Fossil leaf economics quantified: calibration, Eocene case study, and implications

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Abstract.—Leaf mass per area (M_A) is a central ecological trait that is intercorrelated with leaf life span, photosynthetic rate, nutrient concentration, and palatability to herbivores. These coordinated variables form a globally convergent leaf economics spectrum, which represents a general continuum running from rapid resource acquisition to maximized resource retention. Leaf economics are little studied in ancient ecosystems because they cannot be directly measured from leaf fossils. Here we use a large extant data set (65 sites; 667 species-site pairs) to develop a new, easily measured scaling relationship between petiole width and leaf mass, normalized for leaf area; this enables M_A estimation for fossil leaves from petiole width and leaf area, two variables that are commonly measurable in leaf compression floras. The calibration data are restricted to woody angiosperms exclusive of monocots, but a preliminary data set (25 species) suggests that broad-leaved gymnosperms exhibit a similar scaling. Application to two well-studied, classic Eocene floras demonstrates that M_A can be quantified in fossil assemblages. First, our results are consistent with predictions from paleobotanical and paleoclimatic studies of these floras. We found exclusively low-M_A species from Republic (Washington, U.S.A., 49 Ma), a humid, warm-temperate flora with a strong deciduous component among the angiosperms, and a wide M_A range in a seasonally dry, warm-temperate flora from the Green River Formation at Bonanza (Utah, U.S.A, 47 Ma), presumed to comprise a mix of short and long leaf life spans. Second, reconstructed M_A in the fossil species is negatively correlated with levels of insect herbivory, whether measured as the proportion of leaves with insect damage, the proportion of leaf area removed by herbivores, or the diversity of insect-damage morphotypes. These correlations are consistent with herbivory observations in extant floras and they reflect fundamental trade-offs in plant-herbivore associations. Our results indicate that several key aspects of plant and plant-animal ecology can now be quantified in the fossil record and demonstrate that herbivory has helped shape the evolution of leaf structure for millions of years.

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Introduction

Many leaf traits strongly influence ecosystem function (Díaz et al. 2004; Wright et al. 2004; Poorter and Bongers 2006; Shipley et al. 2006; Parton et al. 2007), but few have been quantifiable from the fossil record. Among these traits, leaf dry mass per area (M_A; also commonly abbreviated as LMA; M_A is the in-

verse of specific leaf area) is a key variable representing the dry mass cost of deploying photosynthetic surface (Reich et al. 1997; Westoby et al. 2002). Species investing in a high M_A tend to have lower mass-based photosynthetic rates but longer leaf lifetimes (LL), such that their lower revenue (fixed carbon) per time may be compensated by a longer-lasting revenue stream (Reich et al. 1997; Westoby et al. 2002; Wright et al. 2004). Leaves with higher M_A are more expensive to construct per unit area, generally operate at lower nitrogen and phosphorus concentrations per unit mass, have slower rates of dark respiration, and are better defended against herbivory owing to their greater thickness and/or toughness (Small 1972; Reich et al. 1997; Westoby et al. 2002; Díaz et al. 2004; Wright et al. 2004). These coordinated trade-offs form a "leaf economics spectrum" (Wright et al. 2004), which represents one component of a general continuum running from specialization for rapid resource acquisition ("fast-return" species) to a strategy that maximizes resource retention ("slow-return" species) (Grime 1974; Grubb 1998). Leaf mass per area is also correlated with growth rates and the turnover of plant parts, and the influence of M_A persists through leaf "afterlife effects" into ecosystem processes including decomposition of litter (Kazakou et al. 2006) and mineralization of nitrogen and phosphorus (Kobe et al. 2005).

Insect herbivory can be measured directly from leaf fossils (Beck and Labandeira 1998; Labandeira 1998; Wilf and Labandeira 1999; Wilf et al. 2001, 2005), but the fundamental leaf economic traits that influence herbivory have been difficult to quantify from fossils. Several methods for estimating LL for fossil species exist, but three of these, comparison with nearest living relatives (Chaloner and Creber 1990), leaf thickness (Chaloner and Creber 1990), and presence of leaf mats (Spicer and Parrish 1986), are qualitative; at best they can distinguish deciduous from evergreen leaf habits (e.g., Wolfe 1987; Wolfe and Upchurch 1987). A fourth method quantifies LL from the wood anatomy of conifers (Falcon-Lang 2000a,b; Brentnall et al. 2005), but this method is not yet applicable to angiosperms.
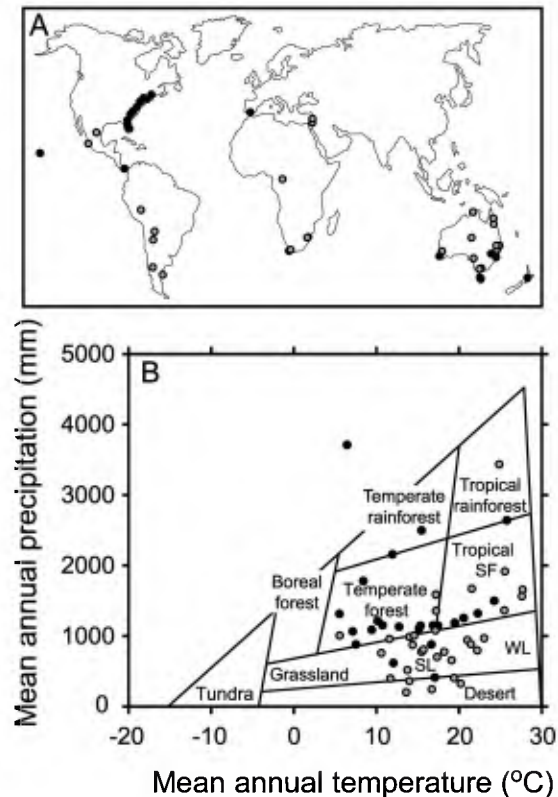
Here we analyze an extant data set drawn

FIGURE 1. Geographic and climatic distribution of calibration sites. A, Geographic distribution of the 65 sites used in the calibration data. Black symbols represent sites where five or more species were sampled; gray symbols represent sites where four or fewer species were sampled (see "Materials and Methods"). B, Climate information and major biome type (Whittaker 1975) for the calibration sites. SF = seasonal forest; WL = woodland; SL = shrubland. Biome boundaries are only approximate and do not encompass all samples. Symbols follow panel A. See Appendices 1 and 2 for further details about sites.

