Specific leaf area: a predictive model using dried samples

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Abstract. Specific leaf area (SLA; fresh-leaf area/dry mass) describes the amount of leaf area for light capture per unit of biomass invested. The standard protocol is simple; however, it requires recently collected sun-exposed leaves to determine fresh-leaf area, limiting where and which samples can be studied. A protocol to predict SLA for fresh leaves from herbarium-dried leaves was developed from samples collected in a dry forest in Bolivia. Leaf area was measured both fresh and dried on the same leaf samples to generate two general mixed-effects models, varying in their inclusion of the position in the crown where the leaf developed. As a test of the potential generality of the models for other systems, we applied them to samples collected in an oak–hickory forest in Missouri, USA. Both models performed well. A recommended protocol for studies predicting SLA from dry leaves was developed. These predictive models and protocols can extend the temporal, geographic, ecological and taxonomic scope of SLA studies.

Introduction

Plants allocate 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). Optimal leaf-tissue allocation varies within and among individuals and species. Inter- and intraspecific variation is driven in part by climatic and topographic differences, such as altitude (Whittaker 1967), latitude (Wright *et al*. 2004), climatic variation (McDonald *et al*. 2003; Ackerly 2004), geology (Whittaker 1967) and soil fertility (McDonald *et al*. 2003; Ackerly and Cornwell 2007), whereas intra-individual variation is driven by microclimatic differences, such as light and wind exposure within the crown (Marshall and Monserud 2003; Koch *et al*. 2004; Sack *et al*. 2006). For instance, leaves exposed to direct sunlight are often small and thick, with a low surface area to mass ratio and high photosynthetic capacity, with the reverse being the case for shade leaves (Rozendaal *et al*. 2006; Hulshof and Swenson 2010; Markesteijn 2010).

Variation in allocation strategies can be understood through measuring morphological and physiological characteristics thought to influence plant performance, i.e. 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 (SLA, or its inverse = leaf mass per area), photosynthetic capacity, nitrogen and phosphorus 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 lifespans and a high SLA are at the quick-returns end of the spectrum, with the converse being true of species at the slow-returns end (Reich *et al*. 1997; Westoby *et al*. 2002; Wright *et al*. 2004).

Among the traits in the leaf-economics spectrum, SLA (fresh-leaf area/dry mass) is one of the easiest to measure and can be readily determined for numerous samples. SLA describes the amount of leaf area for light capture per unit of biomass invested. Although the standard protocol for measuring SLA is simple, it requires access to recently collected sun-exposed leaves to determine fresh-leaf area (Pérez-Harguindeguy *et al*. 2013). This requirement limits the types of samples that can be used to measure SLA; previously collected and dried leaves, such as ecological vouchers and herbarium specimens, are excluded.

Herbarium vouchers are further complicated because microenvironment (e.g. position of the leaf in the crown) is seldom recorded but may influence SLA values. A few studies have recently determined SLA by using the dry-leaf area (SLA_{dry}; Christianson and Niklas 2011; Maharjan *et al*. 2011; Juneau and Tarasoff 2012); however, none of these studies estimated the SLA for fresh-leaf area (SLA_{fresh}), meaning that these measures cannot be compared with the wealth of SLA studies on fresh leaves.

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

We made the following hypotheses: (1) leaf area will be reduced after the leaf is dried; this expectation follows from the fact that ~70% of leaf mass is water (Hopkins 1999); (2) SLA decreases as leaves dry out because water content influences leaf area and SLA variation; SLA from dried samples will be smaller than SLA from fresh samples (Garnier *et al*. 2001); (3) an accurate predictive model for SLA using dried samples should have, as covariates, information about the microenvironment where the leaf developed (e.g. position of the leaf in the crown), because SLA is known to vary with environment (Ackerly 2004; Rozendaal *et al*. 2006; Sack *et al*. 2006).

We developed two models to test the relationship between SLA from fresh and dried leaves. One model is for studies where leaf position in the canopy is known, whereas the second model is for studies where this position is unknown. We tested the generality of our models with an independent dataset from trees in an oak–hickory forest in Missouri, USA.

