

BRIEF COMMUNICATION

Herbarium-based measurements reliably estimate three functional traits

Timothy M. Perez^{1,2,5} , Jessica Rodriguez³, and J. Mason Heberling⁴ 

Manuscript received 19 March 2020; revision accepted 25 June 2020.

¹ Department of Biology, University of Miami, Coral Gables, FL 33146, USA

² Fairchild Tropical Botanic Garden, Coral Gables, FL 33156, USA

³ Department of Earth and Environment, Florida International University, Miami, FL 33199, USA

⁴ Section of Botany, Carnegie Museum of Natural History, Pittsburgh, PA 15213, USA

⁵ Author for correspondence (e-mail: t.more.perez@gmail.com)

Citation: Perez, T. M., J. Rodriguez, and J. M. Heberling. 2020. Herbarium-based measurements reliably estimate three functional traits. *American Journal of Botany* 107(10): 1–8.

doi:10.1002/ajb2.1535

PREMISE: The use of functional traits has surged in recent decades, providing new insights ranging from individual plant fitness to ecosystem processes. Global plant trait databases have advanced our understanding of plant functional diversity, but they remain incomplete because of geographic and taxonomic biases. Herbarium specimens may help fill these gaps by providing trait information across space and time. We tested whether herbarium specimen-derived measurements are reliable estimates of three important, commonly measured functional traits—specific leaf area (SLA), branch wood specific gravity, and leaf thickness.

METHODS: Leaves and branches were collected from species cultivated at Fairchild Tropical Botanic Garden and Florida International University in Miami, FL, USA. Fresh components of SLA (area), branch wood specific gravity (volume), and leaf thickness were measured following standard trait measurement protocols. We compared these trait values to corresponding measurements using plant tissues dried in a plant press following standard herbarium plant collecting protocols.

RESULTS: Herbarium-derived trait measurements (dried tissues) were highly correlated with those measured using fresh tissues following standard protocols (SLA: $R^2 = 0.72–0.97$, $p < 0.01$; wood specific gravity: $R^2 = 0.74–0.75$, $p < 0.01$; leaf thickness: $R^2 = 0.96$, $p < 0.01$). However, except for leaf thickness, linear model slope or intercept coefficients differed from 1, indicating herbarium-derived trait measurements may provide biased estimates of fresh traits without the use of correction factors.

CONCLUSIONS: Herbarium-derived traits cannot always be used interchangeably with those measured from fresh tissues because of tissue shrinkage. However, herbarium-derived trait data still have the potential to drastically expand the temporal, geographic, and taxonomic scope of global trait databases.

KEY WORDS functional traits; museum; shrinkage; plant physiology; plant ecology; climate change; wood specific gravity; specific leaf area; leaf thickness.

Functional traits refer to any morpho-physio-phenological characters that serve as proxies for understanding individual fitness and can be scaled up to study ecosystem processes (Violle et al., 2007). Because of the ecophysiological information these functional traits portray, they have broad ecological applications and are aggregated into databases (e.g., TRY [Kattge et al., 2020; www.try-db.org]; and

Botanical Information and Ecology Network (BIEN) [Maitner et al., 2018; <http://bien.nceas.ucsb.edu/bien/>]). These databases are often used in large-scale analyses to study plant functional diversity (Díaz et al., 2015), and to parameterize global vegetation models that predict patterns in species distributions and biogeochemical cycles (Scheiter et al., 2013).

Despite the rise of functional ecology as a discipline, the coverage of functional trait databases is far from complete, with geographic and taxonomic biases that limit our understanding of global functional plant diversity (Kattge et al., 2020). A recent analysis showed that at least one functional trait is available for 45–50% of equatorial species, and up to 65% of species in temperate regions (Cornwell et al., 2019). When one common, relatively easily measured trait like SLA is considered, however, the proportion of species with available data drops to merely 3.3% in Neotropical species, and 10.4% of New World temperate species (Perez et al., 2019). Moreover, trait databases are only likely to possess a few trait estimates per species, which limits trait database utility for understanding intraspecific variation that is fundamental to ecological and evolutionary theory (Violle et al., 2012; Siefert et al., 2015). Clearly, there is a need for additional functional trait data to advance our understanding of global plant functional diversity at inter- and intraspecific levels across space, time, and phylogeny.

With more than 390 million specimens collected from every continent dating back to over 300 years (Thiers, 2020), herbaria are potentially rich sources of functional trait data that could fill existing data gaps. Herbarium specimens were first collected for the primary purpose of documenting and describing plant taxonomic diversity, but are increasingly used in novel ways, including studying functional traits (Heberling et al., 2019). Although a growing number of studies have used specimens to measure functional traits across space and time (Heberling and Isaac, 2017), it is unclear if functional traits measured using dried tissues (i.e., herbarium specimens) can be reliably used alongside standard trait measurements using fresh tissues. Blonder et al. (2012) found that leaf area and thickness shrinkage can occur upon drying, but fresh trait estimates can be partially predicted from leaf size, thickness, the possession of evergreen leaves, and woodiness. Similarly, Queensborough and Porras (2014) found leaf shrinkage in dried leaves, but suggested SLA measurements using dried leaves could be used cautiously. The immense diversity of plant forms likely affects which trait estimates are robust to dry- vs. fresh-measurement and the reliability with which herbarium-derived traits should vary by species and traits. Additional bias may be introduced into trait estimates simply because of the nature of herbarium specimens (e.g., limited tissue size, quantity, and quality), which were not initially intended for trait sampling. Given that herbarium specimens are likely tissue limited and that preserving specimens is a priority for herbarium staff, tissue fragments (e.g., partial leaf specimen samples) may provide the only source material for functional trait measurement. Typically, leaf fragments lead to overestimates of SLA (Pérez-Harguindeguy et al., 2013), which might be compounded by leaf area shrinkage of dried herbarium specimens, but effects of both on trait measurements have not been investigated.

