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RESEARCH ARTICLE

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Sources and consequences of mismatch between leaf disc and whole-leaf leaf mass per area (LMA)

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Abstract

Premise: Leaf mass per area (LMA), which is an important functional trait in leaf economic spectrum and plant growth analysis, is measured from leaf discs or whole leaves. Differences between the measurement methods may lead to large differences in the estimates of LMA values.

Methods: We examined to what extent estimates of LMA based on whole leaves match those based on discs using 334 woody species from a wide range of biomes (tropics, subtropics, savanna, and temperate), whether the relationship varied by leaf morphology (tissue density, leaf area, leaf thickness), punch size (0.6- and 1.0-cm diameter), and whether the extent of intraspecifc variation for each species matches.

Results: Disc-based estimates of species mean LMA matched the whole-leaf estimates well, and whole-leaf LMA tended to be 9.69% higher than leaf-disc LMA. The ratio of whole-leaf LMA to leaf-disc LMA was higher for species with higher leaf tissue density and larger leaves, and variance in the ratio was greater for species with lower leaf tissue density and thinner leaves. Estimates based on small leaf discs also inflated the ratio. The extent of the intraspecific variation only weakly matched between whole-leaf and disc-based estimates ($R^2 = 0.08$).

Conclusions: Our results suggest that simple conversion between whole-leaf and leaf-disc LMA is difficult for species obtained with a small leaf punch, but it should be possible for species obtained with a large+ leaf punch. Accurately representing leaf traits will likely require careful selection between leaf-disc and whole-leaf traits depending on the objectives. Quantifying intraspecific variation using leaf discs should be also considered with caution.

KEYWORDS

intraspecific variation, leaf density, leaf economic spectrum, leaf heterogeneity, leaf punch, leaf size, leaf thickness, petiole, specific leaf area, within-leaf variation

The primary function of leaves is to return photosynthetic revenue on the resource investment that was used to construct the leaf (i.e., leaf economic spectrum, Wright et al., 2004; Westoby et al., 2013). Leaf mass per area (LMA or 1/SLA [specific leaf area]), determined by lamina thickness and leaf tissue density (LD), describes how much biomass is invested into a given photosynthetic leaf area, which is a key trait in the leaf economic spectrum (Wright et al., 2004; Poorter et al., 2009; Onoda et al., 2017). Generally, resource-acquisitive species (fast-growing species) tend to have low LMA values, high photosynthetic rates and nutrient levels, and often, fast leaf turnover (Wright et al., 2004). In contrast, resource-conservative species (slow-growing species) often have higher LMA values with slower photosynthetic rates, lower nutrient concentrations, and slower leaf turnover (Garnier and Laurent, 1994; Wright et al., 2004; Reich, 2014; Díaz et al., 2016). The LMA is also frequently used to analyze plant growth (Evans, 1972; Poorter et al., 2014; Falster et al., 2018) because relative growth rates (RGR) can be decomposed into the product of net assimilation rate (NAR), leaf mass ratio (LMR) and LMA (i.e., $\mathrm{RGR} = \mathrm{NAR} \times \mathrm{LMR} \times \mathrm{LMA}^{-1}$).

Phisamai Maenpuen and Masatoshi Katabuchi contributed equally to this work.

Another appealing feature of LMA is that it is relatively easy to measure for a large number of species (i.e., "soft" trait; Díaz et al., 2004). Therefore, LMA has been of interest to ecologists and widely used since the first report more than a century ago (Hanson, 1917). Actually, for species coverage among leaf traits in the TRY plant data database (16,460 species), LMA has one of the best species coverage and is also the most often requested leaf trait (2977 of 7330 requests), followed by leaf nitrogen (N) content per leaf dry mass (12,238 species, 1938 requests, Kattge et al., 2020 [TRY version 5, status 1 October 2019]).

Estimates for LMA can be obtained by either measuring whole-leaf (including or excluding petioles) or leaf-disc samples (Pérez-Harguindeguy et al., 2013). According to the TRY database version 5, recorded numbers of observations and species are as follows: (1) whole leaf including (88,490 observations and 7068 species) or excluding petioles (64,838 observations and 7558 species; note that these could include leaf-disc samples), and (2) leaf disc excluding all major veins or petioles (645 observations and 403 species) (Kattge et al., 2020). Not many records explicitly indicate leaf-disc LMA in the TRY database, but leaf-disc LMA has been commonly used for ecological and biochemical studies (e.g., Kraft et al., 2008; Poorter, 2009; Onoda et al., 2011; Osada et al., 2014; Sastry and Barua, 2017; Serbin et al., 2019; Campany et al., 2021). Although using whole-leaf traits seems to be more straightforward in the logic of investment costs and returns on investment (Westoby et al., 2013), Poorter (2009) found that leaf-disc LMA was more highly correlated than whole-leaf LMA with shade tolerance, suggesting that lamina traits are more important than whole-leaf traits in certain ecological contexts. Many studies do not clarify the protocol used (146,315 observations and 13,101 species), but differences in the tissue used may lead to large discrepancies in the estimates of LMA values because the major vein allocation (major vein, which includes first to second or third-order veins, volume per area) has been reported to be one of the main determinants for the variation in LMA (John et al., 2017). Previous works that compared estimates of whole-leaf LMA and leaf-disc LMA using tropical tree species from different forests showed good correlations between the two (e.g., $R^2 = 0.92$ [Kraft et al., 2008]; $R^2 = 0.92$ [Onoda et al., 2011]; both studies used whole leaves without petioles). However, the two estimates for other biomes have rarely been compared, even though LMA responds to climate (Poorter, 2009).

