

## Biomass and nitrogen distribution ratios reveal a reduced root investment in temperate lianas vs. self-supporting plants

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- **Background and Aims** The reliance on external support by lianas has been hypothesized to imply a reduction in the biomass cost of stem construction and root anchorage, and an increased investment in leaves, relative to self-supporting plants. These evolutionary trade-offs have not been adequately tested in an ontogenetic context and on the whole-plant scale. Moreover, the hypothesis may be extended to other potentially limiting resources, such as nitrogen (N.)
- **Methods** Plants belonging to five con-familiar pairs of temperate liana/shrub species were cultivated in 120 L barrels and sequentially harvested over up to three growing seasons. To account for the ontogenetic drift, organ biomass and nitrogen fractions were adjusted for plant biomass and N pool, respectively.
- **Key Results** Lianas invested, on average, relatively less biomass in the root fraction in comparison with shrubs. This was offset by only insignificant increases in leaf or stem investment. Even though liana stems and roots showed higher N concentration in comparison with shrubs, plant N distribution was mostly driven by, and largely matched, the pattern of biomass distribution. Lianas also showed a greater relative growth rate than shrubs. The differences between the growth forms became apparent only when ontogenetic drift was controlled for. These results were confirmed regardless of whether reproductive biomass was included in the analysis.
- **Conclusions** Our results suggest that temperate lianas, in spite of their diverse, species-specific resource distribution patterns, preferentially allocate resources to above-ground organs at the expense of roots. By identifying this trade-off and demonstrating the lack of a general trend for reduction in stem investment in lianas, we significantly modify the prevailing view of liana allocation strategies and evolutionary advantages. Such a resource distribution pattern, along with the cheap unit leaf area and stem unit length construction, situates lianas as a group close to the fast acquisition/rapid growth end of the life strategy spectrum.

**Key words:** Climbing plants, lianas, shrubs, growth forms, resource allocation, allocation trade-offs, leaf mass fraction.

### INTRODUCTION

The diversity of growth forms is a manifestation of the multitude of ways in which plants exploit their habitat. Understanding the adaptive evolution of growth forms requires elucidation of the fitness benefits as well as the accompanying physiological constraints. Lianas are a morphologically heterogeneous group of woody plants that have emerged in numerous lineages and achieved often spectacular morphological specialization for reliance on support provided by stems of neighbouring plants or other external structures (Isnard and Silk, 2009). The evolutionary success of lianas has been attributed to relaxation of the biomass allocation trade-off, whereby greater leaf area may be formed due to a reduced demand for structural support (Darwin, 1865; Putz, 1983; Isnard and Field, 2015). As a corollary, lianas should be capable of faster biomass gains than self-supporting plants (Isnard and Field, 2015).

The idea of increased biomass distribution to leaves in climbing plants, although intuitive and commonly accepted

(Schnitzer and Bongers, 2002; Kazda *et al.*, 2009; Toledo-Aceves, 2015; Isnard and Field, 2015), has received only partial empirical support. For example, in tropical forests, the contribution of lianas to total leaf area exceeded their contribution to woody stem biomass (Hegarty and Caballé, 1991). In contrast, a growth chamber study did not indicate a higher biomass distribution to leaves in climbing vs. self-supporting herbaceous species (den Dubbelden and Verburg, 1996), and a lianescent *Lonicera* had a slightly lower leaf to stem mass ratio than a shrubby congener (During *et al.*, 1994). Importantly, these reports did not consider plant size-related drift in individual biomass distribution resulting from accumulation of supporting biomass with age (Coleman, 1994; Poorter *et al.*, 2015). Meaningful comparisons between growth forms require experimental or statistical removal of the influence of size upon biomass distribution ratios.

Some community-level studies in which allometric control of biomass distribution was applied have indicated that leaf mass in liana individuals was indeed larger than in trees at a

comparable stem basal area (Putz, 1983; Gerwing and Farias, 2000) or at equal above-ground biomass (Wyka et al., 2013; Ichihashi and Tatenno, 2015). A contrasting report, however, demonstrated similarity of allometries of plant biomass vs. leaf or supporting tissue between *in situ* harvested liana *Hydrangea petiolaris* and three co-occurring con-generic shrubs (Kaneko and Homma, 2006). Similarly, shoot-level studies have shown that scaling slopes of leaf vs. stem biomass in extension shoots of woody and herbaceous climbers were indistinguishable from those for gymnosperm shoots (Niklas, 1994), and terminal twigs of canopy lianas supported a similar leaf area to tree twigs at the same biomass (Kazda et al., 2009). These scarce and inconsistent results suggest that trends for biomass allocation in lianas vs. self-supporting plants must be evaluated using additional data sets (Poorter et al., 2012; Campanello et al., 2016).

Even though the root systems constitute a significant fraction of plant biomass, most reports on liana biomass distribution have not accounted for allocation to below-ground parts (Putz, 1983; Daring et al., 1994; Gerwing and Farias, 2000; Gehring et al., 2004; Ichihashi and Tatenno, 2015). Biomass investment in roots may, nevertheless, similarly to investment in stems, underlie trade-offs accompanying evolutionary switches between growth forms. Function-based predictions with respect to root system allocation in lianas are, however, less clear than in the case of the above-ground parts. On the one hand, the utilization of external support may reduce the requirement for structural anchorage in the soil, resulting in low root mass fractions. On the other hand, the widespread re-sprouting ability of lianas may indicate significant storage capacity of the root systems (Mooney and Gartner, 1991). The need for large underground storage compartments in lianas may be especially high given their fast stem turnover, making stems less reliable as a long-term storage location (Fisher and Ewers, 1991; Mooney and Gartner, 1991; Ichihashi and Tatenno, 2015). Moreover, much indirect evidence indicates that lianas produce deep or otherwise extensive roots for efficient water and nutrient extraction. Tropical lianas are generally thought to use deeper water sources than neighbouring trees, as indicated by their favourable water status (Zhu and Cao, 2009), extended photosynthetic activity during the dry season (Cai et al., 2009) and greater predominance of lianas in seasonally dry, as compared with moist, tropical forests (Schnitzer, 2005; Swaine and Grace, 2007; DeWalt et al., 2010; Schnitzer and Bongers, 2011; Brenes-Arguedas et al., 2013). A recent study using stable isotopes, however, did not support this hypothesis, suggesting shallow, but efficient and drought-tolerant, root systems in lianas (De Deurwaerder et al., 2016). Direct excavation of liana roots has rarely been reported and has revealed either considerable root depths (e.g. as much as 10 m in a 1.4 m tall *Davilla kunthii*; Restom and Nepstad, 2004) or, in contrast, shallow root distribution (not deeper than 60 cm in mature canopy lianas; Johnson et al., 2013).

The low requirement for mechanical anchorage on the one hand, and the potential benefits of increased storage function and vertical or horizontal soil penetration by climber roots on the other, places conflicting demands on biomass distribution to root systems. Data on actual root mass fractions in lianas relative to self-supporting plants are, however, scarce. In a field-excavated liana *Hydrangea petiolaris*, the root biomass fraction was smaller than in three shrubby *Hydrangea* species

(Kaneko and Homma, 2006). Similarly, low root mass fractions were reported for pot-grown light-demanding lianescent species of *Bauhinia*, whereas, in a shade-demanding lianescent species, allocation to roots was slightly higher than in arborescent *Bauhinia* species (Cai et al., 2007). In contrast, herbaceous climbers and self-supporting herbs allocated a similar fraction of biomass to roots (den Dubbelden and Verburg, 1996). Unfortunately, field determination of below-ground biomass suffers from intrinsic inaccuracy, while studies using container cultivation have been largely restricted to small-sized plants, highlighting the need for data on whole-plant biomass distribution in variously sized, phylogenetically and functionally diverse climbers.

Carbon, the main constituent of biomass, has traditionally been the principal subject of resource allocation studies, resulting in assembly of large databases and identification of growth form-specific biomass distribution patterns (Poorter et al., 2012, 2015). This focus reflects the direct relevance of biomass to ecological questions and human economy, but is also a result of choosing carbon as the appropriate currency for expressing organ construction costs (Bazzaz, 1997). However, in principle, any of the essential elements may limit growth and become the critical currency underlying allocation trade-offs (Weiner, 2004; Körner, 2015). Although biomass pools may sufficiently represent nutrient pools in comparisons between species in which organ nutrient concentrations are similar, this cannot be *a priori* assumed when comparing plant groups or life forms intrinsically differing in organ nutrient concentrations. Liana leaves tend to be enriched in nitrogen (N) and phosphorus (P) relative to self-supporting woody species (Salzer et al., 2006; Cai et al., 2009; Zhu and Cao, 2010; Asner and Martin, 2011, 2012; Wyka et al., 2013; Kazda, 2015). Similarly, the abundance of stem parenchyma (Carlquist, 1985; Angyalossy et al., 2012) and the often low tissue density in lianescent stems (Putz, 1983) may indicate their greater nutrient concentration in comparison with self-supporting woody plants. Even at similar stem and leaf biomass fractions, relative nutrient costs of liana stems and leaves may thus be larger than in self-supporting plants. However, reports on whole-plant distribution of nutrients are far fewer than those focusing on biomass and, to our knowledge, are not available for lianas. Comparison of nutrient distribution ratios between lianas and self-supporting plants should, analogously to biomass costs, include correction for size of the plant nutrient pool (Coleman et al., 1994).