Here, we test whether three commonly measured functional traits, (1) SLA; cm^2/g), (2) wood specific gravity (G_o ; dimensionless), and (3) leaf thickness (mm), can be reliably measured from herbarium specimens. These three traits define major axes of the global spectrum of plant form and function, and are widely studied in plant ecophysiology (Baraloto et al., 2010; Díaz et al., 2015). SLA is a core trait in the leaf economic spectrum, represents the dry mass invested in leaves, and is proportional to other leaf traits including carbon assimilation rates (Wright et al., 2004; Pérez-Harguindeguy et al., 2013). SLA is typically measured as the fresh one-sided leaf area of whole leaves divided by dry leaf mass. The

G_o , provides information about plant support, carbon storage, and nutrient translocation (Chave et al., 2009); is measured by dividing dried wood mass by dried wood volume; and volume is typically determined with the water displacement method, which requires tissue to be submerged in water (Williamson and Wiemann, 2010). Potential water damage to specimens could be avoided by alternatively calculating wood volume using the dimensions of the wood tissues. Furthermore, most standard herbarium specimens of woody species only include branches. However, branch wood specific gravity is highly correlated with stem wood specific gravity (Swenson and Enquist, 2008). Lastly, leaf thickness modulates leaf temperature (Leigh et al., 2012), influences gas exchange (Parkhurst, 1978), and contributes to leaf toughness—a trait that deters herbivores (Kitajima and Poorter, 2010). Yet, detached leaves rapidly lose the turgidity that causes reductions in leaf thickness, which may make herbarium-derived estimates of leaf thickness subject to bias.

MATERIALS AND METHODS

Study site and species selection

To address our research questions, we collected plant tissues from individuals grown at Fairchild Tropical Botanic Garden (FTBG; 25.677N, –80.275W) in Coral Gables, Florida, USA, and the campus of Florida International University (FIU; 25.758N, –80.373W) in Miami, Florida, USA. The species we studied were selected because they are commonly cultivated at both collection locations and represent a diversity of different plant families (Table 1). All plant tissues were collected from fully sun-exposed branches of each individual, and recently expanded mature leaf tissues were collected from each branch to minimize the influence of environmentally induced trait plasticity.

Specific leaf area

We measured SLA using whole leaves with petioles included and leaf hole-punches for a total of 15 woody species (following Pérez-Harguindeguy et al., 2013). For species with compound leaves, only leaflets and their petiolules were collected. We collected leaves from individuals grown at FTBG and the campus of FIU. Three leaves were sampled from one to three individuals per species and immediately brought back to a lab on FIU's campus. Fresh leaves were scanned and measured using ImageJ (Rueden et al., 2017). After leaves were scanned, one leaf disk $\sim 2.9 \text{ cm}^2$ was taken from half-way between the proximal and distal ends, and half-way between the midrib and margin of each leaf. Leaf-disk area was similarly measured using ImageJ (Rueden et al., 2017). Leaf disks were used in an effort to explore methods that mitigate damage to herbarium specimens when measuring SLA. Fresh leaves and their disks were dried in newspaper within a plant press for at least one week at $\sim 70^\circ\text{C}$. After drying, whole leaves and their disks were scanned a second time to determine dry leaf area. We then measured whole-leaf (including disk) and disk-only mass using a Mettler ME-T Analytical Balance (Mettler-Toledo, Columbus, Ohio, USA). Fresh whole-leaf area and fresh leaf-disk areas were divided by their respective dry weights to determine two different metrics of SLA (i.e., SLA using fresh whole-leaf area and SLA using fresh leaf-disk area). Herbarium-derived SLA measurements were similarly made using the leaf area of dried tissues.

Branch specific gravity

We collected one branch from one to three individuals of 19 different species from FTBG and the campus of FIU. We attempted to standardize our measurements by collecting branches roughly 1 cm in diameter following Swenson and Enquist (2008). In the lab, bark was removed from freshly collected branches and cut to 5 cm in length. Basic branch specific gravity (G_b) was calculated as branch dry mass divided by fresh branch volume (Williamson and Wiemann, 2010). We calculated G_b using two different estimates of fresh volume. First, we used the water displacement method, in which we submerged branches just below the surface of water in a graduated cylinder using a dissecting pin. Using a graduated cylinder placed on a Mettler ME-T Analytical Balance (Mettler-Toledo, Columbus, Ohio, USA), fresh volume was measured once branches were completely submerged. Second, we measured fresh branch volume using the dimensional method, in which the volume of a cylinder was calculated using branch length and width. After fresh branch volumes were measured using both methods, branches were dried in a drying oven (102°C) for several days until we observed no decreases in branch mass. Each dried branch was then weighed to calculate G_b using the displacement method, and G_b using the dimensional method. Once branches were dry, we remeasured their dimensions to calculate a third metric of wood specific gravity, the oven-dry branch specific gravity (G_o ; Williamson and Weiman, 2010). The G_o was calculated by dividing the oven dry branch mass by the oven-dry volume of a cylinder following the dimensional method described above.