Since larger leaves tend to have more of their mass invested in dense midribs and the petiole for support (Niinemets et al., 2006, 2007; Li et al., 2022a,b), and thinner leaves have clearly visible, large-diameter veins with less-uniform leaf structure (i.e., kite-type leaves; [Grubb, 1986]), discrepancies in the estimates of whole-leaf LMA and leaf-disc LMA might be greater for larger and thinner leaves. Higher leaf density (and LMA) is associated with higher vein density (John et al., 2017; Sancho-Knapik et al., 2020); thus, a leaf-disc LMA that avoids major veins might also underestimate whole-leaf LMA for species with higher leaf tissue density. If leaf-disc LMA consistently leads to underestimates of whole-leaf LMA (i.e., differences in means), some calibrations may be required to combine or compare those different estimates (Kraft et al., 2008; Onoda et al., 2011). If divergences between the estimates based on the different methods are large and inconsistent (i.e., differences in variances and low R^2 values), then differences in the estimates will be difficult to calibrate and should inflate errors in the subsequent analyses. To date, however, only a database from a single region, Panama Plant Traits Database (Wright et al., 2010), is available in the TRY database (Kattge et al., 2020) that has both estimates of LMA from small leaf discs and whole leaves including petioles; thus, we have a limited understanding of the conditions needed for estimating LMA from leaf discs to provide reliable estimates of whole-leaf LMA.

Intraspecific trait variation (ITV), which reflects both heritable genetic variation and phenotypic plasticity, leads to differences in plant responses to abiotic and biotic interactions (Westerband et al., 2021). For example, shade leaves tend to have lower LMA than sun leaves do because they have fewer layers of palisade mesophyll cells (Terashima et al., 2001; Onoda et al., 2008). Trait variance explained by intraspecific trait variance within and among communities for LMA can be similar to or greater than that explained by interspecific trait variation (e.g., Messier et al., 2010; Kichenin et al., 2013; Fajardo and Siefert, 2018). The coefficient of variation (CV) is often used to quantify the extent of ITV for each species (Yang et al., 2020; Westerband et al., 2021). Sources of ITV for whole-leaf samples are variation among individuals within the same species and variation among leaves within the same individuals (Messier et al., 2010, 2017). An additional source of trait variation for leaf-disc samples is variation among leaf discs within the same leaves. Given that leaf disc samples have more sources of ITV than whole-leaf samples, the effects of discrepancies between whole-leaf LMA and leaf-disc LMA might be large when ITV is quantified based on CV. Despite the importance of ITV in community ecology (Siefert et al., 2015; Westerband et al., 2021), the effect of sample choice (i.e., whole leaf vs. leaf disc) on the extent of ITV has been largely ignored.

In this study, we aimed to investigate the relationship between whole-leaf (including petiole) LMA and leaf-disc LMA, whether the relationship varied with leaf tissue density, leaf area, and leaf thickness, and whether the extent of ITV for each species matches between the estimates based on the whole leaf and leaf discs. We collected leaves from 1459 individuals of 334 woody species from four biomes (tropics, subtropics, savanna, and warm-temperate) to cover a wide range of geography. We evaluated the following hypotheses: (1) Whole-leaf LMA will be greater than leaf-disc LMA because whole-leaf LMA includes petioles and midribs that have greater dry mass per unit area than laminas do (Niinemets et al., 2007). (2) Species with higher leaf tissue density, larger and/or thinner leaves will have larger variances and differences in the two LMA estimates because whole-leaf LMA should be driven more in those species by veins and petioles, whereas those effects are likely to be ignored in leaf-disc samples. (3) The extent of ITV does not match well between the estimates based on whole leaves and leaf discs because leaf disc samples have more sources of variation than whole-leaf samples. In addition to leaf morphology, we also investigated the effects of leaf punch size on the relationship between whole-leaf and leaf-disc LMA.