The objective of the present study was to identify life form-specific patterns of biomass and N investment, and the associated trade-offs in lianas and self-supporting plants, while controlling for ontogenetic drift of the resource distribution ratios. To ensure a broad representation of typical temperate lianas and self-supporting plants, we selected five pairs of liana and self-supporting woody species, each pair representing a different family. We were interested in intrinsic characteristics of each growth form; therefore, we cultivated plants under common garden conditions with ample growing space and resource supply. Plants were grown in large 120 L barrels to allow recovery of entire root systems, and were harvested sequentially to generate a range of plant biomass. We hypothesized that (1) at equivalent plant biomass, lianas should distribute a larger proportion of biomass to leaves and smaller proportion

to stems and/or roots relative to self-supporting species; (2) at similar plant N pools, the fractions of N distributed to leaves and stems in lianas should be relatively greater than in shrubs; and (3) that lianas should grow faster than shrubs.

## MATERIALS AND METHODS

### Plant material and culture

Seeds of five pairs of, respectively, liana and self-supporting (shrub or small tree) species: *Hedera helix* and *Eleutherococcus senticosus* (Araliaceae), *Lonicera periclymenum* and *L. maackii* (Caprifoliaceae), *Celastrus orbiculatus* and *Euonymus europaeus* (Celastraceae), *Wisteria floribunda* and *Laburnum anagyroides* (Fabaceae), and *Hydrangea petiolaris* and *H. macrophylla* (Hydrangeaceae), with lianas representing two different climbing modes (twinning and adventitious root climbing), were obtained commercially or from stock of natural origin cultivated in local botanical gardens (Table 1). Species selection in each growth form was based on hardiness and availability of seeds. For most species, pre-sowing seed processing was conducted at the Institute of Dendrology Laboratory of Seed Biology according to species requirements. Seeds were germinated indoors in trays filled with a mix of peat and river sand, and maintained under fluorescent light. Seedlings were transferred into multipot trays and, after a few weeks, into 3 L pots filled with peat-based potting compost with the addition of 20 % perlite, and placed outdoors under light shade until final planting. Since our attempts to germinate seeds of *E. senticosus* and both *Hydrangea* species failed, we purchased current-year seedlings of *E. senticosus* in mid summer 2013 and previous-year seedlings of both *Hydrangea* species in spring 2015 from a nursery of Warsaw University of Life Sciences Arboretum in Rogów (Poland). These seedlings had been raised in a leaf mould-based compost with the addition of 25 % sand and 25 % peat. Three species pairs (Caprifoliaceae, Celastraceae and Fabaceae) were planted in the experimental garden in late June 2013, the two Araliaceae in mid-August 2013 and both *Hydrangea* in early June 2015 (Table 1). The garden was located on Institute of Dendrology grounds (55°14'38"N and 17°6'6"E). Mean annual air temperature for the experimental period was 10.0 °C (range –14.7 °C to 37.8 °C) and mean daily temperature for the growing season (April–October) was 14.6 °C, with mean annual precipitation 548 mm (362 mm for the growing season), according to the records from a local weather station.

Plant cultivation was carried out in 120 L barrels (depth 100 cm, outer diameter 0.5 m) each with two 9 cm diameter holes cut in the bottom. Holes were covered by a water-permeable membrane (50 µm mesh) to allow drainage while preventing root penetration outside the barrel. Barrels were buried in the ground such that only the upper one-fifth protruded above the ground level (Supplementary Data Fig. S1A). The bottom of the barrel was covered with a 10 cm layer of pebbles for drainage. Barrels were filled with substrate consisting of pre-sifted and thoroughly mixed top soil from a local forest site enriched with 3 kg m<sup>-3</sup> slow-release fertilizer (Osmocote 16-9-12-2 N-P-K-Mg, with trace nutrients). The barrels were arranged in four sections each consisting of two rows and extending east to west. The distance between barrels in each row and between rows was 0.5 m, and the sections were situated 2 m apart. The ground between barrels was lined with black cloth to suppress weeds. To prevent shading of shrubs by climbing lianas, in each section shrubs were grown in the southerly row, and the northerly row was used for lianas. Harvesting selected plants in consecutive years for this and a companion study (Wyka et al., 2019) allowed us gradually to increase the spacing between the remaining plants. Since species representing the Araliaceae and Hydrangeaceae are shade demanding, they were all planted in a single section, and a 3 m tall shade house covered with a net reducing transmission of solar irradiance by 30 % was erected over the entire section.

Plants were regularly watered to field capacity using a clock-controlled drip irrigation system. Watering was turned off during wet or cool weather and increased during the hottest weather. Each spring in late April, a top-dressing of 160 g of Osmocote (16-9-12-2) was applied to every barrel. Pests and diseases were controlled by spraying with insecticides and fungicides at the earliest symptoms. For wintering, the barrels and bases of plant stems were covered with a 0.1 m thick layer of leaf litter from a nearby tree stand to prevent frost damage. Litter was removed at the onset of spring.

To allow unrestricted climbing, in the beginning of the 2014 season, two 2.5 m long wooden stakes (50 mm diameter and round in cross-section) were placed next to each barrel containing any of the three twinning liana species. For the strongly elongating *C. orbiculatus* and *W. floribunda*, two more such stakes were provided in the following season and an additional pair of 5 m long stakes, 80 × 80 mm thick and square in cross-section, were inserted 80 cm into the ground at 0.5 m distance from the edge of each barrel, giving a total of six stakes per plant (Supplementary Data Fig. S1B). Since from the beginning of the experiment

TABLE 1. List of liana and shrub species used and the timeline of the experiment

Family	Liana	Liana climbing mode	Shrub	Planting date	Harvest year
Araliaceae	<i>Hedera helix</i> L.	Adventitious roots	<i>Eleutherococcus senticosus</i> Maxim.	19 August 2013	2014, 2015, 2016
Caprifoliaceae	<i>Lonicera periclymenum</i> L.	Twinning	<i>Lonicera maackii</i> (Rupr.) Maxim.	23 June 2013	2014, 2015, 2016
Celastraceae	<i>Celastrus orbiculatus</i> Thunb.	Twinning	<i>Euonymus europaeus</i> L.	23 June 2013	2014, 2015, 2016
Fabaceae	<i>Wisteria floribunda</i> (Willd.) DC.	Twinning	<i>Laburnum anagyroides</i> Medik.	23 June 2013	2014, 2015, 2016
Hydrangeaceae	<i>Hydrangea petiolaris</i> Siebold & Zucc.	Adventitious roots	<i>Hydrangea macrophylla</i> (Rupr.) Ser.	10 June 2015	2015, 2016

*L. periclymenum* plants failed to utilize support even though their shoots performed searching movements and came into contact with the stakes, we modified the supporting structure by attaching three evenly spaced horizontal wooden cross-bars to each pair of stakes. We also supplied ten 1.5 m tall bamboo stakes (1 cm in diameter) to each *L. periclymenum* plant, yet, for the duration of the experiment, shoots of the plants did not utilize any of the supplied support. For the root climbers (*H. helix* and *H. petiolaris*), two 2.5 m long stakes lined with a coconut fibre mat were provided in the first year and six additional stakes were added in the following season to each barrel to create a dense palisade of eight stakes. All stakes were placed around the northern side of the barrels. Support was thus freely available to all lianas, but stem growth was unrestricted; thus, occasional stems of each liana species (all stems of *L. periclymenum* but none in *H. petiolaris*) grew prostrate on the ground.

Occasionally plants were eliminated from the experiment as a result of mechanical damage by wind, failure of drainage or visible stress caused by unknown factors. This elimination resulted in unequal sample sizes among species, with the greatest reduction in *E. senticosus* (only four plants were harvested over the course of the experiment, two in 2014 and two in 2016). Otherwise, sample size was typically five plants per species per year (range 2–8); see the Results.

#### Harvest and biomass processing

Plants were harvested at the culmination of each growing season but before the beginning of autumnal leaf senescence. Each year the harvesting campaign started in mid-August and was completed by mid-September. Each pair of liana/shrub species was harvested within the same week to reduce variability within the family. Above-ground parts were cut off at soil level and separated into stems and leaves, and, where present, flowers and fruits. The total length of all stems per plant was measured with tape. Adventitious roots that formed on creeping *H. helix* and *L. periclymenum* stems outside of the barrels were carefully excavated from soil using a hand shovel and eventually included in the general root biomass. Barrels were lifted, cut open and entire root systems were recovered by first washing off soil using a water hose (Supplementary Data Fig. S1C) followed by thorough rinsing of roots in a basin. Roots were then blotted with paper towels and briefly surface-dried in free air.

Stems and roots were cut into segments, and all biomass fractions were placed in forced-ventilation ovens and dried at 65 °C to constant weight for at least 72 h for determination of dry mass. In 2016, when leaf harvest was very bulky, total fresh leaf mass was determined at harvest, and two sub-samples of about 250 g fresh mass were taken for dry mass determination. Total leaf dry biomass was then determined from the dry mass to fresh mass ratio.

#### Laboratory analyses

Ten leaves were randomly chosen from each plant for determination of leaf mass per area (LMA). Petioles (or segments of rachis below the lowermost leaflet in the case of the compound

*Wisteria* leaves) were separated, and laminas were individually scanned with a desktop scanner. Leaf parts were dried at 65 °C for 72 h and weighed. Specific leaf area (SLA; ratio of lamina area to its dry mass;  $\text{m}^2 \text{kg}^{-1}$ ) and the fraction of lamina mass in the whole-leaf mass ( $\text{g g}^{-1}$ ) were determined.