Materials and methods

Study site

The study was primarily conducted in a dry forest in the Madidi National Park (MNP) in north-eastern Bolivia. The dry-forest area 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 characterised by having a wet and dry season each year, with three extremely dry months from June to August. It has a mean annual temperature of 20.5°C (Navarro 2002) and a mean annual precipitation that varies between 1200 and 1400 mm (Mueller *et al*. 2002). The project 'Floristic inventory of the Madidi region' established 16 1-ha plots in 2005. To examine the influence of the drying process on SLA, four of these plots were selected because of their high species richness and accessibility. 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 include a diverse set of species capturing a wide breadth of phylogenetic and functional traits from this forest, we sampled 102 species that belong to 35 families, with up to eight replicates per species per plot (depending on availability) and two replicates per individual. In total, 541 individuals were sampled across the four plots. To capture the largest amount of intra-crown plasticity (Rozendaal *et al*. 2006; Sack *et al*. 2006; Hulshof and Swenson 2010), two within-individual replicates were collected, one from the top and one from the bottom of the crown (i.e. sun and shade leaves within a given crown). Within each plot, we selected robust and apparently healthy trees of all species that had tagged individuals with accessible crown leaves (via tree climbing). In all cases, we sampled mature and fully expanded leaves with minimal symptoms of pathogens, minimal coverage by epiphylls (lichens, fungi, liverworts) and no signs of senescence or herbivory (Pérez-Harguindeguy *et al*. 2013).

After harvesting, leaf area and mass were determined. Petioles were included in leaf measures (Pérez-Harguindeguy *et al*. 2013). In the case of compound leaf species, one leaflet was harvested and treated as a leaf, because a leaflet is functionally equivalent to a simple leaf (Kraft *et al*. 2008; Baraloto *et al*. 2010; Lebrija-Trejos *et al.* 2010). To obtain fresh-leaf area, top- and bottom-crown leaves were flattened between Plexiglas sheets with a scale bar and photographs were taken with a white background. Leaves were treated as herbarium samples (Bean 2010) to replicate the procedure used for plants deposited in a herbaria. After photographs of fresh-leaf areas were taken, leaf samples were pressed and dried in field stoves.

Once leaves were dried, a second photo was taken to obtain dry-leaf area, following the same procedures as for fresh leaves. Finally, leaves were placed in an oven for 24 h at 60°C and then weighed to obtain dry mass, 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 (Rasband 2011). In addition, we measured leaf thickness at the midpoint of the leaf between major veins with calipers, for both fresh and dried leaves. Two SLA values were obtained for each collected leaf, one using the fresh-leaf area and dividing by its dry mass (= SLA_{fresh}) and the second using the dry-leaf area and dividing by its dry mass (= SLA_{dry}). All data are deposited in the Tropicos database (Missouri Botanical Garden 2012).

Model fitting

Values of SLA showed log-normal distributions and were $\log_{10}$ transformed for all analyses. To test whether the slope differed from 1 and intercept differed from 0 between fresh- and dry-leaf areas and between SLA_{fresh} of top-crown leaves and SLA_{fresh} of bottom-crown leaves, reduced major axis (RMA) regressions were run using the package 'smatr' (Warton *et al*. 2012) in the R programming environment (R Development Core Team 2011). RMA regression analyses were developed to fit a line regardless of which variable is treated as X or Y, thus allowing for presence of error along both X and Y axes (Warton *et al*. 2006; Smith 2009).

To evaluate different models that predict SLA_{fresh} from SLA_{dry}, we used the linear mixed-effects (LME) function available in the R package 'lme4' (Bates *et al*. 2011). General

linear mixed-effects models present a statistical framework that allows simultaneous incorporation of fixed effects (SLA_{dry} and crown position) that we hypothesised *a priori* to influence modelled values for SLA_{fresh}, as well as random effects (species and individuals) that may influence values of SLA_{fresh} 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).