Leaf thickness

We collected three leaves from the outer canopies of 11 different species. Leaves were immediately brought back to the anatomy laboratory at FTBG and divided longitudinally along their midribs. One half of a leaf was used to measure fresh leaf thickness and the other half of the leaf was placed in a plant press and dried in an oven at ~70°C for at least 1 week. Leaf cross sections were made on fresh leaves using a disposable razor blade broken in half, and its two sharp edges were held together while gently slicing through leaf tissue placed between two layers of Parafilm (Bemis Company Inc., Oshkosh, Wisconsin, USA) in a Petri dish with water. We wet-mounted and imaged three representative cross sections per leaf. Microphotographs were calibrated with a stage micrometer before thicknesses (i.e., the distance between abaxial and adaxial leaf surfaces) were measured in ImageJ (Rueden et al., 2017). We followed the procedure above to calculate the thickness of the dried leaves after allowing them to rehydrate in a solution of roughly 1:50 household dish detergent and water for approximately 20 min. (Detergent is commonly used to improve rehydration of dried plant materials for anatomical study [e.g., Romero et al., 2019].)

Data analyses

The relatively small size of our dataset precluded us from identifying any meaningful phylogenetic signal in the traits we measured (Blomberg et al., 2003), so we used linear mixed models to account for any nonindependence in our data caused by sampling within families, genera, or species. The influence of family, genus, or species on our results were treated as nested random effects for each trait comparison. For each model, we included terms that allowed

the y -intercept and slope to vary among each nested factor. The importance of these random effects were explored using the lmerTest R package's stepwise model selection procedure to determine final models (Kuznetsova et al., 2017). This procedure iteratively adds or subtracts random and fixed effects using analysis of variation (ANOVA)-like and ANOVA procedures, respectively, to select coefficients that significantly improve model fit while minimizing Akaike information criterion (AIC) values.

Linear mixed effects models were constructed for SLA, branch specific gravity, and leaf thickness. For SLA, we tested the ability of the dry whole-leaf SLA to predict the fresh whole-leaf SLA, and dry leaf-disk SLA to predict fresh-disk SLA. We also tested if fresh leaf-disk area SLA predicted fresh whole-leaf area SLA, and if dry leaf-disk area SLA could predict dry SLA determined with fresh whole leaves. For branch wood specific gravity, we compared potential interchangeability of G_b using the displacement method and G_b using the dimensional method. We then tested the ability of G_o to predict G_b using the displacement method. For both comparisons, our full linear mixed effect model used all branch samples with nested random effects of family, genus, and species. Similarly, we used dried and rehydrated leaf thickness to predict fresh leaf thickness for each leaf with a model that used nested random effects of family and species.

The “lme4” R package was used to construct our linear mixed models (Bates et al., 2015). We used the “sjPlot” R package to generate 95% confidence intervals and p -values for the parameters of each final model (Lüdtke, 2018). All analyses were performed in R (version 3.6.3, R Core Team, 2020).

TABLE 1. Study species and associated trait datasets.

Species and Authorities	Family	Trait Measured
<i>Alvaradoa amorphoides</i> Liebm.	Picramniaceae	LT
<i>Ardisia escallonioides</i> Schlttdl. & Cham.	Primulaceae	LT
<i>Boufferea havanensis</i> (Willd.) Miers	Boraginaceae	LT
<i>Brosimum alicastrum</i> Sw.	Moraceae	SLA, BSG
<i>Bucida buceras</i> L.	Combretaceae	SLA, BSG
<i>Cananga odorata</i> (Lam.) Hook.f. & Thomson	Annonaceae	SLA, BSG
<i>Ceiba pentandra</i> (L.) Gaertn.	Malvaceae	BSG
<i>Chamaedorea seifrizii</i> Burret	Arecaceae	SLA, BSG
<i>Chrysophyllum cainito</i> L.	Sapotaceae	SLA, BSG
<i>Coccoloba diversifolia</i> Jacq.	Polygonaceae	BSG
<i>Coccoloba uvifera</i> (L.) L.	Polygonaceae	SLA, BSG
<i>Cynophalla flexuosa</i> (L.) J.Presl	Capparaceae	LT
<i>Dodonaea viscosa</i> (L.) Jacq.	Sapindaceae	LT
<i>Eugenia rhombea</i> (O.Berg) Krug & Urb.	Myrtaceae	LT
<i>Ficus benghalensis</i> L.	Moraceae	BSG
<i>Guaiacum sanctum</i> L.	Zygophyllaceae	LT
<i>Gymnanthes lucida</i> Sw.	Euphorbiaceae	LT
<i>Hamelia patens</i> Jacq.	Rubiaceae	SLA, BSG, LT*
<i>Krugiodendron ferreum</i> (Vahl) Urb.	Rhamnaceae	LT
<i>Mimusops elengi</i> L.	Sapotaceae	BSG
<i>Pachystachys lutea</i> Nees	Acanthaceae	SLA, BSG
<i>Piper methysticum</i> G.Forst.	Piperaceae	SLA, BSG
<i>Piper neesianum</i> C. DC.	Piperaceae	SLA, BSG
<i>Psychotria nervosa</i> Sw.	Rubiaceae	SLA, BSG, LT
<i>Psychotria viridis</i> Ruiz & Pav.	Rubiaceae	SLA, BSG
<i>Quercus virginiana</i> Mill.	Fagaceae	SLA, BSG
<i>Swietenia mahagoni</i> (L.) Jacq.	Meliaceae	SLA, BSG
<i>Trema micrantha</i> (L.) Blume	Cannabaceae	SLA, BSG

SLA, Specific leaf area; BSG, Branch Specific Gravity; LT, Leaf Thickness; *, intraspecific data