Entire batches of dry stems and roots were cut into 2–3 cm chips using pruners or an electric shredder. Each biomass fraction was then thoroughly mixed to ensure representative sampling of the tissue. A handful of tissue was ground to 1 mm grade powder in a laboratory mill (IKE Labortechnik, Germany) followed by fine grinding in a ball mill (IKE Labortechnik). Nitrogen concentration was determined using an Elemental Combustion System CHNS-O 4010 (Costech Instruments, Italy/USA).

#### Calculations and statistics

Although reproduction occurred in some of the plants, our principal analysis focused on identifying differences between lianas and shrubs with respect to partitioning among vegetative organs (stems, leaves and roots). Vegetative biomass distribution indices, stem mass fraction ( $\text{SMF}_{\text{veg}}$ ), root mass fraction ( $\text{RMF}_{\text{veg}}$ ) and leaf mass fraction ( $\text{LMF}_{\text{veg}}$ ), were calculated by dividing the biomass of the respective fraction by the vegetative biomass. The leaf to stem mass ratio (LSMR) was also calculated. The leaf area ratio ( $\text{LAR}_{\text{veg}}$ ,  $\text{m}^2 \text{kg}^{-1}$ ) was obtained by multiplying  $\text{LMF}_{\text{veg}}$  by SLA and the fraction of lamina in leaf mass. Specific stem length (SSL,  $\text{m kg}^{-1}$ ) was obtained as the ratio of total plant stem length to stem mass (Poorter *et al.*, 2012); see Table 2 for definitions of variables.

The pools of N contained in particular organs were estimated by multiplying organ N concentration by the biomass of each organ type. Fractions of vegetative plant N pool allocated to stems ( $\text{SNF}_{\text{veg}}$ ), roots ( $\text{RNF}_{\text{veg}}$ ) and leaves ( $\text{LNF}_{\text{veg}}$ ) as well as the ratio of leaf N to stem N content (LSNR) were then calculated (Table 2).

To test whether the exclusion of reproductive biomass affected the pattern of investment relationships between vegetative organs,  $\text{SMF}_{\text{wh}}$ ,  $\text{RMF}_{\text{wh}}$ ,  $\text{LMF}_{\text{wh}}$  and  $\text{LAR}_{\text{wh}}$  were additionally calculated on the basis of whole-plant biomass that included flowers and fruits (Table 2). By analogy,  $\text{SNF}_{\text{wh}}$ ,  $\text{RNF}_{\text{wh}}$  and  $\text{LNF}_{\text{wh}}$  were calculated on the basis of whole-plant N pool (i.e. including N contained in reproductive organs). Reproductive mass and N fractions were calculated, based on whole-plant mass or N pool, respectively.

To determine to what extent the distribution ratios are subject to ontogenetic drift, slopes and Pearson's correlation coefficients were estimated for linear regression relationships between log-transformed plant biomass or N pool and, respectively, the biomass or N distribution ratios separately for each species but across all harvests. For group comparison between lianas and shrubs, distribution ratios were predicted for each species at average plant biomass and N pool using these regression equations. Additionally, for each species, average distribution ratios were calculated for three individuals with the largest biomass. Differences between lianas and shrubs were evaluated through analysis of covariance (ANCOVA), using the distribution ratios predicted through

TABLE 2. Indices of biomass and N distribution calculated on the basis of vegetative (combined leaf, stem and root) plant biomass or N pool and on the basis of whole- (combined vegetative and reproductive) plant biomass or N pool

Abbreviation	Variable	Definition	Unit
Based on vegetative biomass			
SMF <sub>veg</sub>	Stem mass fraction (vegetative)	Ratio of stem mass to vegetative plant mass	g g <sup>-1</sup>
RMF <sub>veg</sub>	Root mass fraction (vegetative)	Ratio of root mass to vegetative plant mass	g g <sup>-1</sup>
LMF <sub>veg</sub>	Leaf mass fraction (vegetative)	Ratio of leaf mass to vegetative plant mass	g g <sup>-1</sup>
LSMR	Leaf to stem mass ratio	Ratio of leaf mass to stem mass	g g <sup>-1</sup>
SLA	Specific leaf area	Ratio of leaf area to leaf mass	m <sup>2</sup> kg <sup>-1</sup>
LAR <sub>veg</sub>	Leaf area ratio (vegetative)	Ratio of total leaf area to vegetative plant mass	m <sup>2</sup> kg <sup>-1</sup>
SSL	Specific stem length	Ratio of total stem length to total stem mass	m kg <sup>-1</sup>
SNF <sub>veg</sub>	Stem N fraction (vegetative)	Ratio of stem N pool to vegetative plant N pool	g g <sup>-1</sup>
RNF <sub>veg</sub>	Root N fraction (vegetative)	Ratio of stem N pool to vegetative plant N pool	g g <sup>-1</sup>
LNF <sub>veg</sub>	Leaf N fraction (vegetative)	Ratio of stem N pool to vegetative plant N pool	g g <sup>-1</sup>
LSNR	Leaf to stem N ratio	Ratio of leaf N pool to stem N pool	g g <sup>-1</sup>
Based on whole-plant biomass			
SMF <sub>wh</sub>	Stem mass fraction (whole plant)	Ratio of stem mass to whole plant mass	g g <sup>-1</sup>
RMF <sub>wh</sub>	Root mass fraction (whole plant)	Ratio of root mass to whole plant mass	g g <sup>-1</sup>
LMF <sub>wh</sub>	Leaf mass fraction (whole plant)	Ratio of leaf mass to whole plant mass	g g <sup>-1</sup>
LAR <sub>wh</sub>	Leaf area ratio (whole plant)	Ratio of total leaf area to whole plant mass	m <sup>2</sup> kg <sup>-1</sup>
SNF <sub>wh</sub>	Stem N fraction (whole plant)	Ratio of stem N pool to whole plant N pool	g g <sup>-1</sup>
RNF <sub>wh</sub>	Root N fraction (whole plant)	Ratio of stem N pool to whole plant N pool	g g <sup>-1</sup>
LNF <sub>wh</sub>	Leaf N fraction (whole plant)	Ratio of stem N pool to whole plant N pool	g g <sup>-1</sup>

regression or averaged for the largest plants as individual data points ( $n = 5$  for each growth habit). Mean plant biomass or N pool (calculated, separately, across all harvests and for three largest plants) were used as the respective continuous covariates, and the plant habit as the qualitative predictor. Full ANCOVA models were first used; however, since interaction terms were non-significant in each case, they were dropped and analyses were re-run. Additionally, to check if ontogenetic drift was an important factor to consider in comparing the two growth forms, unadjusted means of vegetative biomass and N distribution variables were compared. Since species were represented at each harvest by unequal numbers of individuals, species means were first calculated for each harvest, averaged across all harvests and then averaged for each growth form. Growth form means were compared using Student's  $t$ -test.

Differences in structural parameters of stems (SSL) and leaves (SLA) were evaluated separately for each harvest (since the 2014 and 2015 harvests did not include all ten species) using factorial analysis of variance (ANOVA) with family and plant habit as fixed factors, followed by pre-planned contrasts.

Relative year to year growth rate ( $RGR_{veg}$ ) was calculated for each interval between harvests (i.e. 2014 to 2015 and 2015 to 2016) using the formula  $RGR_{veg} = (\ln W_{i+1} - \ln W_i)$  where  $W$  is vegetative plant biomass at consecutive harvests.  $RGR_{veg}$  was not calculated for *E. senticosus* because of the small number of individuals available and lack of 2015 data. Differences in  $RGR_{veg}$  between growth forms were evaluated with an ANCOVA model, using geometric means of plant biomass from consecutive harvests as a continuous predictor, as well as with a factorial ANOVA model.

All analyses were conducted using JMP 8.0.2 software (SAS Institute, Cary, NC, USA). Note that individual plant data for distribution ratios and means of SSL, SLA and leaf N for *H. helix*, *C. orbiculatus* and *W. floribunda* from 2015 and 2016 harvests have previously been used in analyses presented in a companion study (Wyka et al., 2019).

## RESULTS

Biomass distribution within species was usually affected by ontogenetic drift. Biomass was generally positively correlated with  $SMF_{veg}$  (significant  $r$  in seven out of ten species) and negatively correlated with  $LMF_{veg}$  (in five species) and  $LAR_{veg}$  (in six species), with the remaining relationships showing non-significant  $r$  (Table 3; Supplementary Data Fig. S2). On the other hand,  $RMF_{veg}$  was significantly related to biomass in only four species, with both Caprifoliaceae showing positive slopes and *C. orbiculatus* and *H. macrophylla* showing negative slopes. The within-species correlations of organ N distribution ratios with the plant N pool were fewer than in the case of biomass, with only six significant correlations for  $SNF_{veg}$ , two for  $RNF_{veg}$  and four for  $LNF_{veg}$  (Table 3; Supplementary Data Fig. S3). Nevertheless, for consistency, predicted means were used in between-group analysis of both biomass and N distribution.

At the inter-specific level, liana and shrub relationships of predicted  $SMF_{veg}$  and  $LMF_{veg}$  with mean vegetative species biomass were not significant (Fig. 1A, E), whereas  $RMF_{veg}$  showed positive slopes against biomass (Fig. 1C). After removing the insignificant interaction terms, adjusted  $SMF_{veg}$  and  $LMF_{veg}$  were statistically indistinguishable between lianas and shrubs (Fig. 1A, E). At the same time, lianas showed significantly lower  $RMF_{veg}$  than similarly sized shrubs (Fig. 1C). The same results were obtained using means of the three largest individuals per species instead of predicted means, although then liana  $SMF_{veg}$  was somewhat, but still non-significantly ( $P = 0.093$ ), higher than shrub  $SMF_{veg}$  (Fig. 1B, D, F). There was, on the other hand, no relationship between LSMR (indicating partitioning of biomass between stems and leaves) and the combined mass of leaves and stems, and no difference between the habits (Fig. 1G, H).

