Leaf size modifies support biomass distribution among stems, petioles and mid-ribs in temperate plants

Ülo Niinemets1,2,3, Angelika Portsmuth1 and Mari Tobias1

1Institute of Environmental and Agricultural Sciences, Estonian University of Life Sciences, Kreuzwaldi 64, Tartu 51014, Estonia; 2Department of Plant Physiology, University of Tartu, Ria 23, Tartu 51010, Estonia; 3Centro di Ecologia Alpina, I-38040 Viote del Monte Bondone (TN), Italy

Summary

• The implications of extensive variation in leaf size for biomass distribution between physiological and support tissues and for overall leaf physiological activity are poorly understood. Here, we tested the hypotheses that increases in leaf size result in enhanced whole-plant support investments, especially in compound-leaved species, and that accumulation of support tissues reduces average leaf nitrogen (N) content per unit dry mass ($N_M$), a proxy for photosynthetic capacity.
• Leaf biomass partitioning among the lamina, mid-rib and petiole, and whole-plant investments in leaf support (within-leaf and stem) were studied in 33 simple-leaved and 11 compound-leaved species.
• Support investments in mid-ribs and petioles increased with leaf size similarly in simple leaves and leaflets of compound leaves, but the overall support mass fraction within leaves was larger in compound-leaved species as a result of prominent rachises. Within-leaf and within-plant support mass investments were negatively correlated. Therefore, the total plant support fraction was independent of leaf size and lamina dissection. Because of the lower $N_M$ of support biomass, the difference in $N_M$ between the entire leaf and the photosynthetic lamina increased with leaf size.
• We conclude that whole-plant support costs are weakly size-dependent, but accumulation of support structures within the leaf decreases whole-leaf average $N_M$, potentially reducing the integrated photosynthetic activity of larger leaves.

Key words: leaf size, leaf structure, nitrogen content, size–function spectrum, support costs.


Introduction

There is tremendous variation in size and in the degree of lamina dissection among the leaves of different plant species, and there is ongoing interest in how variation in leaf size affects the plant capacity for energy capture and physiological activity (Westoby et al., 2002; Pickup et al., 2005). The adaptive significance of variation in leaf size has generally been associated with modifications in leaf energy balance (see Gates, 1980 for a review). As leaf boundary layer thickness for heat exchange decreases with decreasing leaf size and with increasing leaf dissection, smaller leaves are advantageous in hot and dry environments and at high intensities of solar radiation, while large leaves with less efficient energy exchange capacity are advantageous in lower irradiance and cooler and moister environments (Gates, 1980; Givnish, 1984; Ackerly et al., 2002).

Apart from energy balance, leaves of different size require different fractional biomass investments in support for efficient light capture. The gap fraction in the canopy can be diminished by regular leaf arrangement that fills the gaps between neighboring leaves via modifications in petiole length and branching architecture (Pearcy & Yang, 1998; Valladares, 1999). However, efficient light harvesting via structural modifications may become increasingly expensive with increasing leaf size. There is conclusive evidence that large leaves are associated with thicker branches (White, 1983; Westoby et al., 2002; Pickup et al., 2005) that bifurcate less frequently than the branches in small-leaved canopies (White, 1983), suggesting that extensive
costs may limit construction of a far-reaching branch framework that would efficiently fill the gaps in the canopy of a large-leaved species.

Despite thicker branches, recent data demonstrate that, at an equivalent xylem cross-sectional area, large-leaved species possess a greater fraction of shoot biomass in leaves than small-leaved species (Pickup et al., 2005). This has been interpreted as indicative of lower support cost of large-leaved canopies and as a general advantage of increasing leaf size (Pickup et al., 2005). However, as the overall bending moment scales with the cube of leaf length, leaves with common shape are predicted to require increasingly larger fractions of biomass in support within the leaves (Givnish, 1984; Gere & Timoshenko, 1997; Niklas, 1999). Within-leaf support costs have been estimated in only a few cases, but the data do confirm a positive scaling of support costs with leaf size (Givnish, 1984; Niinemets & Sack, 2006). If such scaling exists, there should be an overall trade-off between support investments in branches and stems, which decrease with increasing leaf size, and within-leaf support investments, which increase with leaf size. To date, the complete hierarchy of support investments from leaf to whole plant has not been examined for a large set of species, but the possible compromise between support investments at different hierarchical scales has important implications for energy capture and niche differentiation of species with differing leaf size.

Increases in lamina dissection can further enhance the costs for within-leaf support (Givnish, 1978; Sisó et al., 2001), mainly because neighbouring leaf lobes and leaflets need individual support, and also because leaflets occupy space less efficiently than nondissected lamina of equivalent area, resulting in extended lever arms and greater bending moments (Niinemets, 1998). Given the enhanced within-leaf support costs in compound-leaved species, the compromise between whole-plant investment in within-leaf support and that in stem is expected to be amplified in compound-leaved species.

We studied investment in support tissue at the individual leaf level and at the whole-plant level (above-ground biomass) in 44 temperate herbs and woody seedlings with compound and simple leaves. We used these data to test the main hypothesis that there is an inverse relationship between within-leaf and within-plant support investments. We further predicted that the scaling between within-leaf and within-plant support investments would differ between compound-leaved and simple-leaved species.

Size-dependent variation in within-leaf support investments can also have significant consequences for whole-leaf integrated elemental composition. Increases in the support biomass fraction are likely to reduce mass-weighted average leaf nitrogen (N) content, but potential size-dependent modifications in foliage chemistry have not been examined. As the third goal of our study, we aimed to determine the influence of size-dependent variations in within-leaf support investments on the difference between whole-leaf and lamina N and carbon (C) contents to assess the overall effect of size on integrated leaf elemental composition.