from geographically widespread and climatically diverse sites (Fig. 1) to develop a new method for quantifying M_A rapidly and accurately from the sizes and shapes of leaves. We assess several models for estimating M_A from petiole width (PW), leaf area (A), and leaf length, variables chosen because they can be easily measured on most well-preserved leaf fossils. For example, although petiole length has important biomechanical properties (Niklas 1994), it is much less frequently available from fossils, because of incompleteness, than petiole width. We report results (Fig. 2) using the model
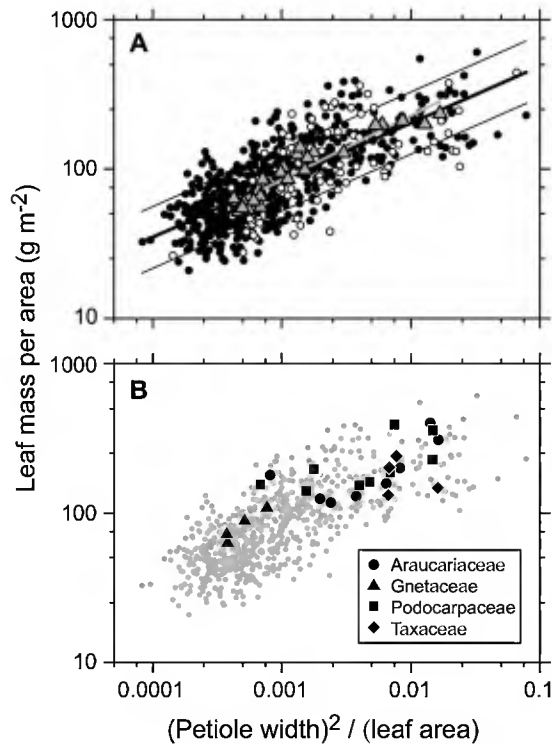
FIGURE 2. Scaling relationship between petiole width (PW) and leaf dry mass per area (M_A) for extant data. A, Calibration data for woody angiosperms. Solid and open symbols represent species-site pairs that came from sites where five or more species and four or fewer species were sampled, respectively (see "Materials and Methods"); triangles represent means for sites where ten or more species were sampled. Linear regression for species (solid black line) is $\log[M_A] = 3.070 + 0.382 \times \log[PW^2/A]$; thin lines represent ±95% prediction intervals (Sokal and Rohlf 1995). Linear regression for sites (gray line) is $\log[M_A] = 3.214 + 0.429 \times \log[PW^2/A]$. B, Preliminary scaling relationship for broad-leaved species with distinct petioles from several gymnosperm families. Species-site pairs are plotted. The following genera are represented: *Agathis*, *Gnetum*, *Podocarpus*, *Phyllocladus* (cladodes), *Saxegothaea*, *Torreya*, and *Taxus*. The gray symbols correspond to the angiosperm data in panel A. All relationships are significant at the family level using log-log linear regression except Taxaceae (Araucariaceae: $r^2 = 0.49$, $F_{1,7} = 5.85$, $p = 0.05$; Gnetaceae: $r^2 = 0.92$, $F_{1,3} = 22.2$, $p = 0.04$; Podocarpaceae: $r^2 = 0.44$, $F_{1,8} = 5.45$, $p = 0.05$; Taxaceae: $r^2 = 0.09$, $F_{1,3} = 0.20$, $p = 0.70$).

$$\log(M_A) = a + b \log\left(\frac{PW^2}{A}\right). \quad (1)$$

This model corresponds to the proposition, based on biomechanical and developmental principles, that the cross-sectional area of the petiole scales with the mass of the leaf. This relationship is expected because the petiole is
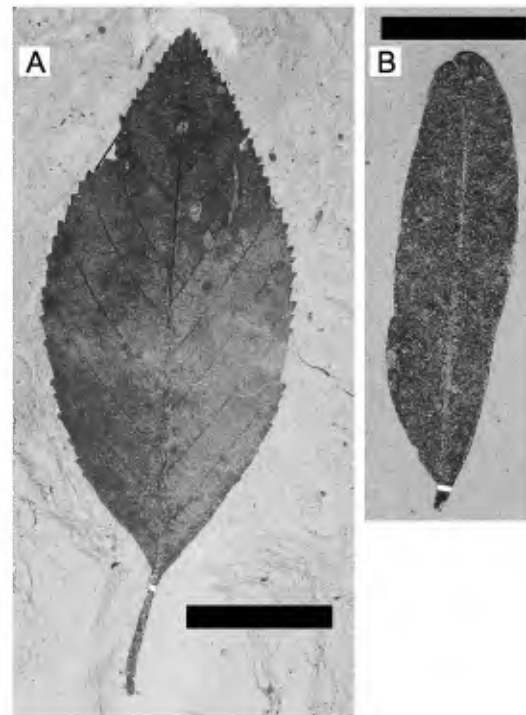

FIGURE 3. Representative examples of fossil specimens used in study. The specimen in panel A (*Alnus parvifolia*, Republic) has a narrower petiole (5.3 mm; see white line) and larger leaf area (442.8 mm²) than the petiolule of the specimen in panel B (*Caesalpinia pecorae*, Bonanza; petiole width = 9.7 mm; leaf area = 191.2 mm²); this results in a lower estimate of leaf dry mass per area for the *A. parvifolia* specimen (70.8 g m⁻²) than the *C. pecorae* specimen (154.3 g m⁻²). Scale bars, 1 cm. The black line in the *A. parvifolia* specimen represents a conservative reconstruction of the leaf-margin segment that was not preserved. See "Materials and Methods" for procedural details on how petioles were measured.

important in the mechanical support of the leaf (Salisbury 1913; Niklas 1994; see "Model Fitting and Justification").

We apply our method to 187 fossil leaves from two Eocene fossil floras (Republic, Washington, Klondike Mountain Formation; and Bonanza, Utah, Green River Formation) (Figs. 3, 4) where well-understood systematics (MacGinitie 1969; Wolfe and Wehr 1987), paleoclimate (MacGinitie 1969; Wolfe and Wehr 1987; Wing and Greenwood 1993; Wilf et al. 1998; Greenwood et al. 2005), and herbivory (Wilf et al. 2001, 2005; Labandeira 2002) allow testable hypotheses. Republic is considered to be dominated by deciduous species (Wolfe and Wehr 1987); thus, our hypothesis is that these species have low reconstructed M_A. Bo-

Insect damage types

16

A
□ Republic
● Bonanza

12

8

4

0

Leaves with insect damage (%)

80

B

60

40

20

0

Leaf area removed by insect damage (%)

3.0

C

2.5

2.0

1.5

1.0

0.5

0.0

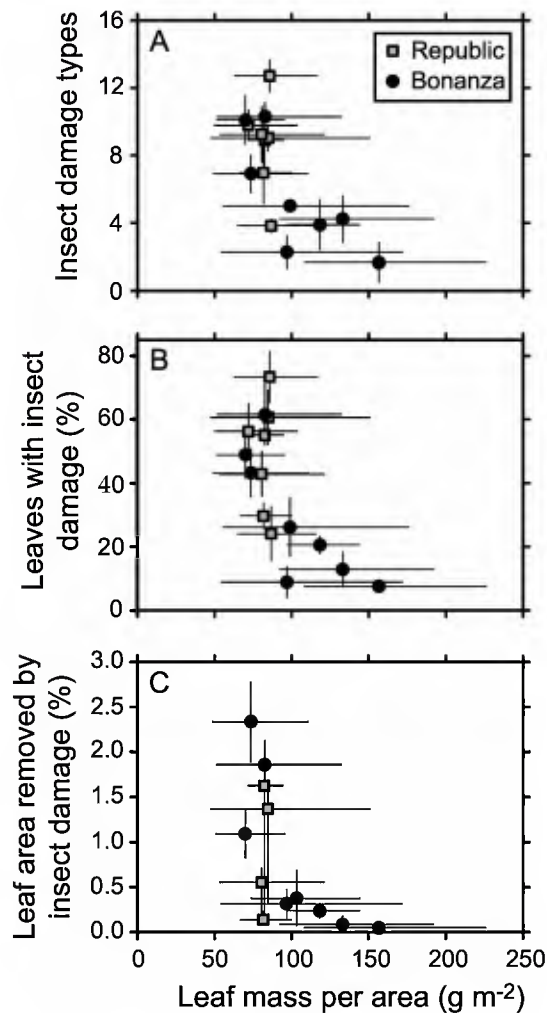0   50   100   150   200   250

Leaf mass per area (g m⁻²)