MATERIALS AND METHODS

Data sources

We used newly compiled individual-level plant data sets from a wide range of biomes in China and Japan (Appendix S1) to examine the relationship between whole-leaf and leaf-disc-based estimates of leaf traits. First, the Yunnan data set is from three forest plots in Yunnan Province, Southwest China, which includes a tropical rainforest (TRF), a tropical hot-dry savanna ecosystem (HDS), and a subtropical evergreen wet forest (STF), with a total of 141 species and 852 individuals. Second, the Yakushima data set is from a warm-temperate forest on Yakushima Island, Japan, with a total of 193 species and 607 individuals. In total, our data set comprised 334 woody species and 1459 individuals from habitats that ranged from 8.1 to 24.7°C in mean annual temperature (MAT) and 732.8 to 4477 mm in mean annual precipitation (MAP) (Appendix S1; Eguchi, 2006; Song et al., 2017; Fei et al., 2018), which covers the wide range in climates where broad-leaved tree species grow. The methodologies for trait sampling and measurement differed slightly between the Yunnan and the Yakushima data sets as described next.

Measurements of leaf-disc and whole-leaf LMA

At the TRF site, we collected leaf samples from trees within reach of a canopy crane (88 m tall with a 60 m long boom). In the HDS, and STF sites, we used a 12-m long pruner to collect samples for most of the target species. For tall individuals that were out of the reach of pruners, we used rope climbing to reach the canopy, then sampled branches with the long pruner. On Yakushima, we used 15-m poles for our leaf sampling. At least six sun-exposed healthy leaves were sampled from each of 3 to 6 individuals for each species. Trait values were averaged at the species- and individual-level for the analyses. For compound-leaved species, we referred to a leaflet (the minimum photosynthetic unit) as a single leaf in our study. We excluded species that had tiny leaves or leaflets (length < 1 cm) because it was not practical to measure leaf-disc LMA.

To determine what extent LMA values were affected by the different methods, we based the LMA on (1) a whole leaf including a petiole or (2) a leaf disc cut to avoid the thick first to second veins. A 0.6-cm-diameter disc was taken from the base, middle, and tip of each leaf using a hole punch in the Yunnan data set. A 1.0-cm-diameter disc was taken from the tip and base of each leaf in the Yakushima data set (see Appendix S2 for more details). Fresh leaf area (LA; cm^2) of leaf materials (whole leaves including petioles and midribs) was measured using a scanner and ImageJ software by the R package LeafArea (Katabuchi, 2015). Fresh leaf thickness (LT; mm) was measured using a micrometer at the base, middle, and tip of the leaf (Mitutoyo 293-240, Mitutoyo Corp., Kawasaki, Japan) with a precision of 0.001 mm. Thickness of each leaf disc was also measured for the Yunnan data set but not for the Yakushima data set, which enabled us to estimate the leaf tissue density (LD; g cm^{-3}) for the whole-leaf and leaf disc separately in the Yunnan data set. Leaf dry mass (whole leaves including petioles and midribs, and leaf discs) was recorded after drying 80°C for over 48 h to constant mass using an electronic scale (Mettler-Toledo MS204TS, Columbus, OH, USA) with a precision at 0.0001 g. In a subset of Yakushima data set, leaf lamina and petiole dry mass were also determined separately (80 of 193 species) to quantify leaf support costs. Dry mass of two or three discs were measured together for each leaf. Based on those measurements of whole-leaf and leaf disc samples, LMA (g m^{-2}) was calculated as the ratio of leaf dry mass to fresh leaf area, and LD was calculated as the ratio of LMA to LT. We focused on LMA in our study, and we do not present results of LD in the main text, because differences between whole-leaf LD and leaf disc LD only depend on the ratio between whole-leaf LMA and leaf-disc LMA and measurement errors of leaf thickness (Appendix S3).

Relationship between whole-leaf and leaf-disc LMA

A bivariate trait relationship between log-transformed LMA for the disc and whole-leaf samples was summarized with variance explained (R^2) based on Pearson correlations and with a standardized major axis (SMA) regression (Warton et al., 2006) across all the species.

The SMA analysis was run using the R package smatr (Warton et al., 2012), which included tests for slope heterogeneity and elevation differences between disc and whole-leaf estimates of LMA values. Confidence intervals for the SMA estimates were based on 2000 bootstraps.

Effects of leaf morphology on the relationship between whole-leaf and leaf-disc LMA

To further quantify the effect of leaf morphology and leaf punch size on the relationship between whole-leaf and leaf-disc LMA, we built a hierarchical Bayesian model. The whole-leaf LMA for species i ($\mathrm{LMA}_{\mathrm{W}i}$) was assumed to follow a normal distribution ($\mathcal{N}$) with the mean of leaf-disc LMA