Materials and Methods

Study sites and plant sampling

The investigation was carried out at the end of June 2000 in Estonia. Within-leaf and whole-plant biomass distributions were studied in 24 herb species and in first-year seedlings of nine woody species (Appendix 1) in a broad-leaved deciduous forest in Ülenurme (58°18′ N, 26°42′ E, elevation 60 m) on a sandy-loam pseudopodzolic soil. This stand was dominated by 16–19 m tall trees of Populus tremula L. and Fraxinus excelsior L. in the overstory, by Corylus avellana L. in the shrub layer, and by Calamagrostis arundinacea (L.) Roth, Fragaria vesca L., Hepatica nobilis Gars., Melampyrum nemorosum L., and Oxalis acetosella L. in the herb layer. Niinemets (1999) provides a detailed description of the site.

Above-ground plant parts were collected for the analysis from at least two individuals of every species [mean ± standard error (SE) = 3.0 ± 0.7]. In herbaceous species, light-driven foliage structural and morphological plasticity saturates at c. 20–30% of full light (Winn & Evans, 1991; Olff, 1992). Consequently, we sampled plants in medium-sized gaps. The mean ± standard deviation (SD)] integrated daily quantum flux density for May and June determined from hemispherical photographs (see Niinemets et al., 2004 for details of light estimation) was 8.0 ± 4.2 mol m−2 d−1, corresponding to c. 25% of full light. Integrated quantum flux density was poorly associated with fractional biomass investment in support (r2 < 0.05, P > 0.2 for different within-leaf and within-plant estimates of support costs defined in the ‘Analysis of above-ground biomass fractions’ section).

The maximum average leaf fresh mass was c. 20 g in the 33 forest species studied. To obtain a more extensive range in average leaf fresh mass and to increase the generality of our results, within-leaf biomass partitioning was studied in 11 herb species with average leaf fresh mass varying between c. 4 and 320 g (Appendix 1) at the Botanical Garden of the University of Tartu (58°22′ N, 26°43′ E, elevation 20 m). The site was an open deciduous woody plantation on brown soil. The mean (± SD) integrated daily quantum flux density at the sampling locations was 18.1 ± 5.2 mol m−2 d−1, corresponding to c. 50% of full light.

After collection, the sampled material was immediately enclosed in plastic bags with wet filter paper and transported to the laboratory for further analyses.

Studied species and species grouping

Species were selected for the study to obtain a wide leaf size range in both simple- and compound-leaved species. Out of 44 sampled species, 33 were simple-leaved and 11 compound-leaved (Appendix 1). For the full dataset, the variation in average leaf
fresh mass was c. 15 000-fold, that in average dry mass was c. 10 000-fold and that in average area was c. 1200-fold (Appendices 1 and 2). The variation was 8-fold for total within-leaf biomass fraction in support structures (Fig. 1).

The compound-leaved species were mostly simple pinnate, but *Anthriscus sylvestris* L. was bipinnate, *F. vesca* and *O. acetosella* were trifoliate and *Geranium palustre* L. was palmately compound. Although *Paris quadrifolia* is a simple-leaved species, the simple leaves are attached as a whorl of four leaves to the base of the peduncle in generative plants and to the apex of the stem in vegetative plants. Thus, the four leaves and the stem to which the leaves are attached functionally represent a peltate tetrafoliate compound leaf. Therefore, *P. quadrifolia* was considered as a compound-leaved species in our analysis.

Widely spread summergreen species were sampled in most cases. In the herbs *Asarum europaeum* L., *H. nobilis* and *O. acetosella*, previous-year leaves may be partly active in the spring of the next year. In all three wintergreen species, leaves are protected from winter frosts by snow cover, and only the leaves of *H. nobilis* have a slightly leathery appearance as is typical for wintergreen broad-leaves. The two other wintergreen species have a mesomorphic foliage habit. Seven of the investigated species were introduced to Estonia either from southern Europe (*Echinops sphaerocephalus* L., *Heracleum sosnowskyi* Manden., and *Rheum rhabarbarum* L.), temperate East Asia [*Ligularia wilsoniana* (Hems.) Green. and *Polygonatum cuspidatum* Sieb. ext Zucc.], North America (*Helianthus annuus* L.) and Central America (*Cucurbita pepo* L.). Except for *C. pepo* and *H. annuus*, all introduced species disperse naturally in Estonia, and may become invasive in suitable sites.

**Analysis of above-ground biomass fractions**

The above-ground plant parts were divided among leaves, stems and flowers, and the total fresh mass of every biomass fraction was estimated. From every plant, a representative leaf was selected for further analyses. In simple-leaved species, the leaf was separated into the petiole, the mid-rib and the rest of the lamina. In compound-leaved species, the leaf was first divided into the leaflets, the petiole (nonleaved support), the rachis (leaved central support carrying leaflets) and the petiolules (petiole analogues that attach leaflets to the rachis). A representative leaflet was selected to measure the biomass distribution between the mid-rib and the rest of the lamina.
After determination of the fresh mass of these leaf fractions, all leaves were photocopied for area measurements.

The dry mass of every plant fraction was estimated after oven-drying at 70°C for at least 48 h, and dry to fresh mass ratios were calculated for all biomass fractions. The biomass partitioning between support and functional tissues was assessed at the lamina, whole-leaf and whole-plant scales. In lamina-scale analyses, the leaflets of compound-leaved species were considered as functional analogues of entire leaves in simple-leaved species. The partitioning in support within the leaf lamina was characterized as the ratio of mid-rib dry mass \( (M_{MR}) \) to the sum of \( M_{MR} \) and the dry mass of the rest of the lamina \( (M_L) \). Leaflet (compound-leaved species) and leaf (simple-leaved species) investment in support was determined as:

\[
F_L = \frac{M_{MR} + M_{PX}}{M_{LS}}, \quad \text{Eqn 1}
\]

\( [M_{PX}, \text{the petiole} (M_P) \text{ or petiolule} (M_{PL}) \text{ dry mass}; M_{LS}, \text{the sum of} M_L, M_{MR} \text{ and} M_0 \text{ or } M_{PL}.] \)

For compound-leaved species, whole-leaf biomass partitioning in support, \( F_{WL} \), was calculated as:

\[
F_{WL} = \frac{n(M_{MR} + M_{PL}) + M_{RP}}{M_{LT}}, \quad \text{Eqn 2}
\]

\( [M_{MR}, \text{the mid-rib dry mass of the selected leaflet}; M_{PL}, \text{the petiolule mass of this leaflet}; n, \text{the number of leaflets on the specific compound leaf}; M_{RP}, \text{the sum of petiole (nonleaved central support) and rachis (leaved central support) dry mass}; M_{LT}, \text{the whole-leaf dry mass (sum of dry masses of all leaflets, petiole and rachis)}.] \)

\( F_{WL} \) and \( F_L \) are numerically equivalent for simple-leaved species.