FIGURE 4. Correlation between insect herbivory and estimated leaf dry mass per area (M_A) for two Eocene fossil floras. Each data point represents a species mean, and errors in M_A represent ±95% prediction intervals (Sokal and Rohlf 1995). Only species where $\geq$23 specimens could be scored for insect herbivory are plotted. A, Insect damage morphotypes (Wilf et al. 2001, 2005) versus M_A; errors in herbivory represent ±1σ. Statistics of log-log linear regression for combined data: $n = 18$; $r^2 = 0.64$; $F_{1,16} = 28.0$; $p < 0.0001$. B, Percentage of leaves with insect damage (Wilf et al. 2001, 2005); errors in herbivory represent ±1σ of the binomial sampling error. Statistics of log-log linear regression for combined data: $n = 18$; $r^2 = 0.67$; $F_{1,16} = 32.6$; $p < 0.0001$. C, Percentage of leaf area removed by insect damage; errors in herbivory represent standard errors. Statistics of log-log linear regression for combined data: $n = 15$; $r^2 = 0.68$; $F_{1,13} = 27.4$; $p < 0.0001$.

nanza putatively contains a mix of species with both short and long LL (MacGinitie 1969; Wilf et al. 2001); these interpretations were based on qualitative methods discussed above

but correctly predicted the bimodal distribution of herbivory observed at the site (Wilf et al. 2001). Thus, we hypothesized a broad range of estimated M_A values at Bonanza. We compare our reconstructions of M_A with qualitative observations of the floras and with direct measurements of insect herbivory, and use them to refine understanding of plant and site ecology as well as forest nutrient cycling rates for these classic fossil floras.

Materials and Methods

Calibration Sites.—To reconstruct M_A from leaf fossils, we first collected leaves to create an extant calibration from 667 species-site pairs representing 468 species of woody angiosperms from 65 geographically and climatically diverse sites (Fig. 1). We sampled 1–20 mature, representative leaves or equivalent photosynthetic organs (phyllodes) (median = 3; 88% of species-site pairs are based on two or more leaves) from each of 5–86 species (median = 21) at 26 sites (Fig. 1A). To broaden our geographical coverage, we also sampled 4–12 leaves (median = 10) from each of one to four species at 39 additional sites (Fig. 1A; Appendices 1–2; Appendix A online at http://dx.doi.org.10.1666/pbio07001.s1). In aggregate, the sites represent most of the major biomes where the foliage of woody angiosperms is likely to be fossilized (Fig. 1B). We collected native, woody angiosperm species exclusive of monocots. We generally restricted our sampling to outer, exposed canopy leaves (Appendices 1, 2) because they constitute the majority of leaf fossils (Spicer 1981). Leaves without obvious, distinct petioles were excluded. Herbaceous species were also excluded because they rarely fossilize (Spicer 1981).

Fossil Sites.—We reconstructed M_A and measured insect herbivory for woody dicot species from two fossil lake floras. The first flora, Republic (Wolfe and Wehr 1987; Radtke et al. 2005), is from the Klondike Mountain Formation in northeastern Washington, U.S.A., and is late early Eocene in age (ca. 49 Ma [reported in Radtke et al. 2005]). The climate at Republic is interpreted as humid and warm temperate (mean annual temperature [MAT] = 13°C; mean annual precipitation [MAP] > 1000

mm) (Wolfe and Wehr 1987; Greenwood et al. 2005). The second flora, Bonanza (MacGinitie 1969), is from the uppermost Green River Formation in northeastern Utah, U.S.A., and is early middle Eocene in age (47.3 Ma [Smith et al. 2007]). In contrast to Republic, the climate at Bonanza has been interpreted as warmer and more seasonally dry (MAT = 15°C; MAP = 840 mm) (MacGinitie 1969; Wing and Greenwood 1993; Wilf et al. 1998). The 187 specimens with measurable leaf area and petiole width were selected from recent unbiased census collections made by K.R.J. of 1019 dicot leaves at Republic and 894 at Bonanza, reported by Wilf et al. (2001, 2005). Both floras were collected from single stratigraphic horizons (thickness of sampled horizons = 1.6 m and 0.1 m for Republic and Bonanza, respectively [Wilf et al. 2001, 2005]).

Leaf Measurements for Quantifying M_A.—We measured petiole width (*PW*) perpendicular to the midvein in the plane of the leaf blade, at the basal-most insertion of the lamina into the petiole. If the position of this measurement corresponded to a locally thickened or winged region of the petiole, *PW* was measured just basal to the feature. Leaflets and petiolules were the units measured for compound leaves, and phyllodes and basal attachments for phyllodes; for our data set, simple leaves and leaflets did not differ in their scaling relationship between M_A and PW^2/A (slope: $p = 0.44$; *y*-intercept: $p = 0.45$; likelihood ratio method of Falster et al. 2003). For a subset of leaves from our calibration data set, we also measured *PW* at the thinnest point and the midpoint of the petiole, but these alternative measurements did not yield improved correlations and tended to be highly correlated across species. Because complete petioles with bases are only rarely preserved, our protocol allows measurement of a greater number of fossils than these alternatives. It is possible that a combination of petiole width and depth correlates more strongly with M_A than does *PW* alone, but original petiole depth is rarely preserved in compressed fossils (Niklas 1978; Rex 1986).

We measured *PW* and leaf length with calipers, often using clear acetate sheets for fossils to protect surfaces, or from high-resolu-

tion digital images (600 dpi minimum); leaf area was determined from digital images. For the extant calibration data, we calculated M_A from the dry mass and area of the leaf blade and petiole (Cornelissen et al. 2003). Leaf mass per area varies 30-fold and leaf area 3.5 orders of magnitude in the calibration data; worldwide, M_A varies about 50-fold and leaf area five orders of magnitude (Wright et al. 2004). Our calibration data thus capture the majority of the known variation in these variables. Given the biomechanical basis for the scaling relationship (see "Model Fitting and Justification"), *PW* could be better optimized for fresh than dry leaf mass. However, in a subset of 98 species-site combinations, there was a strong correlation across species between dry mass and fresh mass ($r^2 = 0.96$; $F_{1,96} = 2068$; $p < 0.0001$). Thus, for this subset, there was little difference in the strength of correlation between PW^2/A and M_A calculated on a fresh or dry mass basis ($r^2 = 0.81$ and 0.77 for fresh and dry mass, respectively); moreover, the slopes of the correlations were not significantly different ($p = 0.72$; likelihood ratio method of Falster et al. 2003).