($\mathrm{LMA}_{\mathrm{D}i}$) and covariates of observed leaf tissue density (LD_i), leaf area (LA_i) and leaf thickness (LT_i) on the log-scale:

$$\ln \mathrm{LMA}_{\mathrm{W}i} \sim \mathcal{N}\left(\ln \mathrm{LMA}_{\mathrm{D}i} + \tilde{\mu}_i, \sigma_i^2\right) \tag{1}$$

$$\begin{aligned}\tilde{\mu}_i = \beta_0 + \beta_1 \ln \mathrm{LD}_i + \beta_2 \ln \mathrm{LA}_i + \beta_3 \ln \mathrm{LT}_i + \beta_4 \mathrm{PS}_i \\ + \mathrm{PS}_i(\beta_5 \ln \mathrm{LD}_i + \beta_6 \ln \mathrm{LA}_i + \beta_7 \ln \mathrm{LT}_i),\end{aligned} \tag{2}$$

where the dummy variable PS_i is set to 1 for samples obtained with a small leaf punch (0.6-cm diameter) and 0 for samples obtained with a large leaf punch (1.0-cm diameter); $\tilde{\mu}_i$ is the effect of leaf tissue density, leaf area, leaf thickness and leaf punch size on the mean estimates for the whole-leaf LMA of species i; σ_i^2 is the variance of whole-leaf LMA for species i; and β are coefficients. Because leaf-disc LMA is used as an offset, this model can be used to see the relationship between whole-leaf to leaf-disc LMA ratio and predictors. The standard deviation of whole-leaf LMA (σ_i) was assumed to follow a normal distribution on the log-scale:

$$\ln \sigma_i \sim \mathcal{N}(\ln \tilde{\sigma}_i, \omega^2) \tag{3}$$

$$\begin{aligned}\ln \tilde{\sigma}_i = \gamma_0 + \gamma_1 \ln \mathrm{LD}_i + \gamma_2 \ln \mathrm{LA}_i + \gamma_3 \ln \mathrm{LT}_i + \gamma_4 \mathrm{PS}_i \\ + \mathrm{PS}_i(\gamma_5 \ln \mathrm{LD}_i + \gamma_6 \ln \mathrm{LA}_\mathrm{i} + \gamma_7 \ln \mathrm{LT}_\mathrm{i}),\end{aligned} \tag{4}$$

where $\tilde{\sigma}_i$ is a hyperparameter for σ_i, ω^2 is a scaling hyperparameter, and all cases of γ are coefficients. For allowing comparisons among parameter estimates, ln LD, ln LA, and ln LT were scaled to a mean of 0 and standard deviation of 1. Note that this model does not include measurement errors of leaf-disc LMA implemented in the previous SMA analysis, because there are too many scaling parameters.

Posterior distributions of all parameters were estimated using the Hamiltonian Monte Carlo algorithm (HMC) implemented in Stan (Carpenter et al., 2017) using the weakly informative priors (Gelman et al., 2008). The Stan code used to fit the models is available from Github at https://github.com/mattocci27/leaf-disc. Convergence of the posterior distribution was assessed with the Gelman-Rubin statistic with a convergence threshold of 1.1 for all parameters (Gelman et al., 2013).

We also performed ordinary least-squares (OLS) regressions to see the relationship between whole-leaf to leaf-disc LMA ratio and petiole to leaf dry mass ratio and between leaf area petiole to leaf dry mass ratio using a subset of the data set in which we measured leaf lamina and petiole dry mass separately.

Relationship between disc-based and whole-leaf estimates of intraspecific variation

The extent of intraspecific variation (ITV) was calculated using Bao's coefficient of variation (CV) estimator (Yang et al., 2020):

$$\mathrm{CV} = \mathrm{CV}_1 - \frac{\mathrm{CV}_1^3}{N} + \frac{\mathrm{CV}_1}{4N} + \frac{\mathrm{CV}_1^2\gamma_1}{2N} + \frac{\mathrm{CV}_1\gamma_2}{8N}, \tag{5}$$

where $\mathrm{CV}_1 = s/\bar{x}$, s is the sample standard deviation and $\bar{x}$ is the sample mean, N is the sample size, and γ_1 and γ_2 are the skewness and kurtosis of the trait distribution. Bao's CV estimator is a robust method to quantify ITV based on the CV when the sample size is small (Yang et al., 2020). We calculated CV for species for which at least five individuals were measured as recommended in Westerband et al. (2021). Ideally, 20 individuals for each species are required for robust estimation of CV (i.e., <5% differences from the true ITV; Yang et al. [2020]).

The SMA analyses were run as described above. We also used a paired t-test to compare the extent of ITV between whole-leaf LMA and leaf-disc LMA. All statistical analyses were conducted in R version 4.1.3 (R Core Team, 2022) using the R package targets version 0.12.0 for workflow management (Landau, 2021).

RESULTS

There was a significant shift in elevation between whole-leaf and leaf-disc LMA (Figure 1, SMA slope: 0.975 [95% CI: 0.938, 1.013], SMA intercept: 0.0881 [95% CI: 0.016, 0.16]), suggesting that the whole-leaf estimates of LMA were 9.69% (95% CI: 8.06, 11.3) greater than disc-based estimates on the arithmetic scale.