The structure of leaf laminas differed significantly between the habits. Within a given harvest, SLA was higher, or at least not lower, in lianas than in the con-familial shrubs (Fig. 2A–C). In spite of this, leaf area ratio ( $LAR_{veg}$ ) was only insignificantly higher in lianas than in shrubs at comparable plant biomass

TABLE 3. Slopes of linear regression and determination coefficients  $r^2$  for relationships between vegetative plant biomass and the biomass distribution ratios:  $SMF_{veg}$ ,  $RMF_{veg}$ ,  $LMF_{veg}$  and  $LAR_{veg}$ , and between vegetative plant N pool and N distribution ratios:  $SNF_{veg}$ ,  $RNF_{veg}$  and  $LNF_{veg}$

Variable	Habit	Araliaceae			Caprifoliaceae			Celastraceae			Fabaceae			Hydrangeaceae		
		Slope	$r^2$	<i>P</i>	Slope	$r^2$	<i>P</i>	Slope	$r^2$	<i>P</i>	Slope	$r^2$	<i>P</i>	Slope	$r^2$	<i>P</i>
Relationships with biomass																
$SMF_{veg}$	L	<b>0.082</b>	<b>0.55</b>	***	0.019	0.00	ns	<b>0.195</b>	<b>0.86</b>	***	<b>0.186</b>	<b>0.79</b>	***	<b>0.186</b>	<b>0.94</b>	**
	S	0.242	0.53	ns	0.068	0.17	ns	<b>0.095</b>	<b>0.48</b>	**	<b>0.126</b>	<b>0.45</b>	**	<b>0.162</b>	<b>0.85</b>	**
$RMF_{veg}$	L	-0.017	0.12	ns	<b>0.072</b>	<b>0.26</b>	*	<b>-0.145</b>	<b>0.48</b>	**	0.004	0.01	ns	-0.105	0.48	ns
	S	0.003	0.00	ns	<b>0.256</b>	<b>0.50</b>	*	-0.014	0.01	ns	-0.002	0.01	ns	<b>-0.147</b>	<b>0.81</b>	*
$LMF_{veg}$	L	<b>-0.065</b>	<b>0.59</b>	***	-0.091	0.22	ns	-0.05	0.18	ns	<b>-0.191</b>	<b>0.77</b>	***	-0.082	0.44	ns
	S	-0.244	-0.55	ns	<b>-0.324</b>	<b>0.71</b>	***	<b>-0.081</b>	<b>0.45</b>	**	<b>-0.124</b>	<b>0.45</b>	**	-0.015	0.02	ns
$LAR_{veg}$	L	-1.515	0.03	ns	<b>-17.4</b>	<b>0.26</b>	*	<b>-16.06</b>	<b>0.34</b>	*	<b>-47.02</b>	<b>0.71</b>	***	-0.158	0.00	ns
	S	-23.27	0.62	ns	<b>-30.37</b>	<b>0.56</b>	**	<b>-12.01</b>	<b>0.66</b>	***	<b>-9.87</b>	<b>0.38</b>	*	3.322	0.03	ns
Relationships with N pool																
$SNF_{veg}$	L	0.039	0.21	ns	0.001	0.00	ns	<b>0.134</b>	<b>0.76</b>	***	<b>0.195</b>	<b>0.54</b>	***	<b>0.044</b>	<b>0.91</b>	**
	S	-0.013	0.00	ns	0.096	0.14	ns	<b>0.100</b>	<b>0.47</b>	**	<b>0.109</b>	<b>0.32</b>	*	<b>0.097</b>	<b>0.67</b>	*
$RNF_{veg}$	L	-0.022	0.11	ns	0.088	0.20	ns	-0.058	0.10	ns	0.003	0.00	ns	-0.080	0.37	ns
	S	0.084	0.29	ns	<b>0.408</b>	<b>0.57</b>	**	0.005	0.00	ns	0.076	0.15	ns	<b>-0.121</b>	<b>-0.89</b>	**
$LNF_{veg}$	L	-0.017	0.06	ns	-0.089	0.11	ns	-0.075	0.24	ns	<b>-0.197</b>	<b>0.57</b>	***	0.036	0.11	ns
	S	-0.071	0.03	ns	<b>-0.505</b>	<b>0.68</b>	***	<b>-0.104</b>	<b>0.39</b>	*	<b>-0.186</b>	<b>0.55</b>	**	0.025	0.06	ns

Associated *P*-values are indicated by asterisks (\*\*\**P* < 0.001; \*\**P* < 0.01; \**P* < 0.01; ns, not significant) and significant relationships are indicated by bold font. Biomass and N pool values were log-transformed for analysis. See [Supplementary Data Figs S2 and S3](#) for plots.

(*P* = 0.079 for predicted means and *P* = 0.082 for the largest individuals; [Fig. 2D, E](#)). This lack of significance was especially influenced by the low, shrub-like  $LAR_{veg}$  in *L. periclymenum*.

The length of the stem produced by a unit of biomass (SSL) was consistently higher in lianas (except for *Hydrangea* in 2015) with, for example, *H. helix* in 2016 showing 17-fold higher SSL than the con-familiar *E. senticosus* ([Fig. 3A–C](#)). In spite of the lack of significant differences in  $SMF_{veg}$  between the growth forms, the high liana SSL resulted in the liana stem system being several-fold longer than in shrubs at a similar biomass (*P* = 0.018 for predicted means and *P* = 0.045 for the largest individuals; [Fig. 3D, E](#)). The greatest total stem length per individual liana plant was 933.5 m in *L. periclymenum* and the longest stem system in shrubs measured 385 m in *L. maackii*. Dead stem mass accounted for, on average, 0–3.85 % of the total stem mass per species at a given harvest, with the highest biomass of dead stems (9.95 %) noted in an individual *L. periclymenum* ([Supplementary Data Table S1](#)).

The concentration of N in stems and roots at a given harvest differed among families and, in 2014 and 2015, also between the habits (see [Supplementary Data Table S2](#) for ANOVA). The stem and root N concentrations in liana species were either higher than, or not significantly different from, those in con-familiar shrubs, except for the higher shrub stem N concentration in Fabaceae in 2016 ([Table 4](#)). On the other hand, leaf N was poorly related to the life form, with most (nine out of 13) within-year contrasts lacking significance ([Table 4](#); [Supplementary Data Table S2](#)). Since variability in N concentration was relatively much smaller than the variability in organ biomass, the interspecific pattern of distribution of the N pool among vegetative organs largely resembled that of biomass distribution, with liana  $RNF_{veg}$  being lower than in shrubs but  $SNF_{veg}$  and  $LNF_{veg}$  of lianas not being differentiated statistically from shrubs ([Fig. 4A–F](#)). Likewise, lianas and shrubs did not differ in N partitioning between leaves and stems ([Fig. 4G, H](#)).

When mean distribution variables were analysed for lianas and shrubs without adjusting for plant biomass, no significant differences between the growth forms were detected ([Supplementary Data Table S3](#)). This result was consistent with the lack of significant ANCOVA differences between lianas and shrubs for  $SMF_{veg}$ ,  $LMF_{veg}$ ,  $LSMR$  and  $LAR_{veg}$ , as well as  $SNF_{veg}$  and  $LNF_{veg}$ , but contrasted with the ANCOVA results showing lower adjusted  $RMF_{veg}$  and  $RNF_{veg}$  in lianas (cf. [Figs 1, 2 and 4](#)). Accounting for ontogenetic drift thus allowed the detection of an otherwise unapparent differentiation in allocation patterns.

Relative year to year growth rates were higher in lianas than in shrubs, and declined proportionally to plant size ([Fig. 5A](#)). Notably, however, the lowest growth rate was found in the liana *L. periclymenum* at the final growth interval. When RGR was not adjusted for biomass, the difference between lianas and shrubs was not significant ([Supplementary Data Fig. S4](#)).