For *PW* measurements of fossils, only specimens where the petiole was clearly and completely preserved at the point of measurement for *PW*, described above, were used (Fig. 3; Appendix B online at http://dx.doe.org.10.1666/pbio07001/s2). One hazard with fossil petioles is longitudinal splitting, creating the false appearance of a thin petiole; thus, fossil petioles were inspected under binocular microscopes to ensure that both petiole margins were preserved before measurement at magnification. For specimens with partially preserved leaf blades, only those specimens whose full leaf areas could be reconstructed with reasonable confidence were considered. Species represented by only one specimen were excluded.

A potential error with fossils is that their morphology can change postmortem. However, previous experiments that mimicked the fossilization process indicated little to no change in the two-dimensional shape of leaf blades and at most a 10% inflation in the width of xylem-rich tissues, such as petioles, that are buried in fine-grained sediment (Wal-

ton 1936; Niklas 1978; Rex and Chaloner 1983; Rex 1986) such as the two fossil localities studied here (MacGinitie 1969; Wolfe and Wehr 1987); a 10% inflation of *PW* would lead to a 7.6% overestimation of M_A.

Leaf Measurements for Quantifying Insect Herbivory.—High rates of insect herbivory generally correlate with trait values towards the "fast-return" end of the leaf economics spectrum, including high foliar nitrogen concentration and short *LL* (Coley 1983; Westoby et al. 2002); herbivory is predicted to inversely correlate with M_A, but this has rarely been directly tested in extant vegetation (Moles and Westoby 2000; Poorter et al. 2004) and never before tested in fossil vegetation. To test for the hypothesized negative correlation between herbivory and M_A, we compared published data on insect herbivory (Coley 1983) to M_A determined from saplings of the same species in a present-day tropical forest on Barro Colorado Island, Panama (Fig. 5).

Insect damage morphotypes and percentage of specimens with insect damage were previously tabulated for the Republic (Wilf et al. 2005) and Bonanza (Wilf et al. 2001) fossil floras. Only species for which ≥23 specimens could be scored for insect herbivory were included here; this sample size represents a compromise between an adequate sampling level for precise results and the inclusion of enough species to establish reliable site-level trends. To account for uneven sampling across species, insect damage morphotype data were randomly subsampled to 23 specimens 5000 times without replacement (Wilf et al. 2001), and the means of these subsamples are reported here (Fig. 4A). Both floras were scored for percentage of leaf area lost to insect damage following the method of Beck and Labandeira (1998) (*n* = 1019 and 894 leaves for Republic and Bonanza, respectively; only those species where ≥23 specimens could be scored for insect herbivory were included in the tally); species means were based on the arcsine transformation of individual leaves (Sokal and Rohlf 1995). We consider these measurements (Fig. 4C) minima because areas of the leaf that were not preserved, and that therefore may have been damaged or entirely removed by insects, cannot be analyzed.
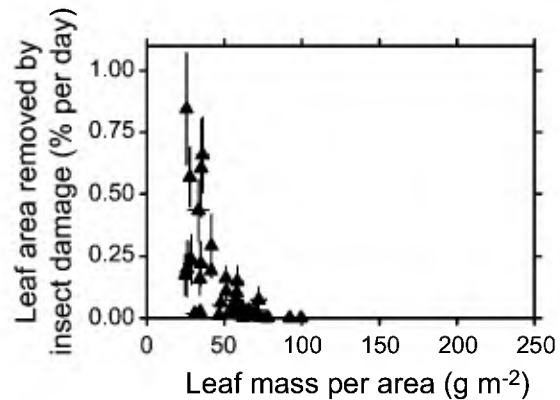

FIGURE 5. Rate of leaf area removed by insect damage (Coley 1983) versus leaf dry mass per area (M_A) for present-day vegetation (saplings) at Barro Colorado Island, Panama. Errors represent standard errors. Statistics of log-log linear regression: $n = 44$; $r^2 = 0.36$; $F_{1,42} = 24.1$; $p < 0.0001$. The offset to lower M_A in these data relative to the fossil reconstructions (Fig. 4) is a consequence of saplings having leaves with a lower M_A than mature plants (Thomas and Winner 2002), and mature plants constitute the bulk of fossil plant deposits (Spicer 1981). The offset in herbivory relative to the fossil measurements (Fig. 4) is a consequence of the fragmentary nature of fossil leaves (see "Materials and Methods" for further details). Importantly, the dividing line in the Panama data between leaves that are highly damaged by insects and those that are not corresponds to an M_A of ~50 g m^{-2}, or a leaf life span (*LL*) of ~12 months (95% of species with an M_A <51.5 g m^{-2} have a *LL* of <12 months, whereas 87% of species with an M_A >51.5 g m^{-2} have a *LL* of >12 months). This relationship between herbivory and *LL* is consistent with the fossil data (see "Results and Discussion") and further emphasizes that the fossil and Panama data sets are compatible.

Model Fitting and Justification

We fit several models (Table 1) to the observed relationships (Appendix A online) between M_A, *PW*, and other leaf dimensions. Previous work in two species has shown that petiole cross-sectional area correlates with supported mass and area within species (Niklas 1991a; Yamada et al. 1999). Additionally, several studies have examined relationships among petiole biomechanical properties within and across species (Niklas 1991a,b, 1994, 1999). Our study is the first to our knowledge to demonstrate general scaling between petiole and lamina dimensions across diverse species, and to develop from these interrelationships a prediction of M_A. Scaling might be influenced by hydraulic supply as well as by mechanical support because the petiole delivers the transpiration stream to the leaf, and peti-

TABLE 1. Models fitted. All models are based on individual species-site samples ($n = 667$). Both leaf dry mass per area (M_A; units in g m^{-2}) and the predictor variables are handled on log scales to allow the use of power law allometries, and because variance increases with the mean whereas after log transformation scatter is more normally distributed (Fig. 2A). All models are fitted using linear regression in order to minimize the sum-of-squares in the y-dimension (i.e., M_A), to facilitate retrodiction with fossils. This contrasts with the standardized major axis (SMA) estimation (also known as Model II regression, geometric mean regression, or reduced major axis), where errors in both the x- and y-dimensions are minimized simultaneously (Falster et al. 2003; Warton et al. 2006); we use SMA to investigate the slopes of allometric relationships (see Model Fitting and Justification). The logic of each model is explained in Model Fitting and Justification; Model E corresponds to equation (1) in the text. PW = petiole width (mm); A = one-sided projected area of leaf (mm^2); L = leaf length (mm); N/A = not applicable because SMA cannot be calculated for multivariate models.