Leaf tissue density, leaf area, leaf thickness, and leaf disc size influenced the relationship between the whole-leaf LMA and leaf-disc LMA (Figure 2; Appendix S4). Species with lower leaf tissue density had greater variance in the estimate of whole-leaf to leaf-disc LMA ratio when a small leaf punch was used (Figure 2A). Leaf-disc and whole-leaf

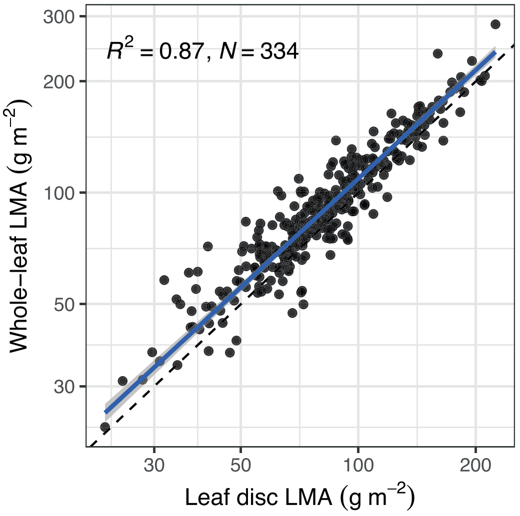

FIGURE 1 Relationship between species mean leaf mass per area (LMA) determined by using whole-leaves and leaf discs. Dashed line indicates 1:1 line. Blue solid line indicates a standardized major axis (SMA) regression. The 95% confidence interval is represented as the shaded area. The correlation is significant ($P < 0.001$).

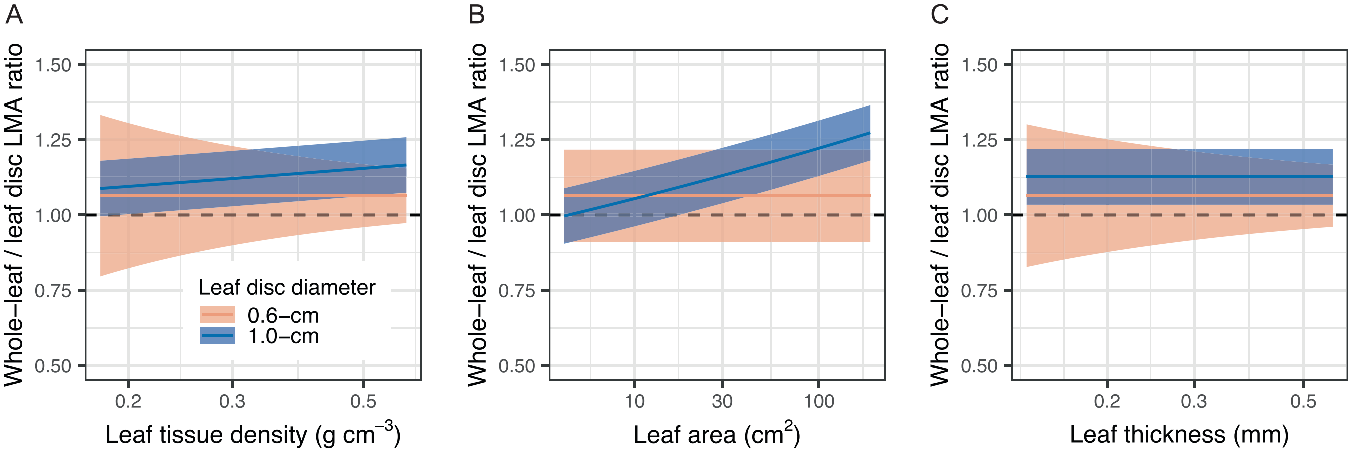

FIGURE 2 Model predictions of the ratio or whole-leaf leaf mass per area (LMA) to leaf-disc LMA as a function of leaf punch sizes and (A) leaf tissue density, (B) leaf area, and (C) leaf thickness. Dashed lines indicate 1:1 ratio of leaf-disc and whole-leaf LMA estimates. Solid lines indicate the posterior means ($\tilde{\mu}$ in Equations 1, 2), and the shaded regions shows ± the posterior means of standard deviations ($\tilde{\sigma}$ in Equations 3, 4).