Whole-plant biomass investment in support, \( F_T \), was determined as:

\[
F_T = \frac{F_{SL} M_{STL} + M_{S}}{M_T}, \quad \text{Eqn 3}
\]

\( (M_{STL}, \text{the dry mass of all leaves on the plant}; M_S, \text{the dry mass of stems and branches}; M_T, \text{total plant dry mass}; F_{SL} \text{ is } F_L \text{ for simple-leaved species for compound-leaved species}.) \)

Analogously, we calculated whole-plant fractional biomass investments in mid-ribs, petiole-like support organs (petiole, rachis and petiolules) and stems.

The photocopied leaf images were scanned with a resolution of 300 dpi, and areas were separately estimated for every leaf and leaflet on the plant with UTHSCSA ImageTool 2.00 alpha (C. Donald Wilcox, S. Brent Dove, W. Doss McDavid and David B. Greer, Department of Dental Diagnostic Science, The University of Texas Health Science Center, San Antonio, TX, USA; http://ddsdx.uthscsa.edu).

Chemical analyses

The percentages of C and N in mid-ribs, petiolules, petioles, rachises, stems, flowers and the rest of the lamina were determined for every plant sampled with a Perkin Elmer series II CHNS/O Analyzer 2400 (Perkin Elmer Life and Analytical Sciences, Inc., Boston, MA, USA). These measurements were employed to determine whole-leaf and whole-plant mass-weighted C and N contents. The relative difference \( (R_N) \) between intercostal lamina areas \( (N_L, \%) \) and whole-leaf N content \( (N_{TL}, \%, \text{mass-weighted average } N \text{ concentration of lamina with support framework}) \) was calculated as:

\[
R_N = \frac{N_L - N_{TL}}{N_L}, \quad \text{Eqn 4}
\]

and the absolute difference \( (R_N, \%) \) as \( N_L - N_{TL}. \)

Values of C and N percentages for all biomass fractions and for the whole leaf and whole plant in all individuals sampled for a given species were averaged to determine species-specific means.

Data analyses

Linear and nonlinear regression analyses were used to examine the statistical relationships between biomass partitioning (within the leaf and within the plant), size of the leaf and of the whole plant, and between the chemical and structural characteristics of the plant fractions. To improve the linearity and normality of these relationships, leaf area \( (A_L) \), fresh \( (M_{FL}) \) and dry \( (M_{DL}) \) mass and whole-plant fresh \( (M_{FL}) \) and dry \( (M_{DL}) \) mass were log-transformed before the statistical analyses. All regressions were considered significant at \( P < 0.05 \).

The statistical relationships between simple- and compound-leaved species were compared with analyses of covariance (ANCOVAs), using log-transformed size estimates (area, dry and fresh mass). A separate-slope ANCOVA model was used first to test the intercept differences among the groups. Whenever the interaction term (grouping variable) \( \times \) covariate was statistically not significant \( (P > 0.05) \), the analysis was continued according to a common slope model to test for intercept differences (Sokal & Rohlf, 1995).

Because the species were sampled from two different sites, we also tested for a site effect on the statistical relationships between biomass partitioning and \( \log(A_L) \), \( \log(M_{FL}) \), \( \log(M_{DL}) \), \( \log(M_{F/L}) \) and \( \log(M_{D/L}) \) using covariation analyses. According to separate-slope and common-slope ANCOVA models, site \( \times \) covariate interactions and site effects were not significant in any of the cases considered \( (P > 0.1) \), indicating that size-dependent modifications of biomass allocation between support and physiological structures occurred similarly at both sites. Therefore, in the following analyses, the data from the two sites were pooled.

We used leaf or leaflet fresh mass, dry mass and area as size estimates. Leaf investments in biomechanical support scale with the extension of lever arms (leaf area) and leaf load (leaf fresh mass). In addition, leaf area also quantifies the size effects on leaf energy balance, while leaf dry mass provides the overall leaf cost for the plant. As all these variables characterize
different leaf functions, we demonstrate the relationships of support investments with both fresh mass and area and also show the correlations between different size estimates in Appendix 2. As area, fresh mass and dry mass were strongly correlated for both leaflets and leaves (Appendix 2), all relationships reported were qualitatively identical with similar proportions of explained variance ($r^2$) for different size estimates.

**Results**

Scaling of within-leaf support costs with leaf size in simple- and compound-leaved species

We observed an extensive variation in the total within-leaf dry mass fraction in support ($F_{WL}$, Eqn 2; Fig. 1). In simple-leaved species this fractional investment in the mid-rib and petiole varied from 0.09 to 0.60 g g$^{-1}$, while in compound-leaved species the fraction of whole-leaf biomass in the mid-ribs and the petiolules of leaflets and rachis varied from 0.19 to 0.70 (Fig. 1). The within-leaf support biomass was almost equally distributed between the leaf mid-rib and petiole in simple-leaved species and the mid-rib and petiole in compound-leaved species (Table 1a). A very large fraction of support biomass was present in the rachis in compound-leaved species (Table 1a). As the leaflet and the petiolule of a compound leaf form a functional analogue of a leaf in a simple-leaved species, we analysed separately the biomass of support investments with both fresh mass and area and in simple-leaved species and with leaflet fresh mass and area in compound-leaved species (Fig. 2a,d). This relationship was stronger for simple-leaved species, for which the range of fractional biomass in the mid-rib was larger than for compound-leaved species (Fig. 2a,d). In simple-leaved species, the petiole fraction in the total leaf dry mass (Fig. 2b,e) and in total within-leaf support ($F_T$, Eqn 1; Fig. 2c,f) also scaled strongly with leaf fresh mass and area. Analogous correlations were found with leaflet fresh mass and area in compound-leaved species (Fig. 2b,c,e,f). Because of strong correlations between area, fresh mass and dry mass (Appendix 2), the relationships were qualitatively identical, with similar proportions of explained variance ($r^2$) with leaf or leaflet dry mass.