	Model	a	b	c	r^2	Slope*	SMA
A	$\log(M_A) = a + \log(PW^2/A)$	4.930			−0.89	0.13	1.00
B	$\log(M_A) = a + \log(PW^{8/3}/A)$	4.870			−0.92	0.001	1.00
C	$\log(M_A) = a + b\log(PW^4/A)$	2.740	0.289		0.47	1.13	0.42
D	$\log(M_A) = a + b\log(PW/A)$	2.870	0.307		0.42	1.09	0.47
E	$\log(M_A) = a + b\log(PW^2/A)$	3.070	0.382		0.55	1.04	0.51
F	$\log(M_A) = a + b\log(PW) + c\log(A)$	3.064	0.983	−0.386	0.58	1.05	N/A
G	$\log(M_A) = a + b\log[(PW)/(L \times A)]$	2.876	0.194		0.39	1.10	0.32

* Slope of measured vs. estimated M_A linear regression fixed through the origin.

ole cross-sectional area correlates with xylem vessel area and with petiole hydraulic conductance per leaf area for leaves of a given species (Salisbury 1913; Sack et al. 2002, 2003). However, across distantly related species, petiole cross-sectional area per leaf area does not necessarily correlate with petiole or leaf hydraulic conductance per leaf area because both the numbers and sizes of xylem conduits within petioles vary strongly (Nardini et al. 2005; Sack and Frole 2006).

Here we discuss the possible underlying biology of the models, the statistical strengths of their fitting to the data, and possible interpretations of the observed scaling coefficients. We note that although our models consider petiole length implicitly as described below, we do not explicitly include petiole length as a predictive variable because complete fossil petioles are rare. Also, we recognize that wind load may affect scaling relationships between M_A and petiole dimensions because plants in windy habitats can have higher M_A and smaller petiole cross-sectional area to allow easier bending and twisting for reducing drag (Niklas 1996, 1998). However, because this adaptation would lead to the opposite trend documented here (Fig. 2), wind load is likely of only minor importance. Lastly, the relationships in this study were determined across diverse species, but they have yet to be tested within species.

Model A is based on a simple scaling rela-

tionship between the ratio of the square of petiole cross-sectional area to leaf area versus M_A. This relationship, $M_A \propto PW^2/A$, where PW = petiole width and A = leaf area, would be expected if the leaf behaved as a mass applied to a vertical petiole that was just sufficient to support it. Preservation of compressive strength to maintain resistance to buckling then leads to the expectation of $PW^2 \propto M$, where M = leaf mass, and thus $M_A \propto PW^2/A$. This scaling treats petiole length as varying little, or at least independently of leaf size. Alternatively, if petiole length and width are co-optimized, a slightly different scaling following "elastic similarity" as for animal legs might be expected (McMahon and Bonner 1983; Peters 1983; Schmidt-Nielsen 1984), with $PW^{8/3} \propto M$ (and $M_A \propto PW^{8/3}/A$; Model B).

Leaf mass is only rarely incident on a vertical petiole; instead, leaves are usually better modeled as end-loaded cantilevered beams (Niklas 1991b, 1999). Under this scenario, if the petiole supports the leaf mass with a fixed deflection distance, at a given wind-load, and without leaf shape and petiole composition and mechanical properties being influential variables, the expected relation is $M \propto 3\ EI/PL^3$, where E is the petiole elastic modulus, I the second moment of area of the petiole, the product EI the petiole flexural rigidity, and PL the petiole length (Niklas 1991b, 1994, 1999). Indeed, previous work has shown that PL^3 scales with EI as expected from the cantilever

model across a diverse range of leaves (Niklas 1991b, 1994, 1999). Model C applies this scenario, assuming PW to be independent of PL, and petiole shape to be relatively invariant; in this case, M would be proportional to EI and I would be related to PW^4, and $M_A \propto PW^4/A$. Model D applies the same scenario, additionally assuming that $PW \propto PL$; in this case, the cantilevered beam model simplifies to $M \propto 3(EI/PL^3) \propto 3(E \times PW^4/PL^3) \propto PW$, and $M_A \propto PW/A$.

Models E–G represent additional scenarios, with greater flexibility. Model E preserves the expectation of the scaling of PW^2 with M, as in Model A (and as observed to hold within given species, as discussed above), but allows an allometric scaling, $M_A \propto (PW^2/A)^b$. Model F modifies Models C–E by allowing the exponents to vary independently. Model G modifies Model D by including leaf length as an additional factor, reflecting the extra leverage of a given mass that is farther from the attachment point of the leaf.

The fits of Models C–G indicate a strong scaling of M_A with petiole and lamina dimensions (Table 1); however, the fitted parameters do not fit simply with many of the expectations discussed above. For example, Models C–E and G show slopes b substantially lower than the expectations for a slope of 1, as determined by a standardized major axis (SMA) estimation (Falster et al. 2003). Further, all models indicate that PW relative to leaf area is inordinately high for leaves of high M_A relative to what simple support requirements would require, under any of the above scenarios. This could be one explanation for the previously demonstrated result that petiole flexural rigidity (EI) increases more strongly with leaf mass (M) than is predicted from the cantilever model ($EI \propto M$ with an exponent of 1.6–2.3 for diverse species sets [Niklas 1991a]). The disproportionate PW relative to leaf area for leaves of larger M_A and the consequently higher petiole flexural rigidity would contribute greater support stability given that the laminar center of mass could be displaced over larger petiolar second moment of area. Such investment in greater safety is consistent with the investment in greater construction cost for leaves of higher M_A, and their gener-

ally longer life spans (Villar and Merino 2001; Wright et al. 2004).

Model E, corresponding to equation (1), was used for estimation because of its relatively high goodness of fit ($r^2 = 0.55$ for species means) and its low bias (the slope of the plot for measured versus estimated M_A is 1.04; Table 1). This model also has the advantage of being a simple expression of the allometric scaling of petiole and lamina dimensions as discussed above. The parameters of Model E indicate that an approximation of petiole cross-sectional area relative to leaf area scales strongly with M_A, with SMA slope of 0.51 (±0.026 95% confidence intervals); this model is most compatible with petioles with circular and square cross-sections (kX^2, where k is a constant and X is the length of the side of a square or the radius of a circle), however a mixture of cross-sectional shapes will decrease somewhat the predictive power of the model. Model F has a slightly higher r^2-value than Model E (0.58; Table 1), but this extra explanatory power is largely due to Model F's having an additional parameter.

Calculating Errors for M_A Estimates.—The calculation of 95% prediction intervals (PI) follows Sokal and Rohlf (1995):

$$\log PI = \left\{ \log M_A \pm \sqrt{s_{Y \cdot X}^2 \left[\frac{1}{k} + \frac{1}{n} + \frac{(X_i - \bar{X})^2}{\sum x^2} \right]} \right\} \times t_{0.05[n-2]},$$

$$(2)$$

where $s_{Y \cdot X}^2$ = unexplained mean square, k = size of unknown sample, n = sample size of calibration data, X_i = mean $\log(PW^2/A)$ of unknown sample, X = mean $\log(PW^2/A)$ of calibration data, $\sum x^2$ = sum of squares of calibration data, and $t_{0.05[n-2]}$ = critical value of Student's distribution for $(n - 2)$ degrees of freedom. Table 2 provides the necessary information for calculating 95% PIs (for species and sites) from the regressions presented in Figure 2A; errors are asymmetric with respect to means because the regressions are based on logarithmic relationships.