Two models were used to predict SLA_{fresh}. Variables included in the models were discrete (position of the leaf in the crown, individuals and species) and continuous (SLA_{dry}). Species and individuals were considered random effects in all models, with individuals being nested within species, and 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. In Model 1, it was considered as a random effect to generate a predictive model for SLA_{fresh} to be applied to dried samples where the position of collection within the crown is unknown (Table 1). Model 1 assumes that to accurately estimate the parameters of the general model on the basis of data from the present study, position of the leaf in the crown should be added as a random-effect term because it influences the variability of leaf traits and, as a consequence, produces variation in SLA_{fresh} (Sack *et al.* 2006; Hulshof and Swenson 2010). This model has species as a random effect because values of SLA_{fresh} have high interspecific variation (Hulshof and Swenson 2010). Additionally, it has individuals within a species as a random effect, assuming that incorporating among-individual variation in SLA_{fresh} will increase model accuracy. In Model 2, crown leaf position was considered as a fixed effect to generate a model to predict SLA_{fresh} for those dried samples where the crown position is known (Table 1), and species and individuals were added as random-effect terms.

Model selection

Bayesian information criterion (BIC) and Akaike information criterion (AIC; Akaike 1974) were used for model selection. AIC is well suited to 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 penalising for adding additional model parameters (Hilborn and Mangel 1997; Burnham and Anderson 2002). 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, and this penalty term is larger in BIC than in AIC (Bhat and Kumar 2010). Both criteria use the lowest value to identify the best model (Hilborn and Mangel 1997; Bhat

and Kumar 2010). In addition to AIC and BIC, we used analysis of variance (ANOVA) to compare fit between nested models (i.e. Model 1 *versus* Model 2).

We 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). We used the package 'lme4' to run 1000 simulations, each having 1000 iterations. Only parameter estimates obtained in the 1000th iteration of each simulation were kept as part of the sampling distribution (Manly 1997).

Model testing

Our objective was to develop an accurate model to predict SLA_{fresh} from dried leaf samples. To determine whether the models could potentially be extended to predict SLA_{fresh} from dried leaf samples for plant species from locations other than the Bolivian dry forests, we sampled plants from a temperate deciduous oak–hickory forest at Washington University in St. Louis' Tyson Research Center located in Eureka, Missouri, USA, as a test dataset. We harvested leaves from five individuals per species (*Quercus alba* L., *Fraxinus americana* L., *Celtis occidentalis* L., *Lonicera japonica* Thunb. ex Murray, and *Juglans nigra* L.). For each individual, we 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. We performed RMA to determine the strength of the linear relationship between the predicted SLA_{fresh} and the actual SLA_{fresh}. We expected that if the predictive models generated were accurate, the intercept of the regression would not deviate significantly from 0 and the slope would not deviate significantly from 1. Additionally, we compared the degree of fit between Models 1 and 2 by using an ANOVA.

Results

Values of SLA_{fresh} from the Bolivian dry forest ranged from $5.3 \, m^2 \, kg^{-1}$ (*Calliandra chulumania* Barneby, Fabaceae) to $23.7 \, m^2 \, kg^{-1}$ (*Phyllostylon rhamnoides* (J.Poiss) Taub., Ulmaceae). Variation in SLA_{fresh} values was mainly explained by interspecific (50.3%) differences, with smaller contributions from among-individual (19.7%) and within-individual (30.0%) differences. Although within-individual variation was high, it should be remembered that samples within an individual were selected to represent the most extreme values. When regressing SLA_{fresh} of top-crown leaves onto SLA_{fresh} of bottom-crown

Table 1. Candidate models and estimated parameters of the candidate models developed for 109 species from Madidi National Park, Bolivia

$SLA_{fresh} =$ fresh-leaf area/dry mass ($m^2 \, kg^{-1}$); $SLA_{dry} =$ dry-leaf area/dry mass ($m^2 \, kg^{-1}$). LP, leaf position in the crown; species, list of species names; individuals, individual number per species

Model	a	b	c
Model 1: $logSLA_{fresh} = a + blogSLA_{dry} + species^A + individuals^A + LP^A$	−0.18	0.88	
Model 2: $logSLA_{fresh} = a + blogSLA_{dry} + cLP + species^A + individuals^A$	−0.16	0.88	0.04

