizes of SLA at 0.1 ha. **A.** SDNN effect size distribution of values in relation to the number of species assemblages of large grain size at 0.1 ha. **B.** Correlation between range effect size values of small and large grain at 0.1 ha. Dashed line indicates a 1:1 relation between SDNN effect size values of small and large grain at 0.1 ha. **C.** Frequency distribution of the difference between SDNN effect size values of small grains and SDNN effect size values of large grains. Dashed line indicates the mean of the difference between SDNN effect size values of small grains and SDNN effect size values of large grains. **D.** SDNN effect size distribution of values in relation to the number of species assemblages of small grain size at 0.1 ha.

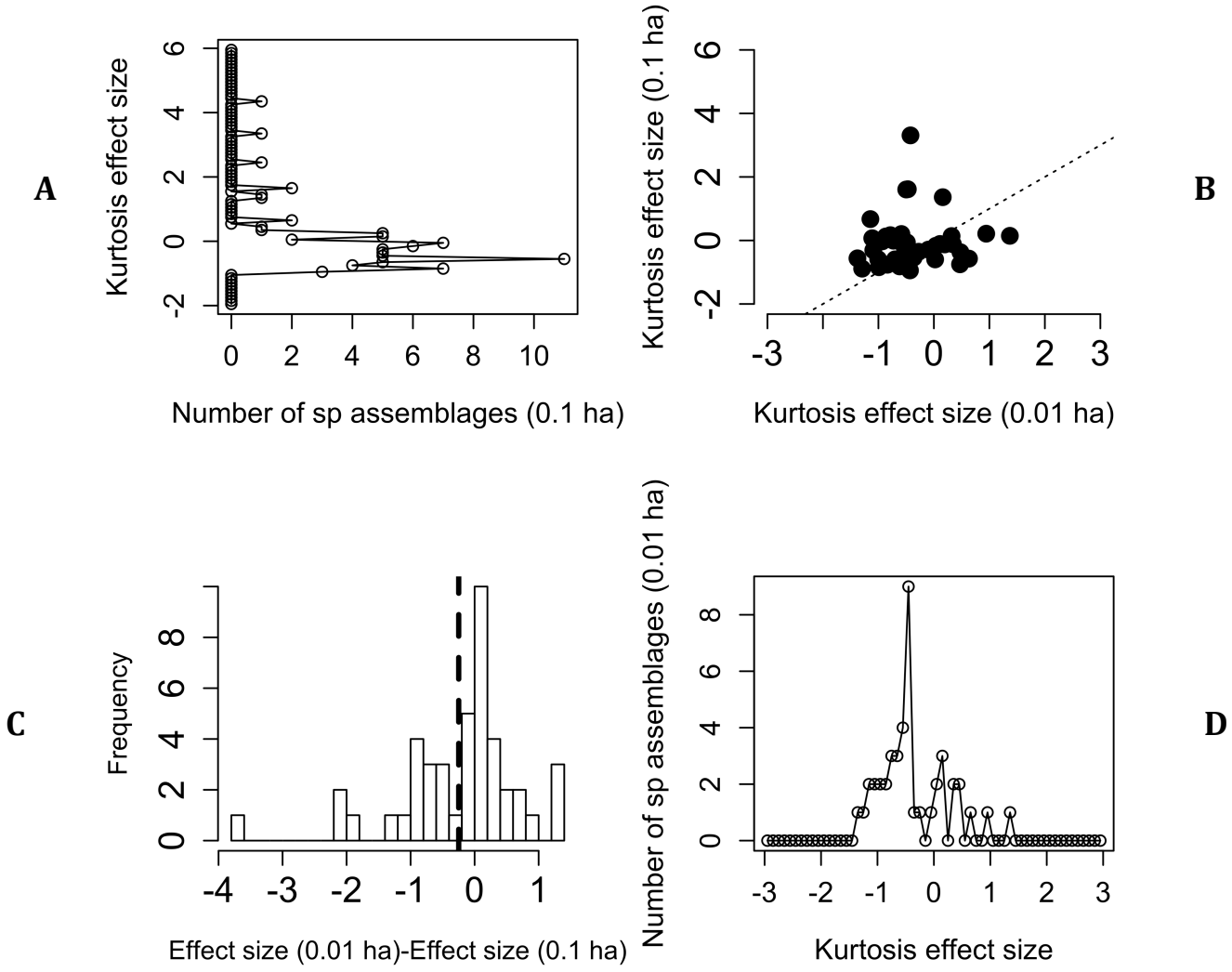

Figure 6. Kurtosis effect size values of small (0.01 ha) and large (0.1 ha) grain sizes of SLA at 0.1 ha. **A.** Kurtosis effect size distribution of values in relation to the number of species assemblages of large grain size at 0.1 ha. **B.** Correlation between range effect size values of small and large grain at 0.1 ha. Dashed line indicates a 1:1 relation between Kurtosis effect size values of small and large grain at 0.1 ha. **C.** Frequency distribution of the difference between Kurtosis effect size values of small grains and Kurtosis effect size values of large grains. Dashed line indicates the mean of the difference between Kurtosis effect size values of small grains and Kurtosis effect size values of large grains. **D.** Kurtosis effect size distribution of values in relation to the number of species assemblages of small grain size at 0.1 ha.

Table 2. *P*-values of Wilcoxon signed sample rank test are reported for the comparison of effect size between small and large grains at 0.1 ha plots. H1 denotes that the analysis was done to test grain size the prediction of habitat filtering from hypothesis 1.

Trait	Plot (ha)	Habitat filtering	Competitive exclusion		
		Range (H1)	SDNDr	SDNN	Kurtosis
SLA	0.1	0.2	**<0.001**	0.5	0.2
H_{max}	0.1	**0.02**			

Discussion

The present study used a functional trait approach to determine whether deterministic assembly processes structure species assemblages in a species-rich Neotropical dry forest. I found evidence that deterministic processes (habitat filtering and competitive exclusion) structured species assemblages in the dry tropical forest of the MNP. Additionally, I found that habitat filtering had a significantly stronger effect at large grain sizes than at small grain sizes, and that competitive exclusion had a significantly stronger effect at small grain sizes than at large grain sizes in structuring species assemblages in the dry tropical forest of the MNP.