The total fraction of support biomass within the leaf (Eqn 2; sum of the mid-rib and petiole in simple-leaved species and sum of the mid-ribs and petiolules of all leaflets and the rachis in compound-leaved species per total leaf dry mass) scaled positively with whole-leaf fresh (Fig. 3a) and dry (data not shown; see Appendix 2) mass, and with leaf area (Fig. 3b) in simple- and compound-leaved species.

According to ANCOVA analyses, the slopes of all these relationships did not significantly differ between the simple- and compound-leaved species ($P > 0.1$; Figs 2, 3). At the same leaf or leaflet fresh mass, the intercepts did not significantly differ for any of the relationships depicted in Fig. 2 ($P > 0.1$; Table 1a), indicating that the size-dependent scalings of support biomass in leaves of simple-leaved species and in leaflets of compound-leaved species are essentially the same. However, compound-leaved species also possess a large fraction of biomass in the rachis, and at the same total leaf mass and area, the fraction of total leaf biomass in support was significantly larger in compound-leaved species than in simple-leaved species (Fig. 3; Table 1a).

### Table 1
Mean (± standard error) dry mass fractions of biomass in support (g g$^{-1}$) within the leaf (a) and within the plant (b) in 33 simple-leaved and 11 compound-leaved species$^1$. (a) Fraction in support within leaf/leaflet. (b) Fraction in support within plant

(a)

<table>
<thead>
<tr>
<th>Leaf type</th>
<th>Mid-rib</th>
<th>Petiole/petiolule</th>
<th>Sum within leaf/leaflet</th>
<th>Rachis$^2$</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>0.130 ± 0.017a</td>
<td>0.157 ± 0.023a</td>
<td>0.287 ± 0.028a</td>
<td>0</td>
<td>0.287 ± 0.028a</td>
</tr>
<tr>
<td>Compound</td>
<td>0.083 ± 0.006a</td>
<td>0.086 ± 0.029a</td>
<td>0.169 ± 0.029a</td>
<td>0.208 ± 0.038</td>
<td>0.378 ± 0.049b</td>
</tr>
</tbody>
</table>

(b)

<table>
<thead>
<tr>
<th>Leaf type</th>
<th>Mid-rib</th>
<th>Petiole analogues$^3$</th>
<th>Total within-leaf</th>
<th>Stem</th>
<th>Total support$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>0.067 ± 0.021a</td>
<td>0.083 ± 0.023a</td>
<td>0.151 ± 0.026a</td>
<td>0.403 ± 0.038a</td>
<td>0.449 ± 0.026a</td>
</tr>
<tr>
<td>Compound</td>
<td>0.0570 ± 0.0054a</td>
<td>0.252 ± 0.051b</td>
<td>0.309 ± 0.050b</td>
<td>0.23 ± 0.10b</td>
<td>0.447 ± 0.048a</td>
</tr>
</tbody>
</table>

$^1$Species as described in Appendix 1. Simple- and compound-leaved species were compared with analyses of covariance (ANCOVAs) using a common-slope model with log(whole-leaf fresh mass) as a covariate. Values with the same letter indicate that ANCOVA models did not have significantly different elevations for simple- and compound-leaved species ($P > 0.05$).

$^2$The rachis of compound-leaved species includes both the nonleafed (petiole) and leaved (rachis sensu stricto) parts of the compound leaf.

$^3$The petiole for simple-leaved species, and the sum of the petiolules, rachis and petiole for compound-leaved species.

$^4$The fraction of total support differs from the sum of support fractions in the leaf and stem because some sampled species did not always have stems during the time of sampling and the specific ontogenetic phase.
Within-plant support costs in relation to leaf and plant size

The fractions of whole-plant dry mass in mid-ribs, petioles and analogues (petioles, petiolules and rachises) were weakly associated with total whole-plant dry and fresh masses. Only in compound-leaved species was the fraction of whole-plant dry mass in mid-ribs positively correlated with both whole-plant dry mass and whole-plant fresh mass \( r^2 = 0.40–0.44 \) for log-transformed masses; \( P < 0.05 \) for both. The sum of
biomass fractions in petioles and mid-ribs was also independent of whole-plant dry and fresh masses in both compound- and simple-leaved species ($P > 0.1$). The fraction of total plant dry mass in stem ($F_S$, $r^2 = 0.29$, $P < 0.01$) and total biomass fraction in support ($F_Y$, Eqn 3; $r^2 = 0.38$, $P < 0.001$) scaled positively with log(whole-plant dry mass), while $F_S$ and $F_Y$ were not associated with dry mass in compound-leaved species ($P > 0.1$). The latter relationships were qualitatively identical to those with log(whole-plant fresh mass) (data not shown).

Whole-plant fractional investment in mid-ribs was similar between simple- and compound-leaved species, but the fraction of petiolar biomass and the total within-leaf support fraction were larger in compound-leaved species (Table 1b). However, the stem mass fraction was larger in simple-leaved species (Table 1b), and there was an overall negative correlation between the biomass fractions in support within the leaf and within the stem (Fig. 4). As the result of this trade-off, total support costs were essentially the same in simple- and compound-leaved species (Table 1b). The total whole-plant support mass fraction (mean ± SE = 0.448 ± 0.023) was independent of leaf size. For regressions with log(fresh leaf mass), $r^2 = 0.00$ and $P > 0.8$ for simple-leaved species and $r^2 = 0.01$ and $P > 0.6$ for compound-leaved species.

### Nitrogen and carbon contents and dry to fresh mass ratios of biomass fractions

Mass-based nitrogen contents of the mid-rib, petirole, rachis and stem were strongly correlated with lamina N contents (Fig. 5a for linear correlations with untransformed data; $r^2 = 0.43–0.69$ for linear correlations with log-transformed data). Significant correlations were also observed between the C contents of support biomass fractions and lamina C content (Fig. 5b), as well as between the dry:fresh mass ratio of support biomass fractions and the lamina dry:fresh mass ratio (Fig. 5c).