Indices of resource distribution reported above were calculated on the basis of vegetative plant biomass, excluding the biomass of reproductive parts. Reproduction occurred in all shrubs and in two out of five lianas (*L. periclymenum* and *C. orbiculatus*), and usually took place in the final one or two growing seasons. On average, in a given year, reproductive effort did not exceed 17 % of biomass and 21 % of plant N (*H. macrophylla* in 2016); however, it varied strongly among individuals and, in a single heavily fruiting shrub of *E. europaeus*, reached 25 % of plant biomass and 27 % of plant N ([Supplementary Data Tables S4 and S5](#)). When the whole-plant biomass (including reproductive organs) was used in calculations, liana  $SMF_{wh}$  and  $LMF_{wh}$  were still not significantly higher than in shrubs, and lianas still showed lower  $RMF_{wh}$  in comparison with shrubs ([Supplementary Data Fig. S5A–F](#)). The predicted  $LAR_{wh}$ , however, became significantly higher in lianas than in shrubs ([Supplementary Data Fig. S6A](#)). The inclusion of reproductive biomass did not alter the distribution

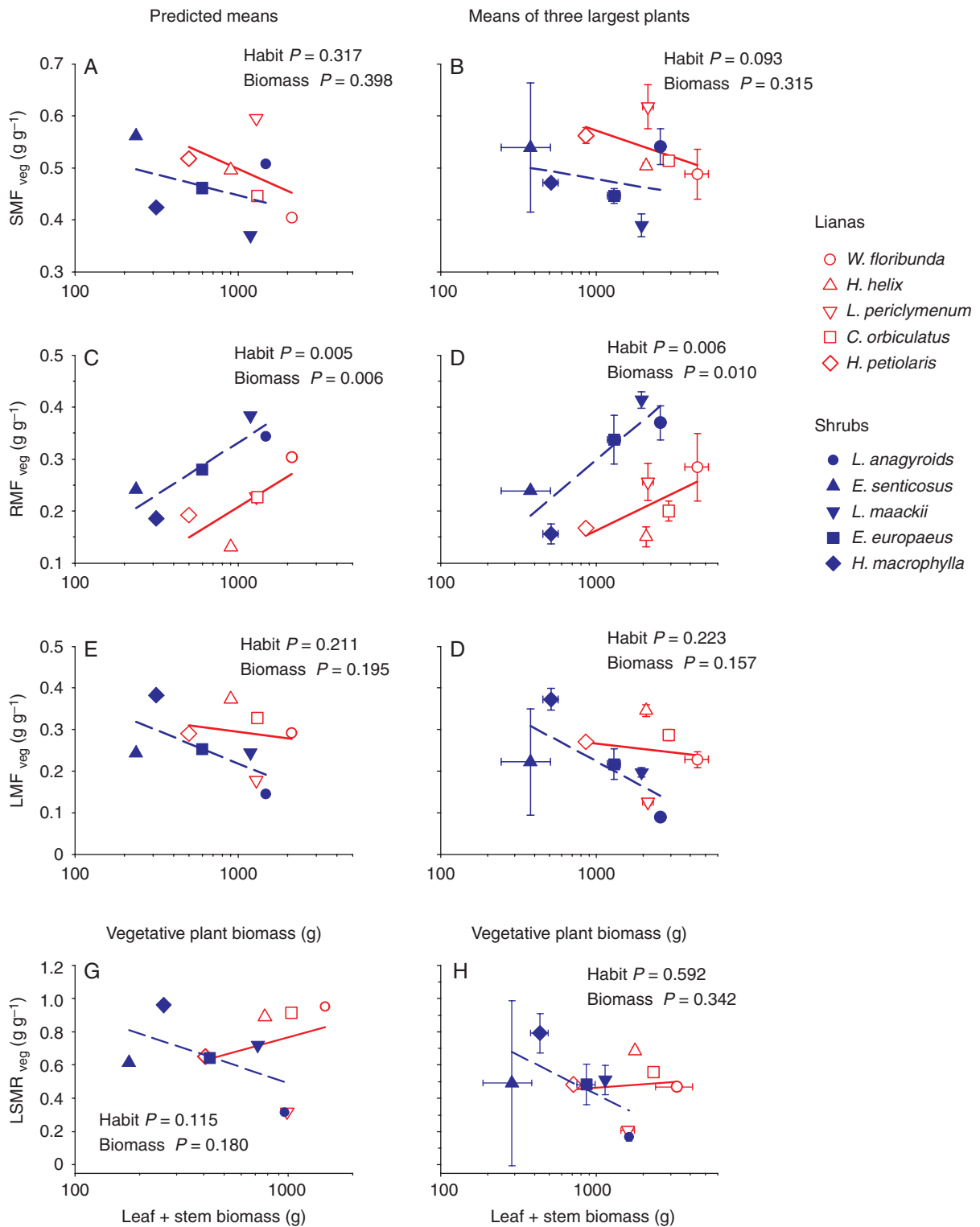


FIG. 1. Vegetative biomass distribution ratios: stem mass fraction ( $SMF_{veg}$ ; A, B), root mass fraction ( $RMF_{veg}$ ; C, D), leaf mass fraction ( $LMF_{veg}$ ; E, F) and ratio of bulk leaf to stem biomass ( $LSMR_{veg}$ ; G, H) shown in relation to vegetative plant biomass (A–F) or combined leaf and stem biomass (G–H) for five con-familiar pairs of liana/shrub species. Symbols represent predicted species means at average biomass (left-hand panels) and means ( $\pm$  s.e.) of the three largest plants per species (right-hand panels). Significance levels  $P$ -values of ANCOVA effects are given. Bold font indicates the effects with  $P < 0.05$ .

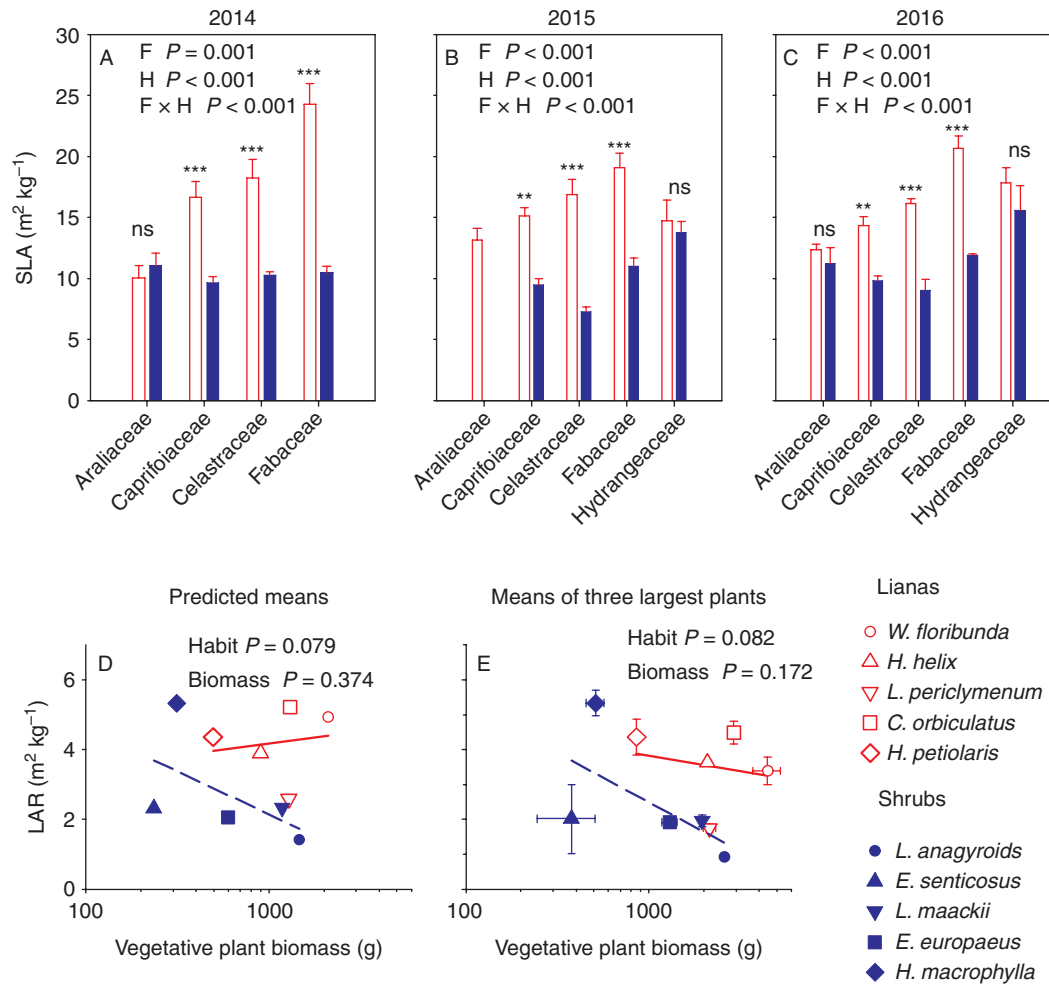


FIG. 2. Biomass allocation to leaf area in five con-familial pairs of liana/shrub species. (A–C) Specific leaf area (SLA; means  $\pm$  s.e.) determined for randomly selected leaves representing various positions throughout the canopy at (A) 2014, (B) 2015 and (C) 2016 harvests. ANOVA results with family (F) and habit (H) effects are given for each harvest, and significance levels for contrasts between species within family are shown above the bars (\*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ ; ns, not significant). (D and E) Relationships between plant leaf area ratio (LAR<sub>veg</sub>) and plant vegetative biomass. Symbols in (D) are predicted species means at average biomass, and symbols in (E) represent means ( $\pm$  s.e.) of the three largest plants per species, and results of ANCOVA are shown. Bold font indicates the effects with  $P < 0.05$ .

patterns of N between shrubs and lianas (Supplementary Data Fig. S7) or the differentiation in growth rates (not shown).

## DISCUSSION

The evolutionary success of lianas has been attributed to a specialized pattern of resource investment favouring the production of leaves over supporting organs (Darwin, 1865; Putz, 1983). In the classical allocation terminology, lianas are expected to feature a high leaf mass fraction but low stem or root mass fraction, relative to self-supporting plants. Although this hypothesis has gained a firm footing in the literature (Ichihashi and Tateno, 2015; Isnard and Field, 2015; Toledo-Aceves, 2015), empirical tests that would account for the ontogenetic drift of biomass ratios and consider whole-plant (as opposed to shoot-level) allocation have been scarce (Kaneko and Homma 2006; see Wyka et al., 2013; Poorter et al., 2015 for reviews). Our data set fills this gap for a selection of temperate lianas.