A restricted trait range of SLA and H_{max} was found. This pattern was consistent with the habitat filtering model. Probably, these trait range patterns found for SLA and H_{max} can best be explained by the topographical variation (ridgetops, slopes, and valley bottoms) in the area. Topography leads to a high variation in soil moisture, light irradiance, and species deciduousness among ridgetops, slopes, and valley bottoms (Torrez 2008). In the area ridgetops are characterized by having high light irradiance with lower soil moisture than slopes and valley bottoms. Generally, woody Cactaceae (such as *Opuntia brasiliensis*) along with mostly deciduous tree species can be found in ridgetops. Most trees are shorter with tough leaves than the trees found in valley bottoms (Torrez 2008). Valley bottoms are characterized by high soil moisture because of the proximity to rivers; valley bottoms also have great diversity of lianas, epiphytes, and herbs. Trees with the tallest and highest dbh in the dry forest are usually found in valley bottoms. Slopes are generally rocky with inclined and thin trees (Torrez 2008). However, further analysis should be done to support that this suggested habitat association is occurring in the dry forest of the MNP.

In addition, I found patterns of even trait dispersion that are consistent with the inter-specific model, which can result from competitive exclusion, natural enemies, or priority effects. The even trait dispersion of the three traits (SLA, D_{max}, H_{max}) that I have analyzed in ecological terms mean that the species assemblages are composed of tree species with different strategies in light acquisition, and with a multistratified canopy given the broad distribution of tree heights. Similar patterns consistent with habitat filtering and competitive exclusion were found in an Ecuadorian moist tropical forest (Kraft et al 2008, Kraft and Ackerly 2010), and in a Costa Rican dry tropical forest (Swenson and Enquist 2010).

It is particularly interesting that I found evidence of competitive exclusion in more than 55% of the individual species assemblages, and that in less than 25% of the individual species assemblages I found evidence of habitat filtering. This finding is striking given that it was reported that metrics that are used to detect even spacing, such as SDNN, SDNNr,

SDNDr, have low power as species richness increases (Kraft et al. 2010). In addition, metrics used to detect habitat filtering have high power and are not influenced by changes in species richness (Kraft et al. 2010), suggesting that analysis of the power of the metrics used to detect deterministic patterns should be done. These analyses can determine if the patterns found were produced by effects of habitat filtering that are difficult to detect..