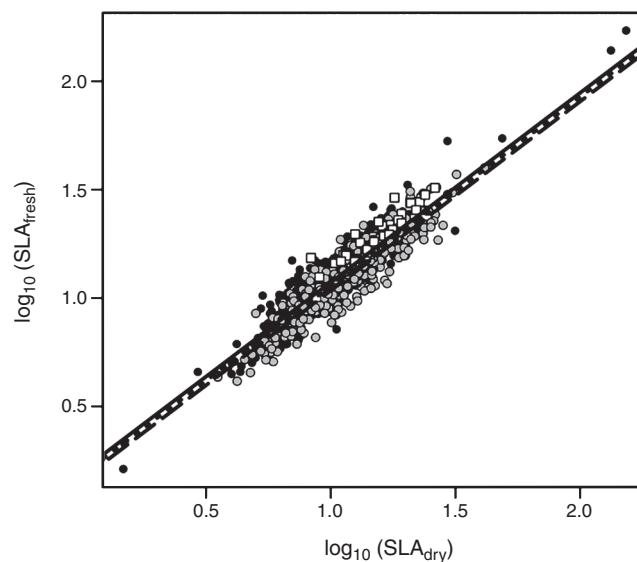[A]The variable was considered a random-effects term in the model.

leaves, the slope ($b = 1.2$) was significantly ($P < 0.001$) different from 1 and the intercept ($a = -0.2$) was significantly ($P < 0.001$) different from 0. Similarly, the slope ($b = 1.1$) of the regression of fresh- onto dry-leaf area was significantly ($P < 0.001$) different from 1 and the intercept ($a = 5.9 \times 10^{-5}$) was significantly ($P = 0.006$) different from 0 (Fig. 1).

Across the dataset, shrinkage during the drying process of the samples was on average 16%. Interestingly, a small proportion (~14%) of the leaves seemingly gained leaf area during the drying process. On average, those leaves that lost area lost 380 ± 587 mm^2 and those leaves that gained area gained 235 ± 397 mm^2. We tested whether variation in shrinkage was associated with other leaf traits, namely leaf thickness, leaf area and SLA$_{fresh}$, and found that variation in shrinkage was unrelated to variation in any of these other leaf traits ($P > 0.05$; data not shown). Leaves that gained in area had both thin and thick leaves, and both big and small leaf areas and SLA$_{fresh}$.

SLA-predictive models

Two candidate models were generated to predict SLA$_{fresh}$. Model 1 was built to predict SLA$_{fresh}$ from dried samples that lack information about where in the crown they were collected (typical for herbarium samples), and Model 2 was built to predict SLA$_{fresh}$ from dried leaf samples that have information about from where in the crown the leaves where collected. We compared the two models to gauge the importance of information on crown position when it is available, using AIC, BIC and ANOVA. Model 2 (AIC = −3269.2, BIC = −3239.2) performed significantly ($P < 0.001$) better than did Model 1 (AIC = −3279.1, BIC = −3249.3). These results suggested that a more accurate prediction of SLA$_{fresh}$ for samples from MNP is obtained from dried leaf samples when it is known from where in the crown the samples were collected. It should be noted, however, that although Model 2 was significantly more accurate, differences in model-parameter estimates were small (Table 1).

We estimated the sampling distribution of the parameters in the models to determine the 95% confidence region. We obtained a sample from the Bayesian posterior distribution of the parameter estimates (a and b) for both selected models by using MCMC methods. For both Models 1 and 2, a high number of points were concentrated near the mean points (Table 2). The slopes did not differ between the models, whereas the intercepts were significantly different, although this difference was small. The bivariate distributions of the 1000 parameter estimates for Models 1 and 2 were positively correlated; the covariance of the parameters was also positive (Table 2), indicating that a increases with an increasing b.

Application of the SLA-predictive models

The third objective of the study was to propose the application of these predictive models to other datasets. To test the potential for extending our models to other systems, we applied the models obtained from data gathered in Bolivia to our test dataset. We determined the degree to which the predicted SLA$_{fresh}$ correlated with the actual SLA$_{fresh}$. The range of SLA$_{fresh}$ for samples collected in the temperate forest was within the range of SLA$_{fresh}$ values for samples collected in Bolivia (Fig. 1).