For all data pooled, the mean (± SE) N content of the lamina without the mid-rib ($N_L$) of 2.52 ± 0.15% was significantly larger than the N contents of support structures (1.43 ± 0.15% for the mid-rib, 1.10 ± 0.11% for the petiole and 0.97 ± 0.10% for the stem; $P < 0.001$ for all comparisons with lamina N content), resulting in significantly lower slopes relative to the 1 : 1 line of N in support structures vs $N_L$ relationships (Fig. 5a).

### Size-dependent variation in whole-leaf N contents

As the fraction of support biomass increased with increasing leaf size (Figs 2, 3), both the relative difference ($R_D$, Eqn 4) between $N_L$ and whole-leaf mass-weighted N content ($N_{FL}$) and the absolute difference ($R_A = N_L – N_{FL}$) increased with increasing leaf size (Fig. 6). In the largest leaves, whole-leaf N content was up to 80% lower than that of the lamina (Fig. 6a,c), and the absolute difference in N percentage was 1.4% (Fig. 6b,d). The slopes of $R_D$ vs log(fresh mass) ($P < 0.01$) and $R_D$ vs log(area) ($P < 0.05$) relationships were larger in compound-leaved species, but the slopes did not differ between simple- and compound-leaved species for the $R_A$ vs log(size) relationship (Fig. 6b,d; $P > 0.7$ for both relationships according to separate-slope ANCOVA models). However, the intercepts of $R_A$ vs log(fresh mass) ($P < 0.03$ according to common-slope ANCOVA model) and $R_A$ vs log(area) ($P < 0.04$) relationships were larger in compound-leaved species. Thus, these statistical comparisons demonstrate an overall larger difference between lamina and whole-leaf N contents in compound-leaved species.
Leaf size is the primary determinant of within-leaf support investments

Biomass partitioning is an important driver of whole-plant net C gain (Körner, 1991; Körner, 1994). Plant growth rate scales positively with the mass fraction in leaves and is negatively associated with the fraction of support tissues (Reich, 1998; Wright & Westoby, 2001). While the fact that within-leaf support investment can be a significant component of overall plant investment in support is well recognized (Givnish, 1984, 1986), these support costs have been estimated only for a limited number of species (Givnish, 1984, 1986; Niklas, 1991, 1993; Garnier et al., 1999; Niinemets & Sack, 2006). Previous studies have shown that the fractional leaf biomass investment in the petiole varies from c. 0.02 to 0.3 (Cao & Ohkubo, 1998; Niinemets, 1998; Rundel et al., 1998; Niinemets & Kull, 1999). Our study demonstrates that when mid-rib investments are further accounted for, the total within-leaf support mass fraction (mid-rib and petiolar elements) may reach values as high as 0.70 in the largest leaves (Figs 1–3).

We found that the within-leaf support investments in simple-leaved species and within-leaflet support investments in compound-leaved species scaled similarly with leaf and leaflet size (Fig. 2), and that, at the same size, leaflets had essentially the same investments in support as leaves of simple-leaved species (Fig. 2; Table 1a). These data collectively underscore the importance of leaf size in determining the within-leaf support costs. Scaling of fractional investments in within-leaf support with leaf size is in accordance with disproportionately greater bending moments of larger mechanical structures of similar shape (Givnish, 1986; Gere & Timoshenko, 1997).

However, on top of these considerable costs, compound-leaved species also possessed a significant fraction of biomass in the rachis such that the overall within-leaf support costs (Eqn 2) were significantly larger in compound-leaved species at the same whole-leaf mass (Fig. 3; Table 1a). The greater overall cost of compound leaves is partly the result of the lower mechanical efficiency of dissected structures than of entire laminae, in which the epidermis and neighbouring lamina areas increase the rigidity of the whole lamina (Niklas & Paolillo, 1997; Niklas, 1999). In addition, only rectangles can be packed along the rachis to completely fill the outline of a compound leaf. Arranging leaflets with their specific ovate or elliptic shapes along the rachis without overlap of neighbouring leaflets to maximize light interception always results in a significant fraction in the leaf outline. This means that leaf mass is located farther from the axis of rotation in compound than in simple leaves, implying larger bending moments of compound leaves than of simple leaves of equal total mass. In agreement with this prediction, the fractional biomass investment in leaf rachis in compound-leaved species increases with the number of leaflets per leaf (Ninemets, 1998), reflecting the enhanced requirements for support.

Hierarchy of support investments: from leaf to whole plant

Support biomass in the mid-rib, in the petiole and in petiole analogues also comprised important biomass fractions from a whole-plant perspective (Table 1b). However, while simple- and compound-leaved species were strikingly different in total
within-leaf support costs (Eqn 2; Fig. 3), the biomass fractions in within-leaf support and in the stem were inversely related (Fig. 4). As the result, the whole-plant support investment was the same in simple- and compound-leaved species (Table 1b) and did not depend on leaf size. Previously, it has been shown that, at the same xylem cross-sectional area, large-leaved species have a greater fraction of shoot biomass in leaves (Pickup et al., 2005), and this has been interpreted as a general advantage of large leaves. Our study confirms that large-leaved species possess a lower fraction of biomass in stems (Fig. 4). However, our study further demonstrates that the apparent advantage of large leaves is offset by greater within-leaf support investments.

The efficiency of light interception increases as the lamina is located farther from the leaf attachment to central support and farther from other potentially shading leaves, modifications that collectively reduce leaf aggregation (Cescatti & Niinemets, 2004; Pearcy et al., 2005). This can occur via extension of the petioles and an increase in the total length of the stem and branches (Brites & Valladares, 2005; Pearcy et al., 2005). The trade-off between within-leaf and whole-plant support investments demonstrates that limited combinations of leaf size and biomass investments in support within the leaf and in the stem are possible. Increases in leaf size inevitably bring about increased support costs within the leaf lamina, leaving less C for construction of the long stem framework. In contrast, increased requirements to form a long branch framework to support small leaves constrain the amount of C that can be invested for construction of long petioles in small leaves. Experimental data demonstrate that the length of branches per unit leaf area supported decreases with increasing leaf size (Cao & Ohkubo, 1998), underscoring the limited branch support in large leaves.