Most of the evidence of deterministic processes was found in 0.1 ha plots, a big difference between both types of plots that I used for the analyses was the minimum dbh used to measure individuals during the establishment of the plots. In 0.1 ha plots individuals with ≥2.5 cm were collected and in 1 ha plot individuals with ≥10 cm were collected. This difference allowed the inclusion of more species in 0.1 ha plots. Apparently, given the results obtained, most of the evidence of deterministic processes was recovered because of the presence of species with individuals with <10 cm of dbh in the dataset of 0.1 ha plots. Studies carried out in Ecuador in a 16 ha plot (Kraft et al. 2008) and in Costa Rica in a 25 ha (Swenson and Enquist 2009), based on plots where stems with dbh ≥1 cm and ≥3 cm were counted, respectively, found evidence of deterministic processes structuring species assemblages. Further analysis should be done to determine the importance of the inclusion of individuals with <10 cm of dbh in analyses of species assemblages.

Through the analysis of trait dispersion of SLA, I found evidence that supports the prediction that the importance of competitive exclusion increases as the grain size used to define the species assemblages decreases. This pattern indicates that at small grain sizes individuals of species assemblages possess traits values more evenly dispersed in the trait space than at large grain sizes. This finding suggests that biotic interactions (e.g., competition), natural enemies, and priority effects may have greater effects in species coexistence at small grain sizes. It also suggests that SLA may be important in promoting species diversity and coexistence in dry tropical forests. Kraft et al. (2010) reported a similar pattern in the moist tropical forest of Yasuni, however their findings were through the analysis of trait dispersion of seed mass and D_{max}.

I found evidence that supports the prediction that the importance of habitat filtering increases as the grain size used to define species assemblages increases. This pattern indicates that at large grain sizes individuals of species assemblages possess traits values restricted within a range. I found support for this hypothesis using H_{max}. A similar study performed in Yasuni found that habitat filtering had a relatively constant effect across grain sizes with the analysis of SLA, leaf nitrogen, life size, and seed size (Kraft and Ackerly 2010). However, they found significant effects at large grains (from 50 to 100 m^2) with wood density and D_{max} (Kraft and Ackerly 2010).

To my knowledge the present study is the first to the role of deterministic processes affecting traits values with non-contiguous plots encompassing a large regional area with great spatial heterogeneity in a species-rich Neotropical dry forest. The results obtained indicate that stabilizing processes promote the patterns of species diversity and co-existence in the dry forest at the MNP in Bolivia. Patterns consistent with competitive exclusion are evident across grain sizes, but their strength is higher at small grain sizes. On the other hand, patterns consistent with habitat filtering are important but diffuse across grain sizes. It will be valuable to perform analysis of the power of the metrics used to detect deterministic patterns of species assembly, and revisit these analyses as more trait data become available.

References

Ackerly, D. 2004. Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. Ecological Monographs 74:25–44.

Anten N.P.R. and T. Hirose. 1999. Interspecific differences in above-ground growth patterns results in spatial and temporal partitioning of light among species in a tall-grass meadow. Journal of Ecology 87: 583–597.

Bell, G., M. J. Lechowicz, A. Appenzeller, M. Chandler, E. Deblois, M. Jackson, B. Mackenzie, R. Preziosi, M. Schallenberg, and N. Tinker. 1993. The spatial structure of the physical environment. Oecologia 96: 114–121.

Bridges, L., A. E. Crompton, and J. A. Schaefer. 2007. Landscapes as gradients: The spatial structure of terrestrial ecosystem components in southern Ontario, Canada. Ecological complexity 4: 34–41.

Cavender-Bares, J., A. Keen, and B. Miles. 2006. Phylogenetic structure of Floridian plant communities depends on spatial and taxonomic scale. Ecology 87(Supplement): S109–S122.

Cavender-Bares, J., K. H. Kozak, P. V. A. Fine, and S. W. Kembel. 2009. The merging of community ecology and phylogenetic biology. Ecology letters 12:693–715.