Results and Discussion

Testing Extant Vegetation.—Fitting Model E to our calibration data shows that the M_A of

TABLE 2.   Parameters used to calculate 95% predictions intervals for estimates of leaf dry mass per area (M_A). $S^2_{Y \cdot X}$ = unexplained mean square, n = sample size of calibration data, $\bar{X}$ = mean log(PW^2/A) of calibration data, Σx^2 = sum of squares of calibration data, and $t_{0.05[n-2]}$ = critical value of Student's distribution for $(n-2)$ degrees of freedom. See "Materials and Methods" for details.

	$S^2_{Y \cdot X}$	n	$\bar{X}$	Σx^2	$t_{0.05[n-2]}$
Species means	0.032237	667	−3.011	182.1	1.964
Site means	0.005285	25	−2.857	5.331	2.069

individual species is estimated to a significant degree (Fig. 2A; $n = 667$, $r^2 = 0.55$, $F_{1,666} = 825$, $p < 0.0001$): 95% prediction intervals (Sokal and Rohlf 1995) are $\sim \pm^{60\%}_{38\%}$ of observed values, assuming a sample size of three leaves. This error is small compared to the ~30-fold range observed across species.

Estimates of M_A are unbiased: the regression slope of the measured versus estimated M_A is close to unity (1.04 ± 0.04 95% confidence intervals). Mean values for sets of species at sites can be estimated very precisely because of the lack of bias and increased sample size (Fig. 2A; $n = 25$, $r^2 = 0.89$, $F_{1,24} = 186$, $p < 0.0001$; 95% prediction intervals for individual sites are $\sim \pm^{16\%}_{14\%}$ of their observed values, assuming a sample size of ten species). Preliminary data from broad-leaved gymnosperms ($n = 25$ species) match the correlation as well (Fig. 2B), suggesting applications that include the pre-angiosperm record.

We tested whether MAT or MAP modulated the relationship between M_A and PW^2/A in our calibration data, using partial correlation. The relationship between PW^2/A and M_A remains significant and largely unchanged (full correlation: $r = 0.74$; correlation after accounting for MAT and MAP: $r = 0.75$ and 0.74, respectively; $n = 667$ and $p < 0.0001$ for both tests). This insensitivity to environmental conditions contrasts with many other paleoecological and paleoclimatological proxies (Royer et al. 2002) and reinforces the notion that PW^2/A is a faithful recorder of M_A. Moreover, interrelationships among leaf economic variables such as M_A and LL are not strongly modulated by phylogeny (Ackerly and Reich 1999); this is important for paleobiological studies, where fossil taxa may be extinct, be only distantly related to taxa in the calibration data, or have unknown affinities.

Because leaf economic traits are strongly in-

tercorrelated (Reich et al. 1997; Westoby et al. 2002; Wright et al. 2004, 2005), our method has the potential to predict other traits. For example, in a worldwide compilation of leaf economic information (Wright et al. 2004), a M_A of 129 g m^{-2} for woody angiosperms corresponded to a mean LL of 12 months. We determined this LL category (<12 or >12 months) for a subset of our data ($n = 496$ species-site pairs). A PW^2/A of 0.0011, corresponding to an estimated M_A of 87 g m^{-2}, correctly predicts the LL category 85% of the time in our calibration data. We adopt these M_A values to broadly distinguish between the short-lived "fast-return" ($<\sim 87$ g m^{-2}) and long-lived "slow-return" ($>\sim 129$ g m^{-2}) ends of the leaf economic spectrum.

Application to Fossil Record.—We hypothesized low M_A at Republic and a broader range of M_A at Bonanza on the basis of previously published, qualitative interpretations (see "Introduction"). Consistent with hypotheses, results from Republic show domination by low-M_A species (57–87 g m^{-2}; Table 3). Consequently, it is likely that most of these species had leaf life spans of <12 months, suggesting the presence of a deciduous forest among the angiosperms. Also consistent with hypotheses, the Bonanza flora shows a broader mix of M_A values (70–157 g m^{-2}; Table 3). The most abundant species at Bonanza have high M_A values (Table 3), suggesting that the vegetation was dominated by species with long-lived leaves. The site mean of M_A among angiosperms is significantly higher at Bonanza than Republic (113.2 $\pm^{14.5}_{12.9}$ versus 76.8 $\pm^{9.2}_{8.2}$ g m^{-2}; errors represent 95% prediction intervals; $t_{1,14} = 3.54$, $p = 0.003$), and Bonanza is associated with a higher coefficient of variation (23.8% versus 12.8%; $t_{1,17} = 2.85$, $p = 0.01$ after arcsine transformation; Sokal and Rohlf 1995).

Thus, these floras had very different ecolog-

TABLE 3. Reconstructions of leaf dry mass per area (M_A) for measurable species in the Republic and Bonanza fossil floras. M_A estimates for the Fabaceae (legumes) may be somewhat too high because of their short, pulvinulate petiolules.

	n	Abundance* (%)	M_A** (g m^{-2})
REPUBLIC			
Alnus parvifolia (Betulaceae)	37	44.2	82.5
Betula leopoldae (Betulaceae)	5	3.1	72.0
Cercidiphyllum obtritum (Cercidiphyllaceae)	17	12.6	81.7
Cornus sp. (Cornaceae)	2	0.8	57.3
aff. Crataegus sp. (Rosaceae)	3	0.5	79.8
Crataegus sp. (Rosaceae)	2	3.2	84.8
Ericaceae sp.	2	0.4	84.0
Itea sp. (Saxifragaceae)	3	2.0	77.9
Macginitiea gracilis (Platanaceae)	2	1.8	69.2
Photinia pageae (Rosaceae)	2	2.0	63.2
Prunus sp. (Rosaceae)	2	0.6	68.4
Rhus malloryi (Anacardiaceae)	7	2.9	85.6
Sassafras hesperia (Lauraceae)	4	4.8	80.6
Spiraea sp. (Rosaceae)	8	2.5	86.7
Ternstroemia sp. (Theaceae)	2	0.2	87.0
Ulmus sp. (Ulmaceae)	8	8.1	67.0
Zelkova sp. (Ulmaceae)	2	0.5	85.9
Zizyphoides flabella (Trochodendraceae)	2	1.7	63.0
BONANZA			
Allophylus flexifolia (Sapindaceae)	4	4.9	73.6
Caesalpinia pecorae[†] (Fabaceae)	5	4.4	133.2
Cardiospermum coloradensis[†] (Sapindaceae)	2	3.8	96.7
Cedrelospermum nervosum[‡] (Ulmaceae)	18	26.7	118.3
Leguminosites lesquereuxiana (Fabaceae)	6	2.0	103.3
Macginitiea wyomingensis[‡] (Platanaceae)	7	5.3	70.0
Parvileguminophyllum coloradensis[†] (Fabaceae)	5	33.1	156.6
Populus tidwellii[‡] (Salicaceae)	2	2.6	98.9
Populus wilmattae[‡] (Salicaceae)	3	2.9	82.7
Rhus nigricans (Anacardiaceae)	13	7.2	115.2
Salix cockerelli[‡] (Salicaceae)	6	2.7	96.1
Styrax transversa (Styracaceae)	4	0.8	72.2
Syzygioides americana (Myrtaceae)	2	1.0	137.2

* Based on unbiased field census collections (Wilf et al. 2001, 2005). Values within floras do not sum to 100% because not all species are represented here (see "Materials and Methods").