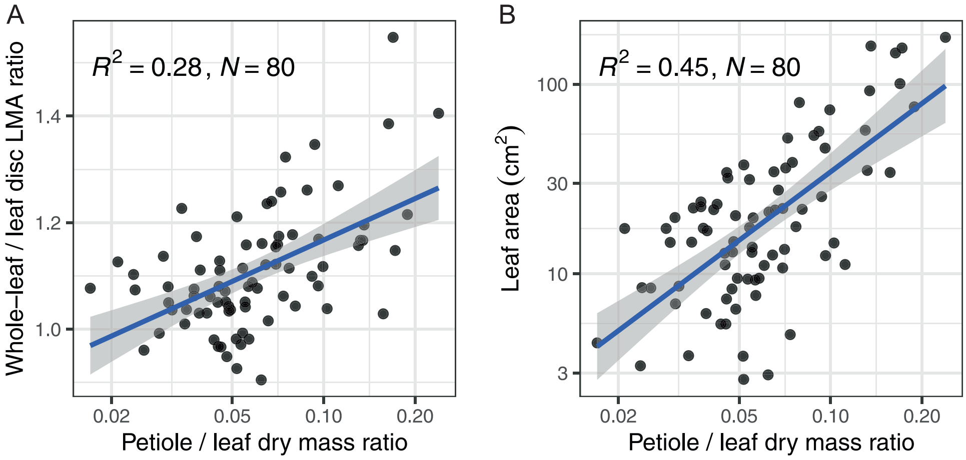

FIGURE 3 Relationships between petiole to leaf dry mass ratio and (A) the ratio of whole-leaf leaf mass per area (LMA) to leaf disc LMA and (B) leaf area. Blue solid lines indicate ordinary least squares (OLS) regressions. The 95% confidence intervals are presented as the shaded area. All the correlations are significant ($P < 0.001$). Note that all the samples were obtained with a 1.0-cm-diameter leaf punch.

LMA estimates diverged for species with higher leaf tissue density when a large leaf punch was used (Figure 2A). Species with larger leaves had greater estimate than small-leaved species for the whole-leaf to leaf-disc LMA ratio (Figure 2B), which was only significant for discs obtained using the large punch. Species with thinner leaves had greater variance in the estimate of whole-leaf to leaf-disc LMA ratio (Figure 2C), which was only significant for discs obtained with the small punch. The small punch also inflated variance in the estimate of the ratio of the whole-leaf LMA to leaf-disc LMA (Figure 2A–C; Appendix S4). Leaf disc and whole-leaf LMA estimates diverged for species with greater investment in petiole dry mass (Figure 3A). Large-leaved species tended to have greater investment for petiole dry mass than small-leaved species (Figure 3B).

The extent of intraspecific trait variation (CV) only weakly matched between whole-leaf and disc-based estimates (Figure 4). Although there were no statistical differences in the SMA slope or elevation (SMA slope: 1.112 [95% CI: 0.951, 1.302], SMA intercept: 0.096 [95% CI: −0.18, 0.372]), leaf-disc LMA had greater CVs than whole-leaf LMA (t = −3.2, df = 143, $P < 0.001$).

DISCUSSION

Leaf-disc LMA explained interspecific variations in whole-leaf LMA ($R^2 = 0.87$, $N = 334$) quite well, and the strength of their relationship was approximately same or slightly weaker than previously reported ($R^2 = 0.92$, $N = 409$, for whole leaves excluding petioles by Kraft et al. [2008]; $R^2 = 0.92$, $N = 364$ for whole leaves excluding petioles by Onoda et al. [2011]). The standardized major axis (SMA) regression showed significantly greater intercepts when all the species were pooled (9.69% differences). Together, these findings suggest that disc-based estimates can be generally

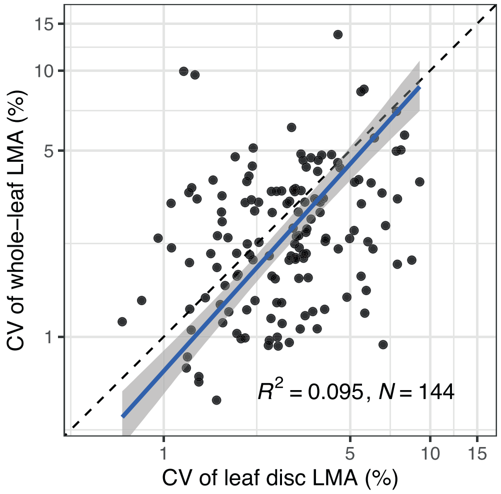

FIGURE 4 Relationship between coefficient of variation (Bao's CV estimator, Equation 5) in leaf mass per area (LMA) values determined by using whole leaves and leaf discs. The correlation is significant ($P < 0.001$). Details as for Figure 1.

used as good proxies for their whole-leaf estimates with appropriate calibration (see discussion below). However, leaf tissue density, leaf area, leaf thickness, and leaf disc size also influenced the relationship between the whole-leaf LMA and leaf-disc LMA (Figure 2; Appendix S4).