Cayola, L., P.M. Jørgensen, A. Araujo-M., and A. Fuentes. 2007. Diversidad y composición florística del bosque seco andino del Parque Nacional y Área Natural de Manejo Integrado Madidi. Vol 3 pp. 1433–1441, In: J. Feyen, L. Aguirre, and M. Moraes (eds.). Memorias del Congreso Internacional sobre Desarrollo, Medio ambiente y Recursos naturales: Sostenibilidad a múltiples niveles y escalas.

Cayola L., A. Fuentes, and P. M. Jørgensen. 2010. Plantas vasculares del bosque seco de la Región Madidi. Poster. X Congreso Latinoamericano de Botánica, 4–10 October 2010.

Chase, J. M. and M. A. Leibold. 2003. Ecological niches: linking classical and contemporary approaches. University of Chicago Press, Chicago, Illinois, USA.

Cornwell, W. K., D. W. Schwilk, and D. D. Ackerly. 2006. A trait-based test for habitat filtering: Convex hull volume. Ecology 87: 1465–1471.

Cornwell, W. K. and D. Ackerly. 2009. Community assembly and shifts in the distribution of functional trait values across an environmental gradient in coastal California. Ecological monographs 79: 109–126.

Cornwell, W.K. and D.D. Ackerly. 2010. A link between plant traits and abundance: evidence from woody plants in coastal California. Journal of ecology 98: 814–821.

Diamond, J. M. 1975. Assembly of species communities. Pp 342–444, In M. L. Cody and J.M. Diamond (eds.). Ecology and evolution of communities. Harvard University Press, Cambridge, Massachusetts, USA.

Falster, D. S. and M. Westoby. 2005. Alternative height strategies among 45 dicot rain forest species from tropical Queensland, Australia. Journal of ecology 93: 521–535.

Grime, J.P. 1979. Plant strategies and vegetation processes. Wiley, Chichester.

Hardin, G. 1966. The competitive exclusion principle. Science 131: 1292–1297.

Hubbell, S. P. 1997. A unified theory of biogeography and relative species abundance and its application to tropical rain forests and coral reefs. Coral Reefs 16: S9–S21.

Hubbell, S. P. 2001. The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton, New Jersey, USA.

Hulshof, C. and N. Swenson. 2010. Variation in leaf functional trait values within and across individuals and species: an example from a Costa Rican dry forest. Functional ecology 24: 217–223.

Ingram, T. and J. B. Shurin. 2009. Trait-based assembly and phylogenetic structure in northeast Pacific rockfish assemblages. Ecology 90: 2444–2453.

Keddy, P. A. 1992. Assembly and response rules—2 goals for predictive community ecology. Journal of vegetation science 3: 157–164.

Kessler, M. and N. Helme (1999). Floristic diversity and phytogeography of the central Tuichi Valley, an isolated dry forest locality in the Bolivian Andes. Candollea 54: 341–366.

King, D. A., S. J. Davies, and N. S. M. Noor. 2006. Growth and mortality are related to adult tree size in a Malaysian dipterocarp forest. Forest ecology and management 223: 152–158.

Kraft, N. J. B., R. Valencia, and D. Ackerly. 2008. Functional traits and niche-based tree community assembly in an Amazonian forest. Science 322: 58–582.

Kraft, N. J. B. and D. Ackerly. 2010.Functional trait and phylogenetic test of community assembly across spatial scales in an Amazonian forest. Ecological monographs 80: 401–422.

MacArthur, R. H. and R. Levins. 1967. Limiting similarity, convergence and divergence of coexisting species. American naturalist 101: 377–385.

Maharjan, S., L. Pooter, M. Holmgren, F. Bongers, J. Wieringa, and W. Hawthorne. 2011. Plant functional traits and the distribution of West African rain forest tress along the rainfall gradient. Biotropica 43: 552–561.

McGill, B.J., B.J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. Trends of ecology and evolution 21: 178–185.

Müeller, R., S. Beck, and R. Lara. 2002. Vegetación potencial de los bosques de Yungas en Bolivia, basado en datos climáticos. Ecología en Bolivia 37: 5–14.