The hypothesis of increased LMF or LAR in lianas vs. self-supporting plants received limited support in our study. While LMF values in two of the five lianas (*L. periclymenum* and *H. petiolaris*) were aligned with those of shrubs, the remaining three species showed higher LMF than shrubs, not resulting, however, in significant group differences. These results are consistent with the few literature reports using whole plants in which climbing species did not differ from self-supporters with respect to LMF (den Dubbelden and Verburg, 1996 for non-leguminous species; Kaneko and Homma, 2006) or showed higher LMF (den Dubbelden and Verburg, 1996 for leguminous species; Cai et al. 2007). Moreover, even at the shoot scale, we found no evidence for increased distribution of biomass to leaves in lianas, similarly to Daring et al. (1994) and Selaya et al. (2007), but contrasting with reports showing higher LSMR of lianas (Putz, 1983; Wyka et al., 2013; Ichihashi and Tateno, 2015). Perhaps significantly, the latter studies included shoots of larger biomass than those in our study, suggesting that the ontogenetic reduction in LMF may be stronger in



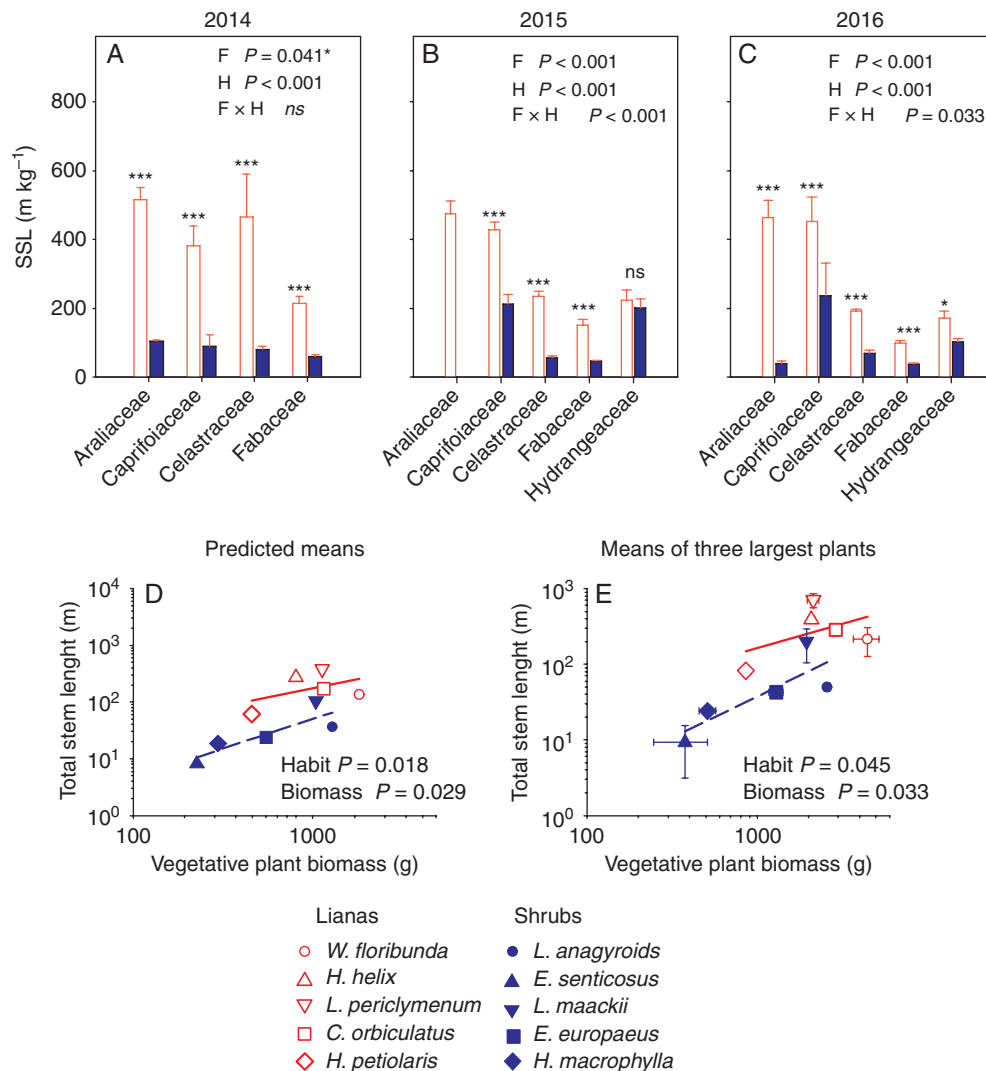


FIG. 3. Structural characteristics of stems in five con-familial pairs of liana/shrub species. (A–C) Specific stem length (SSL) calculated on the basis of total length of plant stems for (A) 2014, (B) 2015 and (C) 2016 harvests. ANOVA results with family (F) and habit (H) effects are given for each harvest, and significance levels for contrasts between species within family are shown above the bars (\*\* $P < 0.001$ ; \* $P < 0.01$ ;  $P < 0.05$ ; ns, not significant). (D and E) Relationships between total stem length and vegetative plant biomass. Symbols in (D) are predicted species means at average biomass, and symbols in (E) represent means ( $\pm$  s.e.) of the three largest plants per species, and results of ANCOVA are shown. Bold font indicates the effects with  $P < 0.05$ .

self-supporting species. However, since no report has shown a lower LMF in climbing vs. self-supporting plants, it may be confidently accepted that the increased distribution of biomass to leaves in lianas is a general rule, but it strongly varies with the selection of species in the sample, plant size and the environmental context.

Further, because lianas showed generally higher SLA than shrubs, the  $LAR_{veg}$  and  $LAR_{wh}$  of lianas were marginally higher than in shrubs (significantly in the case of predicted  $LAR_{wh}$ ). High SLA is a frequently noted feature of lianas both locally (Salzer *et al.*, 2006; Cai *et al.*, 2009; Sánchez-Azofeifa *et al.*, 2009; Han *et al.*, 2010; Zhu and Cao, 2010; Collins *et al.*, 2016) and in global comparisons of lianas vs. trees or lianas vs. shrubs (Asner and Martin, 2012; Wyka *et al.*, 2013). This suggests that the low leaf area construction cost should enable lianas to achieve higher leaf area displays even when biomass allocation to leaves in lianas does not exceed that in self-supporting

species. The disadvantages of high SLA include greater vulnerability to biotic and abiotic stresses and, perhaps, an increased leaf turnover leading to a loss of standing leaf area and reduction in LAR. We are not aware of demographic studies of leaves in temperate lianas vs. self-supporting plants, although we have observed a mid-season abscission of leaves formed in early season in *L. periclymenum*, a low LMF/LAR species (T. P. Wyka, unpubl. obs.).

Lianas did not show an overall reduction of SMF relative to shrubs. In fact, liana SMF values tended to overlap with, or insignificantly exceed, the shrub values, especially in the largest individuals. In the largest lianas, stem biomass accounted for about half or more of plant biomass, with especially high values noted in *L. periclymenum*. The rare studies addressing whole-plant biomass distribution reported a similar lack of reduction in SMF, e.g. in climbing vs. shrubby *Hydrangea* (Kaneko and Homma, 2006), in tropical lianas vs. con-generic trees

TABLE 4. Concentration (mg g<sup>-1</sup>; mean ± s.d.) of total N in dry biomass of stems, roots and leaves of five con-familial pairs of lianas and shrubs at three annual peak-of-season harvests

Family	Year	n	Stems			Roots			Leaves			
			Lianas	Shrubs	P	Lianas	Shrubs	P	Lianas	Shrubs	P	
Araliaceae	2014	5	2	17.48 ± 2.25	16.86 ± 0.63	ns	23.61 ± 5.40	21.53 ± 1.96	ns	21.63 ± 1.94	23.90 ± 3.38	ns
	2015	5	0	15.91 ± 1.25	–		22.00 ± 3.85	–		26.95 ± 2.11	–	
	2016	8	2	14.06 ± 1.76	12.28 ± 3.87	ns	21.20 ± 2.25	23.44 ± 3.74	ns	<b>24.41 ± 1.89</b>	<b>34.44 ± 2.99</b>	***
Caprifoliaceae	2014	5	5	<b>14.99 ± 2.26</b>	<b>11.04 ± 1.18</b>	***	<b>27.44 ± 2.05</b>	<b>19.03 ± 2.81</b>	***	29.84 ± 2.67	27.64 ± 2.90	ns
	2015	5	3	<b>12.91 ± 0.71</b>	<b>9.63 ± 1.96</b>	**	<b>25.22 ± 2.49</b>	<b>19.11 ± 1.47</b>	***	27.06 ± 1.19	23.66 ± 0.92	ns
	2016	7	4	<b>12.55 ± 0.94</b>	<b>10.29 ± 0.46</b>	*	<b>20.85 ± 1.41</b>	<b>16.71 ± 2.61</b>	**	<b>27.86 ± 2.92</b>	<b>23.60 ± 1.96</b>	**
Celastraceae	2014	5	5	<b>18.65 ± 3.64</b>	<b>13.22 ± 2.63</b>	**	21.84 ± 3.84	26.30 ± 4.41	ns	29.86 ± 3.42	26.89 ± 2.40	ns
	2015	5	5	<b>20.58 ± 2.53</b>	<b>14.80 ± 4.36</b>	**	<b>35.27 ± 4.63</b>	<b>27.56 ± 3.66</b>	**	30.82 ± 1.92	28.82 ± 5.71	ns
	2016	4	5	15.16 ± 2.04	13.83 ± 1.83	ns	28.63 ± 6.56	25.76 ± 2.18	ns	25.58 ± 3.51	25.82 ± 1.54	ns
Fabaceae	2014	5	5	20.80 ± 2.50	18.80 ± 2.21	ns	<b>36.08 ± 5.09</b>	<b>25.40 ± 4.40</b>	***	33.16 ± 0.99	33.42 ± 3.04	ns
	2015	5	5	13.96 ± 2.11	16.32 ± 1.20	ns	<b>28.37 ± 7.84</b>	<b>22.76 ± 2.45</b>	*	28.65 ± 2.91	27.72 ± 2.15	ns
	2016	6	4	<b>13.05 ± 1.45</b>	<b>15.81 ± 2.46</b>	*	21.26 ± 0.89	23.31 ± 1.94	ns	<b>24.12 ± 3.26</b>	<b>31.44 ± 3.29</b>	***
Hydrangeaceae	2015	3	3	<b>14.61 ± 2.00</b>	<b>7.86 ± 1.07</b>	***	<b>21.11 ± 3.83</b>	<b>15.16 ± 2.22</b>	*	25.35 ± 1.13	20.83 ± 2.10	ns
	2016	3	3	11.55 ± 0.83	9.36 ± 1.95	ns	22.13 ± 0.71	18.36 ± 0.55	ns	<b>32.02 ± 1.75</b>	<b>24.49 ± 5.16</b>	*