RESULTS

Specific leaf area

Our estimates of SLA (range: ~50–500 cm² g⁻¹) encompassed a large portion of the global variation in this trait (10–1000 cm² g⁻¹, Wright et al., 2004). Herbarium-derived SLA (calculated using dried

whole-leaf area) was highly correlated with standard measurements of SLA based on whole-leaf area of fresh leaves (marginal and conditional R² separated by “/” = 0.96/0.99, *P* < 0.01; Fig. 1A). Because leaf tissue is limited in many herbarium specimens, we also measured SLA using 2.9 cm² leaf disks. Similar to whole leaf measurements, the herbarium-derived SLA of dried leaf disks were similarly highly predictive of fresh leaf-disk area SLA (marginal/conditional

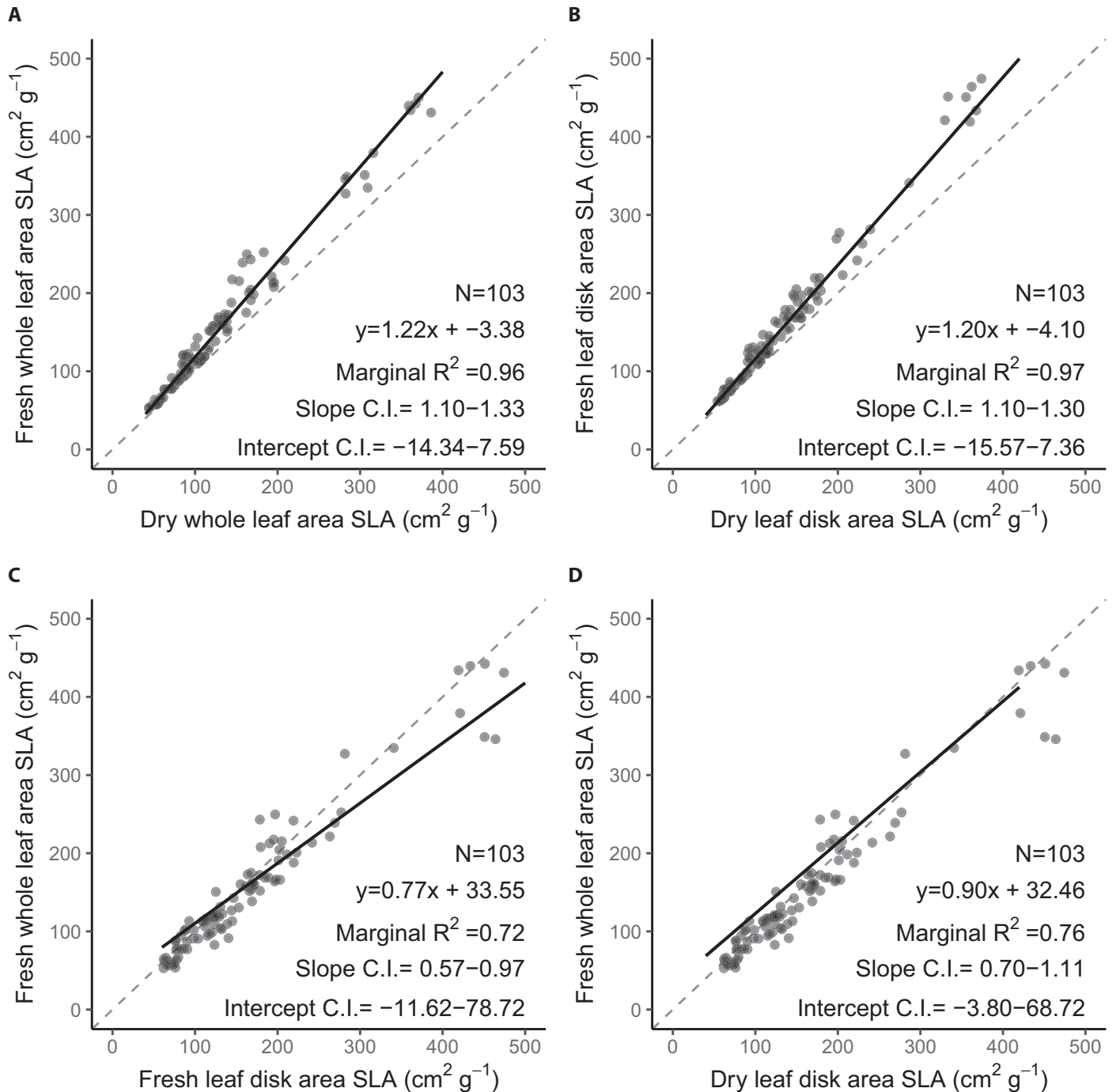


FIGURE 1. Main effects of the linear mixed models comparing different metrics of SLA: (A) fresh whole-leaf SLA vs. dry whole-leaf area SLA (B) fresh leaf-disk area SLA vs. dry leaf-disk area SLA (C) fresh whole-leaf area SLA vs. fresh leaf-disk area SLA, and, (D) fresh whole-leaf area SLA vs. dry leaf-disk SLA. Lines appear offset because of the random effects (see Supplemental Information). Dashed lines indicate the 1:1 and each point shows SLA for one leaf or leaf disk.

$R^2 = 0.97/0.99, P < 0.01$; Fig. 1B). Despite the high correlations, using both whole leaves and disks, herbarium-derived SLA overestimated SLA using the standard method with fresh leaf area (Fig. 1A, B). We also found that fresh areas of whole leaves and disks, which were used to calculate SLA, were highly correlated (marginal/conditional $R^2 = 0.71/0.96, P < 0.01$; Fig. 1C). Lastly, herbarium-derived SLA using dried leaf disks predicted nearly as much variation in SLA calculated using fresh whole leaves (marginal/conditional $R^2 = 0.76/0.95, P < 0.01$; Fig. 1D) as SLA determined with fresh leaf disks. The global fixed effects of each modeled SLA comparison indicated that y -intercepts were not statistically different from zero and that the slopes were statistically different from one except for dry leaf-disk SLA and fresh whole-leaf SLA (Fig. 1A–C). The global y -intercept and slope estimate for dry leaf-disk SLA and fresh whole-leaf SLA was not statistically different than zero and not statistically different from one, respectively (Fig. 1D). However, the random effects included within each model that compared different estimates of SLA indicated that the y -intercept and slope may vary among plant families or species, but explain or help explain 3–20% of the overall variation in a given trait relationship (Fig. 1A–C; Appendix S1, Tables S1–S4; Figs. S1–S5).