**Ecological implications for variation in support investments**

The trade-offs between within-leaf and within-stem support investments result in overall convergence of the light harvesting efficiencies of crowns of different design (Valladares et al., 2002). However, what is the ecological significance of having small or large leaves with different distribution of support costs inside leaves and in stems and branches? In trees and shrubs, large leaves have been suggested as a strategy to cope with low-light environments, because large leaves can be arranged in a planar layer that efficiently harvests light from vertical inclination angles and captures most light-flecks (Horn, 1971). In agreement with this prediction, temperate late-successional understory woody species tend to possess large leaves (Niinemets & Kull, 1994). However, large leaves coupled with a small gap fraction are expensive in terms of support costs, because they require an enhanced branch framework together with larger support investment within the leaves – a combination that was actually not observed in our study. To resolve this apparent discrepancy, it is important that, in temperate trees, the costs of support can be amortized over several seasons, and the overall compromise between within-leaf and stem support investments can probably be relaxed. In temperate herbs, the above-ground biomass costs need to be renewed every year, resulting in conflicting within-leaf and within-plant support investments. In the tropics, fast-growing pioneering trees with rapid leaf turnover often have
large leaves attached to pole-like stems (Tomlinson, 1987), again indicating that having simultaneously large leaves, extensive branching and rapid leaf turnover is not possible.

As suggested by Givnish (1978), the rachises of compound leaves function as throwaway branches. In trees, construction of cheap throwaway rachises can enhance rapid height growth as less biomass is needed for expensive woody branches (Givnish, 1978). The situation can be similar in herbs in which older leaves at lower canopy positions are continuously replaced by younger leaves at higher canopy positions. However, in contrast to woody species, the N and C costs of stem and rachis are similar in herbs (Fig. 5), and the cost advantages of support biomass distribution between the rachis and stem for rapid height growth are less clear. In fact, compound-leaved perennial herbs such as those from the family Umbelliferae (Appendix 1) do not form stem and generative organs every year. In these compound-leaved herbs, the rachis functions as an analogue of the stem. Saving the C and N costs of stem formation allows the plants to accumulate C below-ground in rhizomes and roots for production of generative organs in subsequent years. Again, amortization of the costs of stem formation over several years, especially in dense herbaceous canopies and forest understories, allows the compound-leaved species to loosen the general trade-off between within-leaf and stem support investments.

Another important difference between small and large leaves is in vulnerability to herbivory damage. Moles & Westoby (2000) observed that smaller leaves expanded faster and were less damaged by herbivore attacks. Distribution of total biomass between many small leaves or dissection of lamina into leaflets can also reduce plant vulnerability to accidental damage of single leaves or leaflets. At the same time, after expansion, large leaves with greater fractions of biomass in support can be less palatable to herbivores.

**Whole-leaf N content vs N contents of leaf parts: importance of varying support investments**

Because support structures had lower N contents per unit dry mass than the lamina (Fig. 5a), and the overall fraction of support biomass increased with increasing leaf size (Figs 2, 3), the discrepancy between lamina and whole-leaf N contents (Fig. 5a) increased with increasing leaf size (Fig. 6). This difference was further amplified in compound-leaved species that had a larger biomass fractions in support at the same leaf fresh mass (cf. Figs 2, 3 and 6).

The relative difference between lamina and whole-leaf N contents \( R_{\text{DL}} \) (Eqn 4) was almost two-fold for the largest leaves, and \( R_{\text{DL}} > 0.2 \) for 19 species out of 44 (Fig. 6a,c). The absolute difference in lamina N was \( > 0.4\% \) for 18 species (Fig. 6b,d), demonstrating that the variation in the fraction of support tissues may significantly modify leaf N content. As a large part of foliar N is invested in the photosynthetic apparatus, there is a positive scaling of leaf photosynthetic activity with leaf N content (Field & Mooney, 1986; Wright et al., 2004). Accordingly, leaf-size-dependent variation in support biomass can have important implications for whole-leaf integrated photosynthetic activity. Petioles and veins are photosynthetically active to a certain degree, but their photosynthetic rates are likely to be very small compared with the intercostal areas of leaf laminas (Niinemets, 1999; Hibberd & Quick, 2001). In particular, fractional N investment in enzymes limiting photosynthetic carbon gain appears to be smaller for veins and petioles than for lamina (Niinemets, 1999), and the photosynthetic activity of within-leaf support elements is even less than predicted on the basis of their N content. A significant fraction of foliar N may also be invested in protective compounds (Coley, 1986; Bazzaz et al., 1987), and the fractional N investment in protection can vary for lamina, mid-rib, and petiole. Such a possible variation in fractional N investment in defence among leaf compartments can further bias the general correlations between foliage photosynthetic activity and N when generalizations are sought across a set of species with greatly varying leaf size.

These findings indicate that leaf-size-dependent shifts in the distribution of support biomass between the stem and within-leaf elements (petioles and mid-ribs) do not necessarily modify the overall distribution of above-ground biomass between support and intercostal lamina biomass. However, this evidence also demonstrates that, at an individual leaf scale, the variation in size can substantially modify whole-leaf integrated photosynthetic activity. Depending on the fractional investment of foliar biomass in the petiole and rachis, plants investing a constant fraction of biomass in leaves can possess widely differing photosynthetic activities per unit total foliage dry mass. Given that there are strong positive correlations between leaf photosynthetic activity and leaf and stem hydraulic conductance (Brodrribb et al., 2002, 2005; Sack et al., 2003), the observed negative scaling of the fraction of shoot biomass in leaves with leaf size at a common xylem cross-section area (Pickup et al., 2005) may partly result from the overall lower photosynthetic activity of larger leaves rather than greater efficiency of stem support in large-leaved species.