** We interpret leaves with M_A values of <87 g m^{-2} to have leaf life spans of <1 year, and leaves with M_A values >129 g m^{-2} to have leaf life spans of >1 year (see "Results" and "Discussion").

† Inferred by Wilf et al. (2001) to have long leaf life spans.

‡ Inferred by Wilf et al. (2001) to have short leaf life spans.

ical structuring among woody angiosperms. Republic was dominated by species associated with relatively rapid mass-based rates of gas exchange and more rapid litter decomposition, whereas Bonanza was dominated by "slow-return" species, but with an important secondary component of "fast-return" species. This difference may have been driven by the seasonally drier climate at Bonanza, a pattern consistent with observations in present-day vegetation of greater variance in M_A in seasonally dry forests relative to moister forests (Niinemets 2001; Wright et al. 2005). Because litter decomposition rates influence nutrient turnover rates (Kobe et al. 2005) and regional biogeochemical cycling (Chapin 2003),

we infer that forest-wide nutrient cycling among woody angiosperms was probably more rapid at Republic than Bonanza.

By quantifying the frequency, amount, and diversity of insect damage on leaves from Republic and Bonanza, we directly correlated M_A (and LL by extension) to insect herbivory (Fig. 4). We hypothesized a negative relationship because leaves with high M_A are typically associated with greater thickness and/or toughness, higher amounts of chemical toxins and/or other chemical deterrents, and lower foliar nitrogen concentrations (Coley 1983; Westoby et al. 2002); all of these characteristics help to minimize insect damage. At Republic, where all species have an estimated M_A of <87

$g\ m^{-2}$, insect damage ranges from moderately high to very high (Fig. 4). In contrast, at Bonanza there is a greater range in M_A and herbivory levels, and these properties negatively correlate with one another. For both floras, the dividing line between species that are highly damaged and those that are not corresponds to an M_A of 90–100 $g\ m^{-2}$ (Fig. 4), or an inferred LL of ~12 months. These Eocene results are consistent with our predictions and with patterns observed in an extant forest (Fig. 5), suggesting that strong insect selection of leaf functional traits is of great antiquity.

Conclusions

An extant calibration indicates that leaf mass per area can be easily reconstructed for fossils from the measurement of petiole width and leaf area. This represents, to our knowledge, the first proxy for fossil M_A. Some key advantages of the method include the following: the required measurements can be made quickly and accurately on most well-preserved leaf fossils; the statistical errors for predicting M_A are at most $\pm^{60\%}_{38\%}$ if three or more leaves are measured per species; and the biomechanical scaling relationship is not strongly modulated by factors that can be difficult to evaluate in the fossil record (e.g., temperature, rainfall). Importantly, a preliminary analysis suggests that the method may also be applicable for some gymnosperm groups.

We quantified M_A for 31 species in two well-understood Eocene floras, and we compared these estimates with measurements of insect herbivory and qualitative inferences of other leaf economic variables for the same species. At Republic, where most species have an inferred short LL, we reconstructed low M_A; at Bonanza, where there is a broader range in inferred LL, we reconstructed a broader range in M_A (Table 3). At both sites, there is a statistically significant, inverse correlation between M_A and insect damage (Fig. 4). Together, these results demonstrate a consistent, emergent pattern: Republic was dominated by "fast-return" species, whereas Bonanza was dominated by "slow-return" species but with an important secondary component of "fast-return" species. More broadly, our results highlight the potential for quantifying leaf eco-nomic information, including important aspects of plant-animal interactions and constraints on nutrient cycling rates, from lesser-known floras.

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Appendix 1

Details of calibration sites used in study (five or more species per site). Climate data are from weather stations within 0.1° of the sites.

Site	Lat.	Long.	Species used	MAT (°C)	MAP (mm)	Author responsible for measurements	Further information on sites and sampling protocols
Archbold Biological Station, Florida, USA	27.2 N	81.4 W	15	22.3	1321	Royer, with some species from Harvard Forest by Sack, and some species from Barro Colorado Island by Sack and Moles	Huff et al. 2003; Kowalksi and Dilcher 2003: Royer et al. 2005: images of leaves available at *droyer. web.wesleyan.edu/ DigitalLeafPhysiognomy.htm*; Sack et al. 2005 for Barro Colorado Island leaves measured by Sack, and Sack et al. 2003 for Harvard Forest leaves measured by Sack
Barro Colorado Island, Panama	9.2 N	79.9 W	86	25.8	2640		
Big Hammock Natural Area and Wildlife Management Area, Georgia, USA	31.9 N	82.1 W	25	19.5	1189		
Cockaponset State Forest, Connecticut, USA	41.4 N	72.5 W	24	10.2	1213		
Dilcher's Woods lowland, Florida, USA	29.6 N	82.2 W	24	20.6	1257		
Dilcher's Woods upland, Florida, USA	29.6 N	82.2 W	20	20.6	1257		
Duke Forest, North Carolina, USA	36.0 N	78.9 W	27	15.2	1108		
E. N. Huyck Preserve and Biological Research Station, New York, USA	42.7 N	74.5 W	24	7.6	881		
Florida Panther National Wildlife Refuge, Florida, USA	26.2 N	81.3 W	18	24.3	1500		
Harvard Forest, Massachusetts, USA	42.5 N	72.2 W	34	7.2	1068		
Hawk Mountain Sanctuary, Pennsylvania, USA	40.6 N	75.9 W	23	10.8	1156		
Hubbard Brook Experimental Forest, New Hampshire, USA	43.9 N	71.8 W	15	5.6	1315		
Institute for Ecosystem Studies, New York, USA	41.8 N	73.8 W	29	9.5	1086		
Little Pee Dee State Park, South Carolina, USA	34.2 N	79.4 W	26	16.9	1149		
Smithsonian Environmental Research Center, Maryland, USA	38.5 N	76.3 W	23	12.8	1132		
Alcornocales Natural Park, Malaga, Spain	36.2 N	5.3 W	21	16.7	880	Niinemets & Valladares	Niinemets et al. 2003, 2007
Kuringai Chase National Park (high P), New South Wales, Australia	33.6 S	151.3 E	10	17.5	1148	Lusk	Wright et al. 2001; Wright and Westoby 2002
Kuringai Chase National Park (low P), New South Wales, Australia	33.7 S	151.1 E	14	17.5	1148		
Okataina, North Island, New Zealand	38.8 S	176.4 E	20	12.0	2157		
Round Hill Nature Reserve (high P), New South Wales, Australia	33.0 S	146.2 E	12	17.1	412		
Round Hill Nature Reserve (low P), New South Wales, Australia	33.0 S	146.1 E	5	17.1	412		
Frodshams Pass, Tasmania, Australia	42.8 S	146.4 E	14	8.5	1780	Jordan	Royer et al. 2005 for sampling protocol
Hobart Wet Sclerophyll, Tasmania, Australia	42.9 S	147.3 E	11	12.1	620		
Margaret River, Western Australia	34.1 S	115.1 E	23	15.4	1148		
Mt. Read, Tasmania, Australia	41.8 S	145.6 E	20	6.5	3710		
Koke'e Park, Kaua'i, Hawai'i, USA	22.1 S	159.7 W	10	15.5	2500	Sack	Royer et al. 2005 for sampling protocol

Appendix 2

Details of calibration sites used in study (four or fewer species per site). Climate data are from the model of New et al. (2002) and are typically within 0.2° of the sites.