When regressing the actual SLA$_{fresh}$ onto predicted SLA$_{fresh}$, both predictive models (from Models 1 and 2) had slopes not significantly different from 1, intercepts not significantly different from 0, and $R^2 > 0.80$ (Fig. 2). Additionally, we regressed SLA$_{fresh}$ top-crown leaves onto SLA$_{fresh}$ bottom-crown leaves from the experiment and found that they were not significantly different from a slope of 1 and an intercept of 0 (slope = 1.12, $P = 0.1$; intercept = −0.08, $P = 0.4$). Because the R^2-value was larger for Model 1 than for Model 2 (Fig. 2) and because the slope and intercept of top- and bottom-crown leaves did not differ from isometry, we concluded that Model 1 predicts SLA$_{fresh}$ for dried leaf samples more accurately than does Model 2.

Discussion
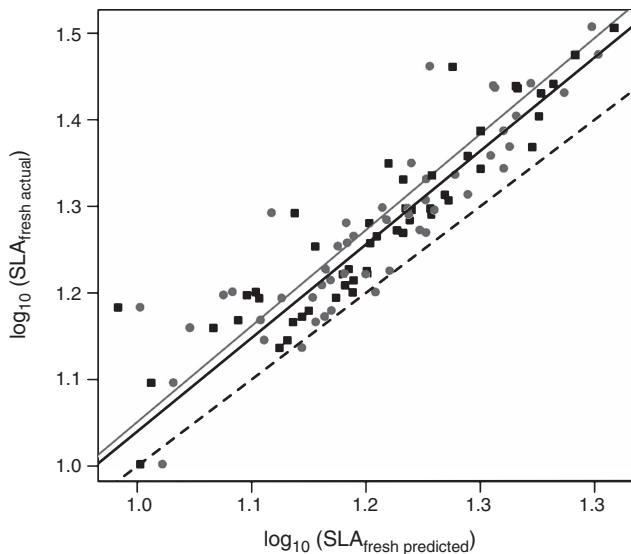
Specific fresh-leaf area, SLA$_{fresh}$, is an easy to measure functional trait that provides insight into leaf allocation and function

Fig. 1. The regression between SLA$_{dry}$ and SLA$_{fresh}$ for 109 species from Madidi National Park, Bolivia, and five species from Tyson Research Center, Eureka, Missouri, USA. For the Bolivian data, grey circles denote bottom-crown leaves, and black circles denote top-crown leaves. The dotted line corresponds to Model 1, the solid line corresponds to Model 2 from top-crown leaves, and the dashed line corresponds to Model 2 from bottom-crown leaves. The USA data are denoted by white squares.

Table 2.   Mean, variance and covariance values of the samples generated from the Bayesian posterior distribution of the parameters (a and b) for Models 1 and 2, using Markov Chain Monte Carlo methods for 109 species from Madidi National Park, Bolivia

Model	Mean of a	Variance of a	Mean of b	Variance of b	Covariance of a and b
Model 1	−0.18	0.005	0.88	0.00016	−0.0001
Model 2	−0.17	0.0002	0.88	0.00017	−0.0002

Fig. 2. The correlation of the actual specific fresh-leaf area (SLA$_{fresh}$) *versus* the predicted SLA$_{fresh}$ for five species from USA. When regressing the actual SLA$_{fresh}$ onto the predicted SLA$_{fresh}$ with Model 1, the slope was 1.09 (confidence intervals: 0.96–1.24), the intercept was –0.05 (confidence intervals: –0.21–0.12), and the R^2 was 0.84. When regressing the actual SLA$_{fresh}$ onto the predicted SLA$_{fresh}$ with Model 2, the slope was 1.12 (confidence intervals: 0.97–1.30), the intercept was –0.08 (confidence intervals: –0.27–0.12), and the R^2 was 0.80. Black squares are values predicted by Model 1 and grey dots are values predicted by Model 2. The black line is the regression line of the relation between the actual log$_{10}$(SLA$_{fresh}$) and the predicted log$_{10}$(SLA$_{fresh}$) that was predicted by Model 1, and the grey line is the regression line of the relation between the actual log$_{10}$(SLA$_{fresh}$) and the predicted log$_{10}$(SLA$_{fresh}$) that was predicted by Model 2. The dashed line shows the 1:1 ratio between the actual log$_{10}$(SLA$_{fresh}$) and the predicted log$_{10}$(SLA$_{fresh}$).