While there are general world-wide relationships between foliage structural and physiological characteristics (Wright et al., 2004), the scatter in specific points along the entire spectrum of leaf functioning is significant. Studies on the photosynthetic activity of foliage do not generally separate the contributions of the lamina and the mid-rib, and often leaf N contents are estimated with or without petioles. For smaller leaves that are entirely enclosed in gas-exchange cuvettes, and in which the fraction of biomass in support is relatively minor, separate consideration of support and assimilative biomass is probably not required. In larger leaves, photosynthesis is often estimated in intercostal areas, especially in species with prominent major veins, while leaf N content is determined as an integrated estimate for the whole leaf, suggesting that
differential investments in support can introduce significant bias. Garnier et al. (1999), carefully distinguishing between support and assimilative tissues within leaf laminas, demonstrated for 14 species that leaf N content and leaf photosynthetic capacity per unit dry mass were negatively associated with the volume fraction of support tissues within the leaf lamina. Our study suggests that part of the variation in the general relationships among leaf N content, structure, and function can be associated with variation in leaf size that modifies the distribution of leaf biomass between support and functional tissues.

Conclusions

Whole-plant above-ground biomass is the sum of investments in support, photosynthesizing lamina and generative organs. Our study demonstrates that a large fraction, up to 70–80%, of total above-ground biomass can be invested in support, whereas the way the costs are distributed between within-leaf and within-plant support depends on leaf size. The trade-off between the investments in stem and within-leaf support significantly affects the possible combinations of crown architectural design and strategies for light capture. Because of extensive stem support costs for efficient distribution of leaves in the canopy, large-leaved species with limited C availability for stem construction are generally confined to open environments, except when the stem support costs can be amortized over several years, as in temperate woody plants. Analogously, perennial compound-leaved species are not forming stem every year and can amortize their larger costs for leaf support over several growing seasons.

While the total support costs (within-leaf and within-plant) appear to be similar in species with varying leaf size, size-dependent variation in within-leaf support investments may significantly affect integrated whole-leaf (lamina intercostal areas, veins, petioles and analogues) chemical characteristics.

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References


Appendix 1

Table A1 shows the leaf habits and sizes of the species included in this study.

**Table A1** Leaf habit and fresh mass ($M_{FL}$) (mean ± standard error) of 33 simple-leaved and 11 compound-leaved species sampled in the broad-leaved mixed deciduous forest (DF) and in the botanical garden (BG)