Navarro, G. and M. Maldonado. 2002. Geografía ecológica de Bolivia: Vegetación y ambientes acuáticos. Centro de Ecología Simón I. Patiño, Departamento de Difusión, Cochabamba–Bolivia.

R Development Core Team.2009. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ricklefs, R. E. and J. Travis. 1980. A morphological approach to the study of avian community organization. Auk 97: 321–338.

Roughgarden, J. 1983. Competition and theory in community ecology. American naturalist 122: 583–601.

Shipley, B. 2010. From plant traits to vegetation structure: chance and selection in the assembly of ecological communities. Cambridge University press. Pp. 36–45.

Storch, D., K. J. Gaston, and J. Cepa´k. 2002. Pink landscapes: 1/f spectra of spatial environmental variability and bird community composition. Proceedings of the Royal Society B: Biological sciences 269: 1791–1796.

Stubbs, W. J. and J. B. Wilson. 2004. Evidence for limiting similarity in a sand dune community. Journal of ecology 92: 557–567.

Swenson, N. G., B. J. Enquist, J. Pither, J. Thompson, and J. K. Zimmerman. 2006. The problem and promise of scale dependency in community phylogenetics. Ecology 87: 2418–2424.

Swenson, N. G., B. J. Enquist, J. Thompson, and J. K. Zimmerman. 2007. The influence of spatial and size scale on phylogenetic relatedness in tropical forest communities. Ecology 88: 1770–1780.

Swenson, N.G., P. Anglada-Cordero, and J.A. Barone. 2010. Deterministic tropical tree community turnover: evidence from patterns of functional beta diversity along an

elevational gradient. Proceedings of the Royal Society B. doi: 10.1098/rspb.2010.1369

Tilman, D. 1988. Plant Strategies and the Structure and Dynamics of Plant Communities. Princeton University Press, Princeton, NJ.

Tilman, D. 2004. Niche tradeoffs, neutrality and community structure: a stochastic theory of resource competition, invasion, and community assembly. Proceedings of the national academy of science (USA) 101: 10854–10861.

Torrez, V. 2008. Estructura y patrones de diversidad en relación a topografía y variables edáficas sector del bosque seco semideciduo subandino (río San Juan, Parque Nacional Madidi, Bolivia). Tesis de Licenciatura. Universidad Mayor de San Andrés.

van der Valk, A. G. 1981. Succession in wetlands: a Gleasonian approach. Ecology 62:688–696.

Webb, C.O., D.D. Ackerly, M.A. McPeek, and M.J. Donoghue. 2002. Phylogenies and community ecology. Annual review of ecology and systematics 33: 475–505.

Weiher, E. and P. A. Keddy. 1999. Ecological assembly rules: perspectives, advances, retreats. Cambridge University Press, Cambridge, UK.

Weiher, E. and P. A. Keddy. 1995. Assembly rules, null models, and trait dispersion: new questions from old patterns. Oikos 74: 159–164.

Weiher, E., G.D.P. Clarke, and P.A. Keddy. 1998. Community assembly rules, morphological dispersion, and the coexistence of plant species. Oikos 81: 309–322.

Westoby, M., D.S. Falster, A.T. Moles, P.A. Vesk, and I.J. Wright. 2002. Plant ecological strategies: some leading dimensions of variation between species. Annual review of ecology and systematics 33: 125–159.

Williamson, M. H. 1987 Are communities ever stable?. pp. 353—371, In Colonization, succession and stability . A. J. Gray, M. J. Crawley, and P. J. Edwards (eds.). Oxford: Blackwell Scientific.

Wright, I.J., P.B. Reich, M. Westoby, D.D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J.H.C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P.K. Groom, J. Gulias, K. Hikosaka, B.B. Lamont, T. Lee, W. Lee, C. Lusk, J.J. Midgley, M. Navas, Ü. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V.I. Pyankov, C. Roumet, S.C. Thomas, M.G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature 428: 821–827.

Appendix

Table 1A. *P*-values of Wilcoxon signed rank test are reported of abundance weighted null model, and absence-presence null model for small and large grain sizes of 1 and 0.1 ha plots for maximum diameter. Numbers in parenthesis are percentage of individual assemblages that had observed metric values below the fifth percentile of the null distribution of metric values.