(Ackerly 2004; Kraft *et al.* 2008; Cornwell and Ackerly 2009; Pérez-Harguindeguy *et al.* 2013). The main drawback of the existing SLA$_{fresh}$ protocol is the requirement for measures of fresh-leaf area, limiting the types of samples that can be used. In the present study, using samples from a Bolivian dry forest, we generated two models to predict SLA$_{fresh}$ from dried leaf samples. One model requires information about from where in the crown the leaf was collected, whereas the other model does not require crown-position knowledge. The generality of the models was tested on data collected in a very different temperate deciduous forest. Although both models performed well, the simpler model – not requiring information about crown position (Model 1) – provided the best prediction of SLA$_{fresh}$ in this forest. We believe that we have established models that should be applicable to other datasets. We have also established an easy-to-follow protocol for researchers wanting to determine equations specific to their study species.

Amendments to the SLA protocol for dry-leaf samples

Although the standard protocol for SLA$_{fresh}$ (Pérez-Harguindeguy *et al.* 2013) provides a useful tool for researchers, we propose the following modifications. These modifications make the protocol more accessible for researchers working with dried leaf samples (e.g. samples

stored in herbaria) to obtain SLA$_{fresh}$ data that they can compare with those from other studies. First, we suggest that 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, we suggest that the measures of the area of the dried leaf be taken as explained in the measurement of fresh-leaf area in the protocol of Pérez-Harguindeguy *et al.* (2013). After the area is measured, the leaf should be placed in an oven at 60°C for 24 h and weighed. SLA$_{dry}$ can be obtained by dividing dry-leaf area by dry-leaf mass. This value can be used in the following equation, which was constructed through general linear mixed-effects models (Table 1), to obtain predicted SLA$_{fresh}$:

$$\log_{10}(\text{SLA}_{fresh}) = -0.17 + 0.88(\log_{10}(\text{SLA}_{dry})) \qquad (1)$$

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 and researchers should determine whether SLA is higher, lower or not significantly different between the top and the bottom of the crown in their study species. Additionally, if top-crown sun-exposed leaves are not accessible for sampling, bottom-crown shade leaves should be collected and differences between the SLA inside and the SLA outside the crown be determined. The samples should be processed as mentioned above. In either case, SLA$_{fresh}$ should be predicted using the following equation, as determined through general linear mixed-effects models (Table 1):

$$\log_{10}(\text{SLA}_{fresh}) = -0.18 + 0.88(\log_{10}(\text{SLA}_{dry})) \\ + 0.04(\text{crown position}) \qquad (2)$$

When SLA is higher at the top, Eqn 2 should be used and a value of 1 input for top leaves and a value of 0 input for bottom leaves for the crown-position term. When SLA is higher at the bottom, Eqn 2 should be used and a value of 0 input for top leaves and a value of 1 input for bottom leaves for the crown-position term. When SLA is not different between the top and bottom of the crown, Eqn 1 should be used.

Effects of drying on leaf area

Because leaves contain a large amount of water, we hypothesised that SLA$_{dry}$ would be smaller than SLA$_{fresh}$. In the dataset, we found that fresh-leaf area was significantly greater than dry-leaf area, which was not surprising. Whereas the predicted tendency was found across the entire dataset, 14% of the collected samples gained rather than lost leaf area during the drying process. Several explanations are possible for this result. For instance, it could be that leaves that gained area were thicker and, when pressed while drying, they added area; however, no relationship between the change in leaf area with drying and leaf thickness was found. Additionally, leaf area increased in both simple and compound leaf species, from leaves collected from the bottom and top of the crown, and from leaves with large and small fresh-leaf areas and SLAs. We did find that image quality was lower in samples that increased in leaf area as they dried. The program ImageJ, which we used to measure the leaf area, has a threshold tool that selects

the object 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 quality 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 avoid such problems is to photograph leaves against a contrasting background with continuous light across the sample.