Effects of leaf disc size and leaf morphology

Leaf disc size was the most important source of the variation (Figure 2; Appendix S4), and samples obtained with a small leaf punch had greater variance in the ratios of whole-leaf LMA to leaf-disc LMA. The use of small leaf discs inflated the size of the trait variation within leaves because dry mass is inflated when major veins are unintentionally included in the samples. Species with lower leaf tissue density and thin leaves had greater variance in whole-leaf LMA to leaf-disc LMA ratio especially when a small punch was used (Figure 2A, C: small leaf punch; Figure 2A: large leaf punch). Kraft et al. (2008) found high R^2 values ($R^2 = 0.92$) between whole-leaf and leaf-disc LMA based on leaf discs of approximately 1.0-cm diameter for sapling leaves under closed canopy that are softer and thinner than adult leaves (Kitajima and Poorter, 2010; Oktavia and Jin, 2020). Although a leaf punch of 1.0-cm diameter was used only in the Yakushima data set in our study, the variance in the whole-leaf LMA to leaf-disc LMA ratio was small for the Yakushima data set, regardless of leaf tissue density or leaf thickness (Figure 2A, C). On the other hand, a previous study found that leaf-disc LMA obtained with a very small leaf punch (0.3-cm diameter) and whole-leaf LMA had different relationships with shade tolerance (Poorter, 2009), suggesting that leaf-disc LMA and whole-leaf LMA can be inherently different when small disc samples are used. The relatively low R^2 values in the Yunnan data set (Appendix S5) compared to the previous study and the Yakushima data set may suggest not only the effect of distinct veins on variance in LMA (John et al., 2017) or distinct veins with kite-type appearances (Grubb, 1986), but also technical difficulty in measuring the tiny mass of discs for leaves with lower LMA. We found that estimates of whole-leaf and leaf-disc LMA diverged when total dry mass of leaf discs was small (Appendix S6), and thus species with lower leaf tissue density and thin leaves, which have smaller dry mass (Appendix S7), tended to have greater variation in the whole-leaf LMA to leaf-disc LMA ratio especially for smaller leaf discs.

Species with higher leaf tissue density and large leaves had a greater whole-leaf LMA than leaf-disc LMA (Figure 2A, B: large leaf punch). We also observed that leaf-disc and whole-leaf LMA estimates diverged for species with greater investment in petiole dry mass (Figure 3A), and large-leaved species tended to have greater investment than small-leaved species for petiole dry mass (Figure 3B). These results are consistent with the pattern of large leaves disproportionately investing in more leaf veins and/or petioles (Niinemets et al., 2006, 2007; Sack et al., 2012) and the pattern of higher leaf tissue density associated with higher vein density (John et al., 2017; Sancho-Knapik et al., 2020) because those driving factors of LMA are likely to be absent for leaf-disc samples. The estimated effects of leaf tissue density and leaf area was also positive for species obtained with a small leaf punch (Appendix S4), but those effects were not significant. The variation in whole-leaf to leaf-disc LMA ratio might be too large to show clear effects of leaf tissue density and leaf area on the mean estimates (β in Equation 2) when a small leaf punch was used.

Conversion between whole-leaf and leaf-disc LMA

When the objective is to use leaf-disc LMA as an alternative for whole-leaf LMA, using an appropriate punch size (e.g., 1.0-cm in diameter) is important for obtaining stable estimates of leaf-disc LMA that matches well with whole-leaf LMA. Our results suggest that simple conversion between whole-leaf and leaf-disc LMA is difficult for species obtained with a small leaf punch. The effects of leaf tissue density and leaf area were predictable when we used a large leaf punch (Figure 2A, B: large leaf punch), suggesting that conversion between leaf-disc and whole-leaf LMA should be possible using leaf area as a covariate (measuring leaf tissue density requires whole-leaf LMA itself). The ordinary least-squares (OLS) regressions with leaf area as a covariate were (1) $\log_{10} \mathrm{LMA_w} = 0.997 \log_{10} \mathrm{LMA_d} + 0.0589 \log_{10} \mathrm{LA}$ and (2) $\log_{10} \mathrm{LMA_d} = 0.15 + 0.945 \log_{10} \mathrm{LMA_w} - 0.063 \log_{10} \mathrm{LA}$ ($R^2 = 0.94$, AIC = –321.7, $n = 193$), where $\mathrm{LMA_w}$ is whole-leaf LMA, $\mathrm{LMA_d}$ is leaf-disc LMA, and LA is leaf area. The OLS regression without leaf area as a covariate was (3) $\log_{10} \mathrm{LMA_w} = 0.124 + 0.958 \log_{10} \mathrm{LMA_d}$ or (4) $\log_{10} \mathrm{LMA_d} = 0.973 \log_{10} \mathrm{LMA_w}$ ($R^2 = 0.93$, AIC = –284.2, $n = 193$). (Note that OLS regressions minimize errors of either whole-leaf LMA or leaf-disc LMA; thus, those four models are not identical, whereas

SMA regressions are not appropriate for predictions [Warton et al., 2006]). Nonetheless, including leaf area only slightly improves the R^2 values for the Yakushima data set where a large punch was used. This result is due to the large discrepancy in the whole-leaf to leaf-disc LMA for large leaves and can be explained by scaling relationships between leaf mass and leaf area called "diminishing returns" (Niklas et al., 2007), whereby increases in leaf dry mass including petioles do not result in a proportional increase in leaf area including petioles (Li et al., 2022a). This effect is generally strong for mature leaves (Jiao et al., 2022). "Diminishing returns" can be either described as the scaling slope of leaf area in Model 1 or the scaling slope of leaf-disc LMA and the intercept in the Model 3. When leaf-disc LMA and whole-leaf LMA needs to be combined in one analysis (e.g., meta-analysis) or leaf-disc LMA needs to be measured quickly as a proxy for whole-leaf LMA, we recommend converting the LMA values using one of the models above.