Grain size (ha)	Habitat filtering	Competitive exclusion			
	Range	Kurtosis	SDNN	SDNNr	SDNDr
Abundance					
1	1 (6.3)	0.68 (12.5)	0.97 (6.3)	0.91(0)	**0.04**(12.5)
0.04	0.95 (0)	0.45(6.3)	0.89 (0)	0.96(0)	0.90(0)
0.1	1 (2.5)	**< 0.001** (8.6)	0.97 (4.9)	1 (4.9)	1 (2.5)
0.01	1 (4.9)	0.17 (4.9)	0.64 (3.7)	0.78 (4.9)	0.21 (2.5)
Absence-presence					
1	1 (0)	**0.0013** (6.3)	**0.042** (6.3)	**0.02 (6.3)**	**0.004** (6.3)
0.04	1 (0)	0.16 (18.8)	0.96 (0)	0.68(0)	0.68(0)
0.1	1 (1.2)	**< 0.001** (3.7)	0.97 (3.7)	0.97 (4.9)	0.35 (3.7)
0.01	1 (1.2)	0.25 (2.5)	0.82 (3.7)	0.23 (8.6)	0.17 (2.5)

Table 1B. *P*-values of Wilcoxon signed rank test are reported of abundance weighted null model, and absence-presence null model for small and large grain sizes of 1 and 0.1 ha plots for maximum height. Numbers in parenthesis are percentage of individual assemblages that had observed metric values below the fifth percentile of the null distribution of metric values.

Grain size (ha)	Habitat filtering	Competitive exclusion			
	Range	Kurtosis	SDNN	SDNNr	SDNDr
Abundance					
1	0.93 (0)	0.51 (0)	0.08 (6.3)	0.11 (0)	0.84 (0)
0.04	0.77 (0)	0.97 (0)	0.87 (6.3)	1 (0)	0.90 (0)
0.1	0.94 (4.9)	**< 0.001** (8.6)	0.07 (8.6)	0.09 (11.1)	1 (1.2)
0.01	0.21 (4.9)	**0.004** (13.6)	0.11 (7.4)	1 (4.9)	1 (2.5)
Absence-presence					
1	**< 0.001** (37.5)	**< 0.001** (68.8)	**< 0.001** (43.8)	**0.01** (12.5)	**0.001** (6.3)
0.04	**0.03** (0)	0.67 (6.3)	0.49 (12.5)	1 (0)	0.98 (0)
0.1	**< 0.001** (7.4)	**< 0.001** (6.2)	**< 0.001** (13.6)	**< 0.001** (14.8)	**< 0.001** (30.9)
0.01	**< 0.001** (1.2)	**< 0.001** (2.5)	**< 0.001** (3.7)	0.96 (17.3)	0.77 (2.5)

Table 1C. *P*-values of Wilcoxon signed rank test are reported for abundance weighted null model and absence-presence null model for small and large grain sizes of 1 and 0.1 ha plots for SLA. Numbers in parenthesis are percentage of individual assemblages that had observed metric values below the fifth percentile of the null distribution of metric values.

Grain size (ha)	Habitat filtering	Competitive exclusion			
	Range	Kurtosis	SDNN	SDNNr	SDNDr
Abundance					
1	1 (0)	0.17 (0)	0.3 (6.25)	1 (0)	0.6 (25)
0.04	0.22 (0)	0.3 (0)	0.9 (0)	1 (0)	0.5 (18.7)
0.1	**< 0.001** (5)	**< 0.001** (8.6)	**< 0.001** (6.2)	1 (0)	1 (11.1)
0.01	**0.02** (6.2)	**< 0.001** (5)	**< 0.001** (3.7)	1 (6.2)	**< 0.001** (48.2)
Absence-presence					
1	0.03 (0)	0.2 (0)	0.14 (6.25)	1 (0)	0.7 (25)
0.04	0.1 (0)	0.4 (6.25)	0.2 (0)	1 (6.25)	0.5 (12.5)
0.1	0.2 (16.05)	**0.008** (15)	0.06 (21)	1 (0)	1 (10)
0.01	0.3 (5)	0.05 (1.2)	0.14 (8.6)	1 (5)	**< 0.001** (54.3)