Leaf crown position

Our third expectation was that an accurate predictive model of SLA_fresh for dried samples should have covariates that describe the environment where the leaf developed, such as position of the leaf in the crown. The model including crown position (Model 2) was a better fit to the data for samples from the tropical Bolivian forest. However, for samples from the temperate deciduous oak–hickory forest, Model 1, which lacked information on crown position, was more accurate in predicting SLA_fresh than was Model 2. Our results showed that these species from this forest did not differ significantly in SLA_fresh between top- and bottom-crown leaves. This finding was surprising because the bottom- and top-crown values of SLA_fresh were significantly different in other datasets (Wright *et al.* 2007; Hulshof and Swenson 2010; the present study from Bolivia). A possible explanation for these differences could be that temperate deciduous forests in USA have crowns that are less stratified in their irradiance. Additionally, it should be noted that even for the Bolivian species, Model 2 did not lead to large shifts in the model parameters, suggesting that crown position had a significant but modest influence on SLA_fresh. When available, we suggest that both models should be tested on data from other ecosystems. We expect that Model 2 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 a lower SLA_fresh for bottom-crown leaves (shade) than for top-crown leaves (sun-exposed). 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 the USA were not significantly ($P = 0.7$) different. These findings do not support the general trend in other studies in which sun leaves had lower SLA_fresh than shade leaves (Rozendaal *et al.* 2006; Sack *et al.* 2006; Hulshof and Swenson 2010). However, studies have reported species with shade leaves with a lower SLA_fresh than that of sun leaves (Talbert and Holch 1957; Niinemets and Kull 1994; Carr 2000; Richardson *et al.* 2000). On the basis of these different results, we recommend that intra-crown SLA_fresh differences should be evaluated when possible.

Top-crown leaves are difficult to collect in tropical forests

Considering that upper-crown sun leaves are difficult to collect in most tropical forests because of their tall stature, standardised methods such as the SLA_fresh protocol proposed by Pérez-Harguindeguy *et al.* (2013) may be difficult to apply. In most situations, it will be easier to collect leaves from bottom-crown branches exposed to sun. Sack *et al.* (2006) reported that the variation in SLA_fresh between top-crown sun leaves and bottom-crown sun leaves is minimal and that more variation is explained by differences in irradiance. They found that bottom-crown

internal leaves (shade) differ strongly from bottom-crown external leaves (sun-exposed). Considering these results and the height of many tropical forest trees, we suggest that bottom-crown external leaves (sun-exposed) may be easier to collect and bottom-crown exterior *versus* interior leaves are likely to represent the extremes in SLA_fresh through the crown of the tree. However, it would be useful to explicitly test this expectation.

Increasing the value of herbaria collections

In our study, we have shown that dried samples from herbaria can be used to predict functional leaf traits, such as leaf size and SLA, allowing researchers to place values of these sample traits into a comparative context. We suggest that such ecological uses of herbaria samples are value adding to both the use of herbaria and studies of functional traits. Herbaria samples capture potential variation in trait values over large ranges in space and time. Such studies should increase the utility of herbaria collections, and hopefully the importance of such studies can be leveraged to increase herbaria budgets. However, to allow ecologists to non-destructively make use of such samples, herbaria should consider other mounting methods besides gluing specimens to paper. Glue can add mass to the leaves and make it difficult to separate samples from paper. We recommend specimens be sewn to paper. In cases where gluing is necessary, we recommend the use of a water-soluble glue or a replicate leaf, with its petiole being placed in an envelope with the specimen.

In the present study, we developed two models that can be used to predict SLA_fresh from dried leaf samples. Both models have been applied to a dry tropical and deciduous temperate forest. On the basis of our results, we believe that they should prove applicable across different study systems; however, we recommend that they be validated when possible in other systems, to test their generality (e.g. the importance of crown position for prediction may differ). Additionally, users should keep in mind when applying the predictive models that they worked well for our study, explaining 80–85% of the variation; however, 15–20% of the variation remained unexplained. Depending on how SLA values are used, these errors have the potential to propagate in further analyses.

A useful application of these SLA models is that they allow data collected from herbarium samples or from samples collected in remote locations, in situations where portable scanners are not available, to be compared with data from other studies around the world. For instance, herbaria samples could be used to ask questions about shifts in SLA over time as a result of climate change. Recently, Guerin and Lowe (2012) used historical herbarium specimens to study the variation of leaf width over time as a result of climate change and compared those records with recently collected samples in South Australia; they found that leaf width had decreased by 2 mm over 127 years. Additionally, herbarium samples can be used to evaluate the amount of intraspecific variation across large geographic ranges. These predictive models extend the temporal, geographic, ecological and taxonomic scope of SLA studies.

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