Branch specific gravity

Our estimates of G_o (range: ~ 0.1 – 1) encompassed a large portion of the global variation in wood density (0 – 1.5 g cm^{-3} , Chave et al., 2009). G_b , calculated with the dimensional method, predicted a significant proportion of the variation in the branch G_b calculated with the displacement method (marginal/conditional $R^2 = 0.74/0.86, P < 0.01$; Fig. 2A). The modeled y -intercept and the slope of this relationship were statistically different from zero and not statistically different

from 1, respectively. For herbarium-derived estimates (i.e., dried branches), G_o , calculated with the dimensional method, predicted a similar proportion of the variation in G_b calculated with the displacement method (marginal/conditional $R^2 = 0.75/0.83, P < 0.01$; Fig. 2B). The confidence interval of the y -intercept included 1, while the confidence interval of the slope was statistically less than 1 (Fig. 2B). The random effects indicate that up to 12% of the variation in the relationship of each branch-specific gravity comparison may be explained by varying the slope and y -intercept among species within families (Appendix S1, Tables S5–S6; Figs. S6–S8).

Leaf thickness

Rehydrated leaf thicknesses predicted a significant portion of the variation in the leaf thickness of fresh leaves (marginal/conditional $R^2 = 0.85/0.97, P < 0.01$; Fig. 3A). The slope and y -intercept of the modeled relationship between these two estimates of thickness did not differ from 1 and zero, respectively (Fig. 3A). Random effects of this model suggest that varying the y -intercept among species and within families can explain an additional 12% variation in the modeled dried rehydrated and fresh leaf thickness relationship (Appendix S1, Table S7; Figs. S9–S10). We also found that dried then rehydrated thickness using ordinary least squares regression predicted a significant portion of the variation in fresh leaf thickness at the intraspecific level ($R^2 = 0.95, P < 0.01$; Appendix S1, Figure S11).

We attempted to compare our herbarium-derived trait values to those from the TRY database (Kattge et al., 2020), but the two datasets had very few species in common. The TRY database lacked any stem wood density (stem specific gravity) and leaf thickness data for our study species. SLA data were available for nine of our study species, but the number of records available per species were limited

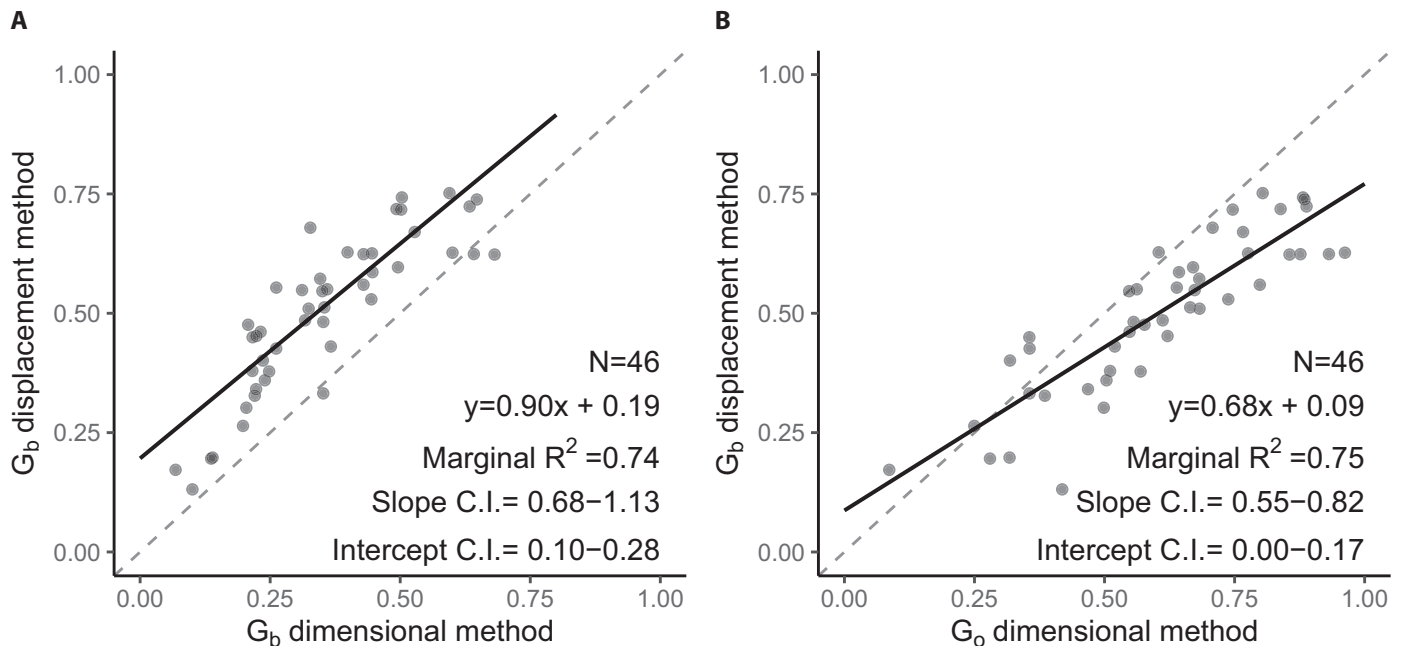


FIGURE 2. Main effects of the linear mixed models comparing different metrics of different metrics of wood specific gravity: (A) Basic specific gravity, G_b , of fresh branches calculated using fresh volume determined with dimensional and displacement methods; and (B) specific gravity of dried branches, G_o , calculated using the dimensional method and G_b of the same branches when using the displacement method. Lines appear offset because of the contribution of random effects (see Supplemental Information). Dashed lines indicate the 1:1 relationship, and each point represents one branch.