Results of Student's *t*-test for significance of differences between liana and shrub means within family and season are shown: \*\*\**P* < 0.001; \*\**P* < 0.01; \**P* < 0.05; ns, not significant.

Significant differences are highlighted by bold font. See [Supplementary Data Table S2](#) for ANOVA results.

(Cai *et al.*, 2007) and in herbaceous climbers vs. self-supporting species (den Dubbelden and Verburg, 1996). The climbing habit may thus be as costly as the self-supporting habit in terms of the biomass needed for stem construction. This counterintuitive observation may be explained by specialization of the liana shoot system for rapid expansion to the forest canopy, resulting in production of greater stem length at a similar plant size (Niklas, 1994). Although liana stems are on a unit length basis cheaper to construct than self-supporting stems, as shown by their high SSL, this did not offset the total biomass investment associated with shoot extension. Even the narrow secondary growth increments typical of lianas (Putz, 1990) may not necessarily imply a reduction of the plant-level cost, as secondary growth in lianas is distributed along a greater stem length than in self-supporting stems. On the other hand, considering that our study included only temperate lianas, it is possible that their cost of stem unit mass (e.g. stem density) may be higher than in the more numerous and abundant tropical liana species, because of the challenges posed by winter stresses (Jiménez-Castillo and Lusk, 2013). Comparative studies of temperate and tropical lianas would be informative.

The general phenomenon of an ontogenetic increase in SMF with accumulating plant mass (Poorter *et al.*, 2015) has been confirmed in individual species of both shrubs and lianas (except in *L. periclymenum* in which SMF was high even in small individuals), without a clear differentiation between habits. This allocation vs. biomass relationship was, however, not reproduced on the interspecific scale, emphasizing the diversity of stem investment within each growth form and yielding further support for the lack of a group difference in SMF between the two habits. Lianas show a remarkable polymorphism of stem morphology. Depending on the species and the climbing mechanism, the stem system may consist of juvenile self-supporting shoots, stiff searcher shoots used for finding support, long extension shoots used for climbing (some later forming permanent trunks) and shorter shoots dedicated to leaf display and used to exploit bright sites within the canopy (Rowe and Speck,

2005; Isnard and Silk, 2009; Ichihashi *et al.*, 2010). Since stem types differ with respect to the ratio of leaf to stem biomass, with, for example, a high proportion of biomass allocated to leaves in leaf display shoots (Ichihashi *et al.*, 2010), the plant SMF will consequently depend on the contribution of each shoot type and this may change with age and accumulation of secondary growth. Further, standing stem biomass in perennial plants may be reduced by the stem turnover, thought to be high in some lianas. The estimated loss of old stems in forest lianas may reach 75 % of the length or 45 % of the biomass before they reach the canopy (Ichihashi and Tateno, 2015). It is not clear, however, whether liana stem turnover is higher in comparison with shrubs, since expendable stems are also part of the shrub design strategy (Götmark *et al.*, 2016). Our data did not indicate a greater propensity of lianas to replace old stems; however, plants were grown under favourable, well-illuminated conditions and over only four seasons.

The key finding of our study was that root investment in lianas was lower relative to that in shrubs. This result extends and confirms the reports by Kaneko and Homma (2006) and Cai *et al.* (2007) on woody species, but contrasts with that by den Dubbelden and Verburg (1996) who failed to find a difference between herbaceous climbers and self-supporters. Notably, in the largest individuals of the two root climbers *H. helix* and *H. petiolaris*, roots accounted for a particularly low fraction of plant biomass (<0.2 g g<sup>-1</sup>, and <0.1 g g<sup>-1</sup> in some smaller *H. helix* individuals). This result suggests that the root climbing mode, in particular, does not require strong anchorage in soil, perhaps because a strong attachment to tree trunks is provided by short, non-absorptive, adventitious roots forming along stems of these lianas (these roots in our study were included with the stem biomass fraction). On the other hand, *W. floribunda*, a liana with the highest RMF, formed a thick rootstock, suggesting a significant storage function of roots in this species. While efficient water uptake and deep root penetration seem to be features of some lianas (Restom and Nepstad 2004; Cai *et al.* 2009; Zhu and Cao 2009), they may be achieved by relatively

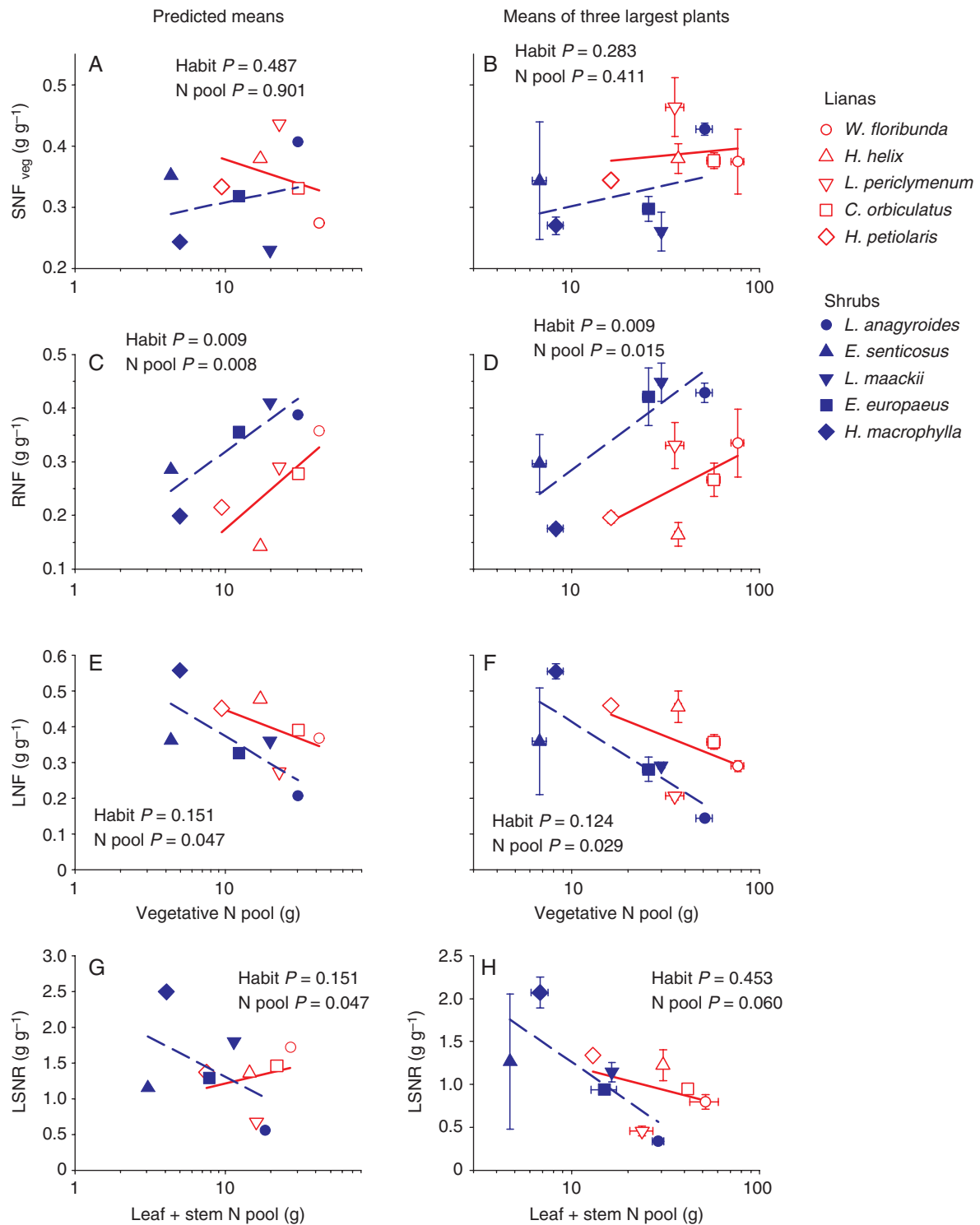


FIG. 4. Nitrogen distribution ratios: stem N fraction (SNF<sub>veg</sub>; A, B), root N fraction (RNF<sub>veg</sub>; C, D), leaf N fraction (LNF<sub>veg</sub>; E, F) and leaf N pool to stem N pool ratio (LSNR; G, H) shown in relation to the vegetative plant N pool (A–F) or combined leaf and stem N pool (G, H) for five con-familial pairs of liana/shrub species. Symbols represent predicted species means at average N pool (left-hand panels) and means ( $\pm$  s.e.) of the three largest plants per species (right-hand panels). Significance levels of analysis of covariance effects are given. Bold font highlights the effects with  $P < 0.05$ .