Site	Lat.	Long.	Species used	MAT (°C)	MAP (mm)	Author responsible for measurements	Further information on sites and sampling protocols
Argentina, Bariloche: *Nothofagus* forest	41.2 S	71.4 W	1	5.6	1008	Moles and the World Herbivory Project (Nigel Andrew, Alejandro Bisigato, Pedro Blendinger, William Bond, Elizabeth Borer, Sarah Boulter, Lucrecia Cella Pizarro, Connie Clark, Philippe Cohen, Moshe Coll, Will Cornwell, Will Edwards, Rasmus Ejrnæs, José Facelli, Alejandro Farji-Brener, Florencia Fernández Campón, Bente Graae, Gilberto Jamangape, Enrique Jurado, Tiffany Knight, Bill Low, Faisness Lumbwe, Benjamin Magaña, Jonathan Majer, Miguel Martinez-Ramos, Peter McQuillan, Hans Meltofte, Ben Moore, Christa Mulder, Pablo Peri, Nigel Pitman, John Poulsen, Lynda Prior, Kate Reardon-Smith, Jorge Rodríguez, Eric Seabloom, James Stegen, Diego Vázquez, Ruan Veldtman, Peter Vesk, An von Coulter, Hugo von Zeipel, Matt Waldram, Charlie Zammit, Zheng Zheng)	*Site selection:* Moles et al. selected areas of natural vegetation, in vegetation types characteristic of each region.
Argentina, Puerto Madryn: Dune grassland	42.8 S	64.1 W	1	13.7	201		
Argentina, Puerto Madryn: Steppe	42.8 S	64.1 W	1	13.7	201		*Species selection:* At each site, Moles et al. studied the four most abundant species (by leaf cover)*. Only woody dicotyledons were included in the present study.
Argentina, Tucuman: Yungas N (seasonally dry forest)	23.7 S	64.8 W	3	13.8	520		
Argentina, Tucuman: Yungas S (seasonally dry forest)	26.8 S	65.3 W	3	11.8	398		
Australia, Adelaide: Chenopod shrubland	34.3 S	139.5 E	2	16.8	243		*Leaf selection:* Canopy access was only available at one site (Daintree, Australia). At all other sites, Moles et al. sampled leaves that could be reached from the ground.
Australia, Adelaide: Sclerophyll shrubland	34.3 S	139.5 E	2	16.8	243		
Australia, Adelaide: Mallee	35.2 S	139.1 E	3	14.0	363		
Australia, Alice Springs: Spinifex (*Triodia*) grassland with emergent eucalypts	23.7 S	133.9 E	3	20.2	324		
Australia, Armidale: *Callitris* woodland	29.8 S	151.1 E	3	15.5	779		
Australia, Brisbane: Subtropical rainforest	28.2 S	153.1 E	4	17.2	1586		*Sampling protocol:* Two mature, but recently expanded, leaves were taken from each of 5 plants from each species. Leaves were kept cool and moist until they could be scanned on a flatbed scanner (failing this, digital photographs were taken on a standard grid). Leaf area was calculated using Image-J (*rsb.info.nih.gov/ij/*). Leaves were dried at 55°C for 2 days, and weighed.
Australia, Daintree: Tropical rainforest	16.1 S	145.4 E	4	25.6	1918		
Australia, Darwin: Vine thicket	12.4 S	130.8 E	4	27.7	1658		
Australia, Darwin: Savanna	12.4 S	131.1 E	3	27.6	1562		
Australia, Melbourne: Grassland with Eucalypt overstorey	38.0 S	145.6 E	1	14.0	989		
Australia, Melbourne: Eucalypt woodland with bracken understorey	38.4 S	144.9 E	2	14.4	877		
Australia, Perth: Open sclerophyll woodland	32.0 S	116.0 E	1	17.2	1080		
Australia, Perth: Sclerophyll shrubland	31.7 S	115.9 E	1	18.3	774		*In cases where one of the selected species did not have enough reachable leaves, we studied the next most abundant species instead.
Australia, Sydney: Sclerophyll shrubland	33.6 S	151.3 E	2	17.3	1355		
Australia, Tasmania: Eucalypt woodland	42.9 S	147.9 E	3	11.6	959		
Australia, Tasmania: *Nothofagus* forest	42.7 S	146.7 E	3	10.7	759		
Australia, Toowomba: Eucalypt woodland	28.1 S	151.7 E	3	17.4	701		
Australia, Townsville: Savanna	19.3 S	146.7 E	1	23.1	968		
Australia, Townsville: Vine thicket	19.3 S	146.8 E	3	23.1	968		
Israel, Adulam: Mediterranean shrubland	31.6 N	34.9 E	4	19.4	407		

Appendix 2 Continued.

Site	Lat.	Long.	Species used	MAT (°C)	MAP (mm)	Author responsible for measurements	Further information on sites and sampling protocols
Israel, Hanadiv: Mediterranean shrubland	32.6 N	34.9 E	3	19.1	656		
Mexico, Chamela: Deciduous tropical forest	19.5 N	105.0 W	3	25.5	1366		
Mexico, Linares: Oak woodland	24.7 N	99.8 W	2	22.2	795		
Mexico, Linares: Thornscrub	24.8 N	99.5 W	2	22.2	795		
Peru, Los Amigos: Tropical rainforest on floodplain	12.6 S	70.1 W	3	24.9	3433		
Peru, Los Amigos, Successional vegetation near river	12.6 S	70.1 W	1	24.9	3433		
Peru, Los Amigos: Tropical rainforest	12.5 S	70.1 W	4	24.9	3433		
Republic of Congo, Kabo: Bai	2.2 N	16.1 E	1	21.6	1672		
Republic of Congo, Kabo: *Gilbertiodendron*-dominated tropical rainforest	2.1 N	16.2 E	3	21.6	1672		
Republic of Congo, Kabo: Mixed tropical rainforest	2.1 N	16.2 E	4	21.6	1672		
South Africa, Stellenbosch: Fynbos	34.0 S	19.0 E	1	14.6	1006		
South Africa, Stellenbosch: Karoo	33.6 S	19.5 E	1	15.8	807		
South Africa, Zululand: Dry forest	28.1 S	32.0 E	4	21.1	943		
South Africa, Zululand: Savanna	28.2 S	31.8 E	2	21.5	885		