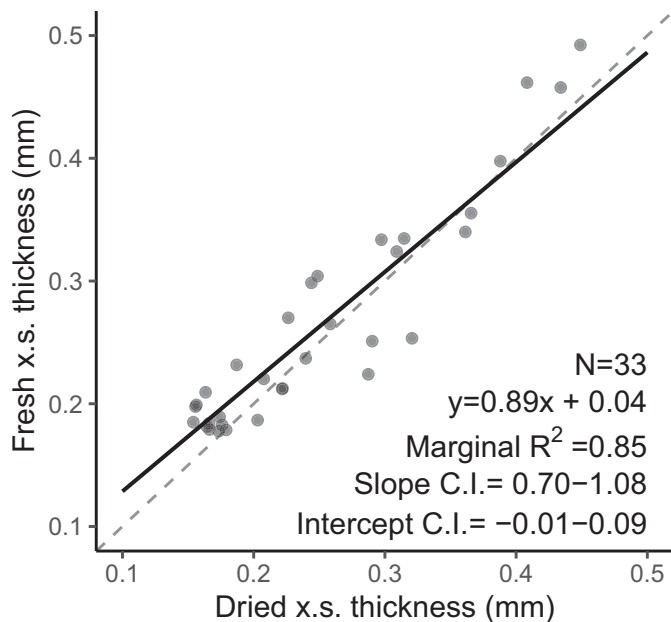


FIGURE 3. Main effects of the linear mixed models comparing the relationship between dried then rehydrated leaf cross-sectional (x.s.) thickness. Dashed line indicates the 1:1 relationship, and each point represents the thickness for one leaf.

and highly variable. Five of the nine species we studied had mean herbarium-derived SLA from leaf pieces that fell within the 95% quantile of TRY's trait values (Appendix S1, Table S8).

DISCUSSION

The use of herbarium specimens has traditionally been limited to taxonomic disciplines, but in recent years has diversified to include the study of phenotypes across time and space (Heberling et al., 2019). Yet, beyond phenological traits (e.g., Davis et al., 2015), the reliability of most herbarium-derived functional trait measurements has not been tested (but see Blonder et al., 2012; Queensborough and Porras, 2014). We found that herbarium-derived estimates of SLA, branch G_o , and leaf thickness strongly correlate to the same traits frequently measured using fresh tissues. Nevertheless, without the use of correction factors, functional traits measured using herbarium specimens are likely to introduce bias if collated with traits measured following standard procedures. Despite these considerations, our results indicate that herbarium specimens can still provide useful estimates of functional traits.

Importantly, our results confirm the promise of herbarium specimens to measure SLA—a trait at the core of current plant strategy theory (e.g., Wright et al., 2004). While our sample sizes were smaller than those of other studies (e.g., Blonder et al., 2012; Queensborough and Porras, 2014), our results show herbarium-derived SLA is a reasonable proxy for SLA collected using standard procedures. That said, our linear models suggest that herbarium-derived estimates of SLA may provide slightly biased estimates of SLA determined using fresh plant tissues. Nevertheless, herbarium-derived SLA may be converted to a standard estimate of SLA with the linear models presented in our results. However, the slope and y -intercepts of these models may vary among species and families, according to

the results of our linear mixed models. Alternatively, rehydrating dried leaves has also been shown to reduce bias in SLA estimates caused by leaf area shrinkage (Blonder et al., 2012). Current trait databases include metadata that documents different methods for estimating SLA (for example, whether the petiole was included or not). We recommend including similar metadata to openly distinguish herbarium-derived measurements, which may differ from those using freshly collected tissues (e.g., see Supplemental Data). Consistent with other observations (e.g., Wright et al., 2007), we found SLA measured using fresh whole-leaf area to be highly correlated with SLA of fresh leaf disks (assuming the petiole mass is negligible or not included). This strong correlation suggests leaf pieces may provide a path forward for obtaining SLA measurements from herbarium specimens while minimizing damage to irreplaceable specimens.

To our knowledge, our study is the first to use herbarium specimens to measure wood specific gravity—a key structural trait that describes the “wood economics spectrum” (Chave et al., 2009). Wood specific gravity can be estimated several ways, and the coefficients from the modeled relationship between the branch G_b using the displacement method, and branch G_b using the dimensional method in this study, suggest a modest effect of measurement method on G_b of small branches. Drying did cause reductions in the branch volume of herbarium specimens (used to calculate G_o), thereby introducing variation to the modeled relationship between G_o and G_b . Ultimately, herbarium-derived G_o predicts a similar proportion of variation in G_b using the displacement as G_b using the dimensional method. This variation suggests each metric of wood specific gravity may need to be treated separately in trait databases. Given that the recommended procedure for determining wood specific gravity is to divide dry wood mass by the oven-dry wood volume (Williamson and Wiemann, 2010), herbarium specimens are ideal sources of branch specific gravity. However, not all herbarium specimens include large branches like those we used to determine G_o and G_b . Some herbarium specimens may only include small branches and it is unclear if small twigs exhibit a similar relationship to stem wood specific gravity as 1-cm-diameter branches.