inexpensive root systems containing abundant absorptive roots. Unfortunately, we were technically unable to quantify the root system morphologies with respect to the ratio of absorptive vs. structural root biomass; however, we noted that at the end

of our experiment, both liana and shrub roots (except for both *Hydrangea* species) penetrated to the bottom of the barrels. Although it is ultimately unclear how the advantages and disadvantages of high root investment differ between climbing and

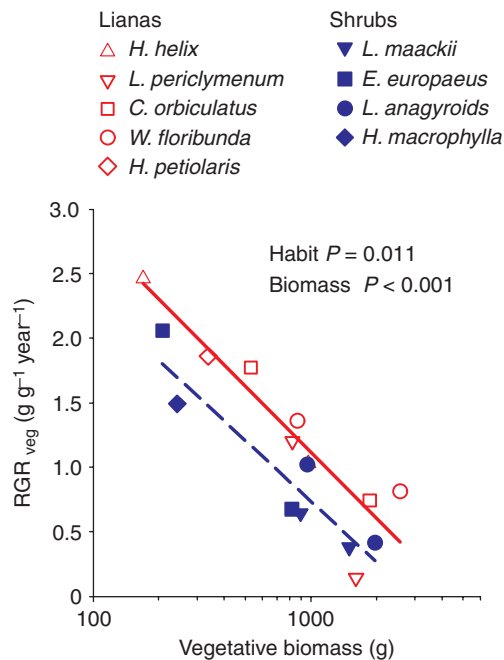


FIG. 5. Vegetative biomass-based relative growth rate ( $RGR_{veg}$ ;  $g\ g^{-1}\ year^{-1}$ ) of lianas and shrubs.  $RGR_{veg}$  values for each species were calculated for intervals between 2014 and 2015, and between 2015 and 2016 harvests; thus, each species contributed two data points, except for both *Hydrangea* species (one point each) and *E. senticosus* for which calculation was not possible ( $n = 9$  for lianas and  $n = 7$  for shrubs). Interval means are shown in relation to geometric means of vegetative biomass from two consecutive harvests. Results of ANCOVA are given, with bold font indicating significant ( $P < 0.05$ ) effects.

self-supporting habits, our data suggest that biomass allocation to roots in lianas is rather low, with a possible differentiation between the climbing modes.

The robustness of our results could potentially be influenced by the fact that our study was performed under adequate nutrient and moisture availability, whereas a deficit of soil resources usually induces an increase in RMF (Poorter *et al.*, 2012) and a decrease in LMF (Klimeš and Klimešová, 1994). Further, plants were grown in full sun or, in the case of two species pairs, in a light shade. Shading is known to induce morphological modification of shoots, such as the formation of longer, less branched extension stems, leading to changes in biomass allocation (During *et al.*, 1994; González and Gianoli, 2004), and induce reduction in RMF (Poorter *et al.*, 2012). However, the response of biomass distribution to such a low degree (30%) of shading is typically very weak (Poorter *et al.*, 2012) and, since our design ensured balanced representation of both lianas and shrubs under shading, the between-group differences were most probably little affected. We acknowledge, at the same time, that reaction norms of lianas and self-supporting plants to environmental factors may not be identical, warranting future manipulative studies using both growth forms (Cai *et al.*, 2009).

Next, the biomass distribution as well as the growth rate in *L. periclymenum* might have been potentially affected by failure to use support. There is no information available on factors controlling the climbing behaviour in *Lonicera*; however, it is known that in high light certain lianas do not enter the climbing

phase (Gallenmüller *et al.*, 2004). According to reports, lack of support restricted growth, increased SMF and reduced RMF in *W. floribunda* (Sakai and Suzuki, 1999; Wyka *et al.*, 2019), modified stem morphology without affecting growth rate in a set of herbaceous vines (den Dubbelden and Oosterbeek, 1995) but had no effect on above-ground biomass of *Toxicodendron obtusilobum* (Gartner, 1991) and *C. orbiculatus* (Wyka *et al.*, 2019). The effects of support use are, however, strongest in shade (Gianoli, 2003); therefore, we presume that in our open, spacious experimental garden, trait expression was not strongly modified. Moreover, if the failure of *L. periclymenum* to climb mimicked the responses of *W. floribunda* (Sakai and Suzuki, 1999), then our main result showing low liana RMF would have been strengthened, and the excess SMF in lianas relative to shrubs would have become closer to significant.

We hypothesized that using N distribution as an alternative to biomass distribution may modify the differences in investment in particular organs between the growth forms. Lianas, relative to shrubs, indeed tended to have a higher N concentration in roots and stems. The latter trend was consistent with the typically high stem parenchyma content in lianas (Angyalossy *et al.*, 2015). However, since variability of biomass distribution was much greater than variability of N concentration, growth form differences in N distribution were mostly driven by those in biomass distribution. As a result, the gross patterns of N and biomass distribution were similar and showed reduced root biomass and N fractions in lianas, without consistent differences in leaf and stem fractions. In our species set, both allocation currencies were thus equivalent in comparisons between lianas and shrubs, whether vegetative parts or whole plants were considered. This occurred in spite of the high N content of reproductive parts.

Climbing growth forms are often interpreted as expressing an acquisitive ecological strategy whereby resources are quickly converted into new growth via a preferential resource allocation to productive parts (Collins *et al.*, 2016). We indeed found greater year to year growth rates in lianas. This elevated growth rate could be attributed to the high LMF and LNF of some lianas, as well as the reduced allocation to roots, especially when soil resources are in high supply, as in our experiment. The high SLA, typical for lianas, is another hallmark of a fast growth rate (Poorter *et al.*, 2012). The co-occurrence of these traits in temperate lianas indicates that they belong to the fast resource uptake/fast growth/quick turnover end of the life strategy spectrum, relative to self-supporting shrubs. On the other hand, similarity of liana and shrub N concentration per leaf mass signals a lack of metabolic specialization in lianas, in contrast to results of other studies that have frequently reported greater leaf N concentrations in lianas than in self-supporting species (Kazda and Salzer, 2000; Cai *et al.*, 2009; Han *et al.*, 2010; Wyka *et al.*, 2013). Moreover, the foliage of our liana species, given their high SLA, would have an overall lower N concentration per leaf area than shrubs, suggesting a lower per area photosynthetic potential as compared with shrubs. Also, globally, the mean per area photosynthetic rate in lianas is lower than in shrubs (but comparable with that of trees; Wyka *et al.*, 2013). Our study thus supports the view that resource allocation plays a greater role than metabolism in giving lianas a growth advantage over self-supporting species.

## CONCLUSIONS

By studying the biomass and N distribution in an assortment of temperate lianas and self-supporting shrubs, we demonstrated some consistent habit-specific features of resource allocation patterns, with biomass patterns recapitulated by those based on N. Foremost they included a reduced root investment in the lianas that in individual species was variously offset by an increased allocation to either leaves or stems, or both. There was, however, no evidence for a trade-off between allocation to leaves and stems; thus, our results were not consistent with the hypothesis of reduced above-ground support cost in lianas. Given the dynamic space exploration by the fast extending liana stems, prioritizing the deployment of new leaf area by reducing SLA seems to be an important part of the liana growth strategy, even if it does not result in decreased cost of investment in stems. Moreover, the partial overlap of SMF, SNF, LMF and LNF in particular between lianas and shrubs showed that the evolution of the lianescent habit may involve very disparate modifications of the resource economy. We cannot, however, exclude that reduced stem investment and increased leaf investment in lianas relative to self-supporting plants may occur in even larger individuals, as suggested by trends for above-ground parts reported by Gerwing and Farias (2000), Wyka et al. (2013) and Ichihashi and Tateno (2015). We also found that lianas as a group grew faster than shrubs, and propose that the reduced costs of the root systems, low stem unit length construction cost, the high LMF and LNF of some species and the high SLA could all contribute to this growth advantage. Finally, our study demonstrates the importance of accounting for ontogenetic drift, or size dependence, of resource distribution patterns, as this approach has revealed an otherwise undetectable differentiation in RMF and RNF, as well as in relative growth rates between the growth forms.

## SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. **Figure S1**: images of the cultivation experiment. **Figure S2**: vegetative biomass distribution ratios for individual plants. **Figure S3**: vegetative N distribution ratios for individual plants. **Figure S4**: mean vegetative biomass-based relative growth rates. **Figure S5**: whole-plant-based biomass distribution ratios. **Figure S6**: relationships between whole-plant-based leaf area ratio and whole-plant biomass. **Figure S7**: whole-plant-based N distribution ratios. **Table S1**: dead stem and leaf mass shown as a percentage of, respectively, total stem and leaf mass. **Table S2**: results of factorial ANOVA for the effects of family and plant habit on N concentration in stems, roots and leaves. **Table S3**: results of Student's *t*-test for differences in unadjusted vegetative biomass and N distribution variables between liana and shrub species. **Table S4**: occurrence of reproduction and concentration of total N in dry mass of flowers and fruits. **Table S5**: fractions of biomass and N contained in reproductive structures (flowers and fruits) relative to, respectively, whole-plant biomass or N pool.

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