<table>
<thead>
<tr>
<th>Species</th>
<th>Site</th>
<th>Life form</th>
<th>Compound-leaved</th>
<th>Leaf/leaflet shape</th>
<th>Venation type</th>
<th>$M_{FL}$ (g) ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aegopodium podagraria L.</td>
<td>DF</td>
<td>Herb</td>
<td>Yes</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>2.09 ± 0.24</td>
</tr>
<tr>
<td>Angelica sylvestris L.</td>
<td>BG</td>
<td>Herb</td>
<td>Yes</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>30 ± 17</td>
</tr>
<tr>
<td>Anthriscus sylvestris (L.) Hoffm.</td>
<td>DF</td>
<td>Herb</td>
<td>Yes</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>0.323 ± 0.011</td>
</tr>
<tr>
<td>Arctium tomentosum Mill.</td>
<td>BG</td>
<td>Herb</td>
<td>No</td>
<td>Cordate</td>
<td>Pinnate</td>
<td>7.7 ± 3.2</td>
</tr>
<tr>
<td>Armoracia rusticana (Lam.) Gaer.</td>
<td>BG</td>
<td>Herb</td>
<td>No</td>
<td>Elliptic</td>
<td>Pinnate</td>
<td>66.8 ± 5.2</td>
</tr>
<tr>
<td>Asarum europaeum L.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Reniform</td>
<td>Parallel</td>
<td>0.61 ± 0.07</td>
</tr>
<tr>
<td>Cirsium oleraceum (L.) Scop.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>19.8 ± 2.2</td>
</tr>
<tr>
<td>Convallaria majalis L.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Elatic</td>
<td>Parallel</td>
<td>0.48 ± 0.15</td>
</tr>
<tr>
<td>Corylus avellana L.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Oval</td>
<td>Pinnate</td>
<td>0.27 ± 0.13</td>
</tr>
<tr>
<td>Crepis paludosa (L.) Moench</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Obovate</td>
<td>Pinnate</td>
<td>0.44 ± 0.20</td>
</tr>
<tr>
<td>Cucurbita pepo L.</td>
<td>BG</td>
<td>Herb</td>
<td>No</td>
<td>Orbicular</td>
<td>Palmate</td>
<td>107 ± 15</td>
</tr>
<tr>
<td>Echinops sphaerocephalus L.</td>
<td>BG</td>
<td>Herb</td>
<td>No</td>
<td>Obovate</td>
<td>Pinnate</td>
<td>10.0 ± 3.0</td>
</tr>
<tr>
<td>Epilobium montanum L.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>Fragaria vesca L.</td>
<td>DF</td>
<td>Herb</td>
<td>Yes</td>
<td>Oval</td>
<td>Pinnate</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>Fraxinus excelsior L.</td>
<td>DF</td>
<td>Tree</td>
<td>Yes</td>
<td>Elliptic</td>
<td>Pinnate</td>
<td>0.123 ± 0.048</td>
</tr>
<tr>
<td>Galeobdolon luteum Huds.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>0.12 ± 0.06</td>
</tr>
<tr>
<td>Geranium palustre L.</td>
<td>DF</td>
<td>Herb</td>
<td>Yes</td>
<td>Palmate</td>
<td>Palmate</td>
<td>1.6 ± 1.4</td>
</tr>
<tr>
<td>Helianthus annuus L.</td>
<td>BG</td>
<td>Herb</td>
<td>No</td>
<td>Cordate</td>
<td>Palmate</td>
<td>56 ± 10</td>
</tr>
<tr>
<td>Hepatica nobilis Gars.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Orbicular</td>
<td>Palmate</td>
<td>0.580 ± 0.042</td>
</tr>
<tr>
<td>Heracleum sosnowskyi Manden.</td>
<td>BG</td>
<td>Herb</td>
<td>No</td>
<td>Orbicular/ovate</td>
<td>Pinnate</td>
<td>38.6 ± 5.5</td>
</tr>
<tr>
<td>Ligularia wilsoniana (Hems.) Green.</td>
<td>BG</td>
<td>Herb</td>
<td>No</td>
<td>Cordate</td>
<td>Pinnate</td>
<td>45.2 ± 6.0</td>
</tr>
<tr>
<td>Loniceria xylostueum L.</td>
<td>DF</td>
<td>Shrub</td>
<td>No</td>
<td>Oval</td>
<td>Pinnate</td>
<td>0.13 ± 0.05</td>
</tr>
<tr>
<td>Maianthemum bifolium (L.) Schm.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Cordate</td>
<td>Parallel</td>
<td>0.24 ± 0.022</td>
</tr>
<tr>
<td>Melampyrum nemorosum L.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>0.063 ± 0.046</td>
</tr>
<tr>
<td>Oxalis acetosella L.</td>
<td>DF</td>
<td>Herb</td>
<td>Yes</td>
<td>Obovate</td>
<td>Parallel</td>
<td>0.071 ± 0.049</td>
</tr>
<tr>
<td>Padus avium Mill.</td>
<td>DF</td>
<td>Tree</td>
<td>No</td>
<td>Elliptic</td>
<td>Pinnate</td>
<td>0.23 ± 0.07</td>
</tr>
<tr>
<td>Paris quadrifolia L.</td>
<td>DF</td>
<td>Herb</td>
<td>Yes</td>
<td>Obovate</td>
<td>Parallel</td>
<td>1.17 ± 0.34</td>
</tr>
<tr>
<td>Polygonatum multiflorum (L.) All.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Oval</td>
<td>Parallel</td>
<td>0.49 ± 0.11</td>
</tr>
<tr>
<td>Polygonum cuspidatum Sieb. et Zucc.</td>
<td>BG</td>
<td>Herb</td>
<td>No</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>4.5 ± 1.1</td>
</tr>
<tr>
<td>Populus tremula L.</td>
<td>DF</td>
<td>Tree</td>
<td>No</td>
<td>Orbicular</td>
<td>Pinnate</td>
<td>0.23 ± 0.08</td>
</tr>
<tr>
<td>Prunella vulgaris L.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>0.071 ± 0.015</td>
</tr>
<tr>
<td>Ranunculus cassubicus L.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Reniform</td>
<td>Palmate</td>
<td>1.30 ± 0.32</td>
</tr>
<tr>
<td>Rheum rhabarbarum L.</td>
<td>BG</td>
<td>Herb</td>
<td>No</td>
<td>Cordate</td>
<td>Palmate</td>
<td>318 ± 160</td>
</tr>
<tr>
<td>Ribes alpinum L.</td>
<td>DF</td>
<td>Shrub</td>
<td>No</td>
<td>Ovate</td>
<td>Palmate</td>
<td>0.1667 ± 0.0044</td>
</tr>
<tr>
<td>Ribes nigrum L.</td>
<td>DF</td>
<td>Shrub</td>
<td>No</td>
<td>Ovate</td>
<td>Palmate</td>
<td>0.33 ± 0.12</td>
</tr>
<tr>
<td>Rubus idaeus L.</td>
<td>DF</td>
<td>Shrub</td>
<td>Yes</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>0.22 ± 0.06</td>
</tr>
<tr>
<td>Rubus saxatilis L.</td>
<td>DF</td>
<td>Herb</td>
<td>Yes</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>0.43 ± 0.07</td>
</tr>
<tr>
<td>Rumex crispus L.</td>
<td>BG</td>
<td>Herb</td>
<td>No</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>10 ± 8</td>
</tr>
<tr>
<td>Sorbus aucuparia L.</td>
<td>DF</td>
<td>Tree</td>
<td>Yes</td>
<td>Elliptic</td>
<td>Pinnate</td>
<td>0.32 ± 0.23</td>
</tr>
<tr>
<td>Stellaria holostea L.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Ovate/lanceolate</td>
<td>Pinnate</td>
<td>0.051 ± 0.030</td>
</tr>
<tr>
<td>Taraxacum officinale Web. ex Wigg.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Obovate</td>
<td>Pinnate</td>
<td>0.80 ± 0.40</td>
</tr>
<tr>
<td>Tussilago farfara L.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Cordate</td>
<td>Palmate</td>
<td>7 ± 7</td>
</tr>
<tr>
<td>Verbascum nigrum L.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>7.7 ± 4.5</td>
</tr>
<tr>
<td>Veronica chamaedrys L.</td>
<td>DF</td>
<td>Herb</td>
<td>No</td>
<td>Ovate</td>
<td>Pinnate</td>
<td>0.035 ± 0.012</td>
</tr>
</tbody>
</table>

For compound-leaved species, leaflet mean ± standard error (SE) fresh mass ($M_{FL/LL}$) was 0.176 ± 0.014 in A. podagraria; 0.22 ± 0.12 in Angelica sylvestris; 0.0224 ± 0.0039 in Anthriscus sylvestris; 0.065 ± 0.013 in F. vesca; 0.031 ± 0.010 in F. excelsior; 1.07 ± 0.35 in G. palustre; 0.033 ± 0.026 in O. acetosella; 0.20 ± 0.06 in P. quadrifolia; 0.041 ± 0.012 in R. idaeus; 0.19 ± 0.13 in R. saxatilis; 0.024 ± 0.019 in S. aucuparia. The full dataset is available from the authors upon request (ylon@ut.ee).
Appendix 2

Fig. A2 Correlations between dry mass, area and fresh mass for leaflets and the whole leaf in 11 compound-leaved and 33 simple-leaved species are presented (see Appendix 1 for species names and study sites). Data were fitted by power functions in the form of \( y = ax^b \) for both compound-leaved (dashed lines) and simple-leaved (solid lines) species, and all regressions are significant at \( P < 0.001 \). Error bars denote ± standard error.