Lastly, we found that the thicknesses of dried then rehydrated leaves is an accurate proxy for fresh leaf thicknesses. These results corroborate those of Blonder et al. (2012), which show that shrinkage in leaf thickness (and leaf area) can be mitigated through hydration. Similar to other traits we studied, converting dried and rehydrated leaf thickness to fresh leaf thickness can be accomplished using the linear model we presented in our results, although family, genus, and species-specific relationships may improve conversion accuracy. Our intraspecific comparison for leaf thicknesses of *Hamelia patens* provides additional support for the use of herbarium specimens to analyze within-species trends in leaf thickness across spatiotemporal gradients (see Appendix S1, Fig. S11).

Without validation against standard trait procedures, studies that have leveraged herbarium-derived traits to test for changes in species' ecological and evolutionary strategies (e.g., SLA in Buswell et al. [2011]; leaf thickness in Parkhurst [1978]; see Heberling and Isaac [2017] for a more complete list) are likely met with skepticism. Our study provides empirical support for the careful use of herbarium-derived measurements in functional ecology, and we propose that they be included in functional trait databases. However, to fully embrace the potential of herbaria as big sources of functional trait data, validations like those in the present study are needed for additional traits and many more species.

CONCLUSIONS

Herbarium specimens show promise, particularly for novel research programs that assess climate change–driven shifts in intra- and interspecific trait variation (Meineke et al., 2018). Yet, with over 390 million specimens worldwide (Thiers, 2020), herbaria are often overlooked as rich repositories for trait data, and even extensive trait databases like TRY and BIEN currently contain no herbarium-derived traits (though permitted). Our study illustrates that herbarium-derived traits could vastly expand the coverage of global trait databases across time, space, and phylogeny, and we advocate for their inclusion in global trait databases.

We found that herbarium-derived SLA, branch specific gravity (G_0), and leaf thickness measurements are reliable proxies for traits measured following standard protocols, with some caveats. For example, depending on the level of precision necessary for a given research question, the use of correction factors may be needed, especially if directly comparing herbarium-derived traits to those measured using fresh tissues. Such conversions may not be possible for some traits like isotopes, which may be biased by alcohol, adhesives, or other chemicals used in specimen preservation.

Our functional trait data encompass much of the global variation in SLA and G_0 such that conversions from herbarium-derived traits to standard trait measurements may be broadly applicable to angiosperms. However, our linear mixed models indicate the accuracy of these conversions may be improved if they are calculated for individual families, genera, or species. Conversions may vary among different ranges of trait values, in relation to environmental conditions or in other ways that we did not explore in this study. Additional “fresh” and “dry” trait comparisons are needed to uncover appropriate conversions, and we recommend that researchers perform such comparisons for their plant groups of interest when needed. However, we note that corrections may not be necessary when comparisons are made using herbarium-derived values alone.

Importantly, the recognition of herbaria as reservoirs of functional information can mutually benefit functional ecologists and herbarium-based researchers. Herbarium-derived traits can invigorate interest in museum collections and strengthen their role in biological research.

Furthermore, collaboration among functional ecologists and herbarium-based researchers is likely to promote standardized data collection procedures that are congruent with open-science principles and can accelerate trait-based science (Gallagher et al., 2020).

ACKNOWLEDGMENTS

We thank Xristian Carvajal and Paola Lopez for their help collecting data used in this study, and Dr. Benjamin Blonder and an anonymous reviewer for their helpful comments that improved our manuscript. We would also like to thank Fairchild Tropical Botanic Garden for providing us with access to their plant collections and lab facilities.

AUTHOR CONTRIBUTIONS

T.M.P. designed the study; T.M.P. and J.R. collected and analyzed the data. T.M.P., J.R., and J.M.H. wrote the manuscript. All authors

have approved this article for publication and are accountable for all aspects of this work.

DATA AVAILABILITY

All data used in this study have been uploaded to the TRY database and is available for download in Appendix S2.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Supplemental Tables S1–S8 and Figs. S1–S11 illustrate the fixed and random effects for each trait relationship presented in the main text.

APPENDIX S2. Trait Data.