Intraspecific variation

Even though we used the same species, the same individuals, and the same trait (LMA) to estimate the extent of intraspecific trait variation (ITV), the explained variance of the relationship between whole-leaf and disc-based estimates was surprisingly small (Figure 4; $R^2 = 0.08$). Leaf-disc samples have more sources of ITV than whole-leaf samples (i.e., variation among leaf discs within the same leaves), which may be responsible for the small explained variance. On average, ITV based on leaf-disc LMA was greater than ITV based on whole-leaf LMA as we expected. Although many studies have quantified trait variation within a single leaf, a previous study on a giant-leaved species, *Alocasia macrorrhiza*, suggests that LMA varied within leaves due to reduced water supply and demand from midrib to outer regions (Li et al., 2013). Because calculating the mean values generally diminishes the effect of within-sample variation, the relationship between disc-based estimates of individual mean LMA and its whole-leaf estimates will be stronger than the relationship between leaf-disc CV and whole-leaf CV. Thus, leaf-disc and whole-leaf estimates of species means will show the strongest relationship. The mismatch of ITV between whole-leaf and disc-based estimates can be ignored in the type of ITV studies that attempt to determine sources of trait variation (e.g., site, across species, within species) and often use mixed linear models and variance partitioning (e.g., Messier et al., 2010; Lepš et al., 2011; Siefert et al., 2015). However, patterns are likely to change significantly in the type of ITV studies that attempt to quantify the extent of ITV, usually based on the CV (e.g., Albert et al., 2010; Bastias et al., 2017; Yang et al., 2020). This caveat might apply to other leaf chemical traits such as leaf N contents because those traits have both disc and whole-leaf based estimates, but the sample type for those two estimates are usually not clarified in trait databases (e.g., TRY). Because we used limited sample numbers (at least 5 samples per species) and most were obtained with a small punch, our study design may inflate errors in estimating ITV (Yang et al., 2020). Future studies should include more samples to improve the comparisons of ITV based on leaf discs and whole leaves.

CONCLUSIONS

The present study was designed to examine the extent to which whole-leaf and leaf-disc LMA matched. Although our study was not designed to evaluate the effects of diameter sizes of leaf punches, the sizes of the leaf discs and leaf morphology were important sources of variance between the whole-leaf LMA and the leaf-disc LMA. Our study shows that mean values of leaf-disc LMA are generally a good proxy for mean values of whole-leaf LMA when an appropriate size of leaf punch (e.g., 1.0 cm diameter) is used. Those LMA values can be converted using OLS regressions. Additionally, although the mean values of leaf-disc LMA and whole-leaf LMA matched well, quantifying trait variation using leaf discs should be done with caution because variation among leaf discs within the same leaves (including measurement errors) seems to be considerably large but cannot be avoided with the typical sampling numbers (2–3 discs per leaf). Leaf discs, which can be targeted to avoid leaf veins, are useful and essential for biochemical determinations such as measurements of Rubisco activity (Parry et al., 2002) because photosynthesis occurs mostly in lamina rather than in veins for C_3 plants (Hibberd and Quick, 2002; Gao et al., 2018). Leaf-disc LMA is also the preferred method for quantifying leaf lamina density, which avoid effects of veins. Accurately representing leaf traits will probably require careful selection between leaf-disc and whole-leaf traits depending on the objectives.

AUTHOR CONTRIBUTIONS

P.M., M.K., and Y.J.C. conceived the study; P.M., Y.O., and Y.J.C. collected data; M.K. and C.Z. performed the analysis; M.K. and Y.J.C. led the writing of the paper; and all authors contributed to revisions.

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DATA AVAILABILITY STATEMENT

Data, codes, and computing environments to reproduce this manuscript are archived on Zenodo at https://doi.org/10.5281/zenodo.6665083 (Maenpuen et al., 2022) and also available on Github at https://github.com/mattocci27/leaf-disc.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Site information.

Appendix S2. Detailed sampling protocol for whole-leaf and leaf-disc leaf mass per area (LMA).

Appendix S3. Generalization for a relationship between ratios of disc-based and whole-leaf-based estimates of leaf mass per area (LMA), leaf tissue density (LD), and leaf thickness (LT).

Appendix S4. Standardized regression coefficients modeling the effects of leaf tissue density, leaf area, leaf thickness, punch size, and their interactions on (a) the mean estimates of whole-leaf-based leaf mass per area (LMA) and (b) the estimated variance of whole-leaf LMA.

Appendix S5. Relationships between species mean leaf mass per area (LMA) determined by using whole leaves and leaf discs obtained with leaf punches of different diameters.

Appendix S6. Relationships between the ratio of whole-leaf leaf mass per area (LMA) to disc-leaf LMA and total dry mass for the leaf disc.

Appendix S7. Summary of a linear mixed model for log-transformed total dry mass of leaf discs.

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