LITERATURE CITED

- Baraloto, C., C. E. T. Paine, L. Poorter, J. Beauchene, D. Bonal, A. M. Domenach, B. Hérault, et al. 2010. Decoupled leaf and stem economics in rain forest trees. *Ecology Letters* 13: 1338–1347.
- Bates, D., M. Maechler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48.
- Blomberg, S. P., T. Garland, and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57: 717–745.
- Blonder, B., V. Buzzard, I. Simova, L. Sloat, B. Boyle, R. Lipson, B. Aguilar-Beaucage, et al. 2012. The leaf area shrinkage effect can bias paleoclimate and ecology research. *American Journal of Botany* 99: 1756–1763.
- Buswell, J. M., A. T. Moles, and S. Hartley. 2011. Is rapid evolution common in introduced plant species? *Journal of Ecology* 99: 214–224.
- Chave, J., D. Coomes, S. Jansen, S. L. Lewis, N. G. Swenson, and A. E. Zanne. 2009. Towards a worldwide wood economics spectrum. *Ecology Letters* 12: 351–366.
- Cornwell, W. K., W. D. Pearse, R. L. Dalrymple, and A. E. Zanne. 2019. What we (don't) know about global plant diversity. *Ecography* 42: 1819–1831.
- Davis, C. C., C. G. Willis, B. Connolly, C. Kelly, and A. M. Ellison. 2015. Herbarium records are reliable sources of phenological change driven by climate and provide novel insights into species' phenological cueing mechanisms. *American Journal of Botany* 102: 1599–1609.
- Díaz, S., J. Kattge, J. H. C. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, et al. 2015. The global spectrum of plant form and function. *Nature* 529: 167–171.
- Gallagher, R. V., D. S. Falster, B. S. Maitner, R. Salguero-Gómez, V. Vandvik, W. D. Pearse, F. D. Schneider, et al. 2020. Open science principles for accelerating trait-based science across the tree of life. *Nature Ecology and Evolution* 4: 294–303.
- Heberling, J. M., and B. L. Isaac. 2017. Herbarium specimens as exaptations: New uses for old collections. *American Journal of Botany* 104: 963–965.
- Heberling, J. M., L. A. Prather, and S. J. Tonsor. 2019. The changing uses of herbarium data in an era of global change: an overview using automated content analysis. *BioScience* 69: 812–822.
- Kattge, J., G. Bönsch, S. Díaz, S. Lavorel, I. C. Prentice, P. Leadley, S. Tautenhahn, and G. D. A. Werner. 2020. TRY plant trait database—enhanced coverage and open access. *Global Change Biology* 26: 119–188.
- Kitajima, K., and L. Poorter. 2010. Tissue-level leaf toughness, but not lamina thickness, predicts sapling leaf lifespan and shade tolerance of tropical tree species. *New Phytologist* 186: 708–721.
- Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest Package: Tests in linear mixed effects models. *Journal of Statistical Software* 83: 1–26.

- Leigh, A., S. Sevanto, M. C. Ball, J. D. Close, D. S. Ellsworth, C. A. Knight, A. B. Nicotra, and S. Vogel. 2012. Do thick leaves avoid thermal damage in critically low wind speeds? *New Phytologist* 194: 477–487.
- Lüdecke, D. 2018. sjPlot: Data visualization for statistics in social science. website: <https://CRAN.R-project.org/package=sjPlot> [accessed 30, March 2020].
- Maitner, B. S., B. Boyle, N. Casler, R. Condit, J. Donoghue, S. M. Durán, D. Guaderrama, et al. 2018. The BIEN R package: A tool to access the Botanical Information and Ecology Network (BIEN) database. *Methods in Ecology and Evolution* 9: 373–379.
- Meineke, E. K., C. C. Davis, and T. J. Davies. 2018. The unrealized potential of herbaria for global change biology. *Ecological Monographs* 88: 505–525.
- Parkhurst, D. F. 1978. The adaptive significance of stomatal occurrence on one or both surfaces of leaves. *Journal of Ecology* 66: 367–383.
- Perez, T. M., O. Valverde-Barrantes, C. Bravo, T. C. Taylor, B. Fadrique, J. A. Hogan, C. J. Pardo, et al. 2019. Botanic gardens are an untapped resource for studying the functional ecology of tropical plants. *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences* 374: 20170390.
- Pérez-Harguindeguy, N., S. Díaz, E. Garnier, S. Lavorel, H. Poorter, P. Jaureguiberry, M. S. Bret-Harte, et al. 2013. New handbook for standardized measurement of plant functional traits worldwide. *Australian Journal of Botany* 61: 167–234.
- Queenborough, S. A., and C. Porras. 2014. Expanding the coverage of plant trait databases - A comparison of specific leaf area derived from fresh and dried leaves. *Plant Ecology and Diversity* 7: 383–388.
- R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Romero, A., M. Florencia, R. Manuel, A. Maria, F. Romero, R. M. Salas, and A. M. Gonzalez. 2019. Taxonomic and ecological implications of foliar morphoanatomy in *Cephalanthus* (Naucleaeae, Rubiaceae). *Systematic Botany* 44: 387–397.
- Rueden, C. T., J. Schindelin, and M. C. Hiner. 2017. ImageJ2: ImageJ for the next generation of scientific image data. Website: <https://www.R-project.org/> [accessed 30, March 2020].
- Scheiter, S., L. Langan, and S. I. Higgins. 2013. Next-generation dynamic global vegetation models: learning from community ecology. *New Phytologist* 198: 957–969.
- Siefert, A., C. Violle, L. Chalmandrier, C. H. Albert, A. Taudiere, A. Fajardo, L. W. Aarssen, et al. 2015. A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. *Ecology Letters* 18: 1406–1419.
- Swenson, N. G., and B. J. Enquist. 2008. The relationship between stem and branch wood specific gravity and the ability of each measure to predict leaf area. *American Journal of Botany* 95: 516–519.
- Thiers, B. 2020. The World's Herbaria 2019: *The World's Herbaria 2019: A Summary Report Based on Data from Index Herbariorum*: 1–20. Website: <http://sweetgum.nybg.org/science/ih/> [accessed 30, March 2020].
- Violle, C., B. J. Enquist, B. J. McGill, L. Jiang, C. H. Albert, C. Hulshof, V. Jung, and J. Messier. 2012. The return of the variance: Intraspecific variability in community ecology. *Trends in Ecology and Evolution* 27: 244–252.
- Violle, C., M. L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the concept of trait be functional! *Oikos* 116: 882–892.
- Williamson, G. B., and M. C. Wiemann. 2010. Measuring wood specific gravity correctly. *American Journal of Botany* 97: 519–524.
- Wright, I. J., D. D. Ackerly, F. Bongers, K. E. Harms, G. Ibarra-Manriquez, M. Martinez-Ramos, S. J. Mazer, et al. 2007. Relationships among ecologically important dimensions of plant trait variation in seven neotropical forests. *Annals of Botany* 99: 1003–1015.
- Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, et al. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821–827.