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Research paper

Scaling relationships among twig size, leaf size and leafing intensity in a successional series of subtropical forests

En-Rong Yan[1,2,4], Xi-Hua Wang[1,2], Scott X. Chang[3] and Fangliang He[3]

[1]Department of Environmental Sciences, East China Normal University, Shanghai 200241, China; [2]Tiantong National Station of Forest Ecosystem, Chinese National Ecosystem Observation and Research Network, Ningbo 315114, China; [3]Department of Renewable Resources, University of Alberta, Edmonton, Alberta, Canada T6G 2E3; [4]Corresponding author (eryan@des.ecnu.edu.cn)

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Scaling relationships among twig size, leaf size and leafing intensity fundamentally influence the twig–leaf deployment pattern, a property that affects the architecture and functioning of plants. However, our understanding of how these relationships change within a species or between species as a function of forest succession is unclear. We determined log–log scaling relationships between twig cross-sectional area (twig size) and each of total and individual leaf area, and leafing intensity (the number of leaves per twig volume) for 78 woody species along a successional series in subtropical evergreen forests in eastern China. The series included four stages: secondary shrub (S1), young (S2), sub-climax (S3) and climax evergreen broadleaved forests (S4). The scaling slopes in each of the three relationships did not differ among the four stages. The *y*-intercept did not shift among the successional stages in the relationship between twig cross-sectional area and total leaf area; however, the *y*-intercept was greatest in S4, intermediate in S3 and lowest in S2 and S1 for the relationship between twig size and individual leaf area, while the opposite pattern was found for the twig size-leafing intensity relationship. This indicates that late successional trees have few but large leaves while early successional trees have more small leaves per unit twig size. For the relationship between twig cross-sectional area and total leaf area, there was no difference in the regression slope between recurrent (appear in more than one stages) and non-recurrent species (appear in only one stage) for each of the S1–S2, S2–S3 and S3–S4 pairs. A significant difference in the *y*-intercept was found in the S2–S3 pair only. In the relationship between twig cross-sectional area and individual leaf area, the regression slope between recurrent and non-recurrent species was homogeneous in the S1–S2 and S3–S4 pairs, but heterogeneous in the S2–S3 pair. We conclude that forest succession caused the shift in the intercept, but did not affect scaling slopes for relationships among twig size, leaf size and leaf intensity. For recurrent species, the invariant scaling slope in the twig–leaf size relationship between adjacent pairs of successional stages may be related to their phenotypic plasticity by adjusting their twig and leaf deployment strategy to similar to what the non-recurrent species display.

Keywords: allometric scaling, evergreen broad-leaved forests, recurrent species, scaling slope.

Introduction

As one of the leading dimensions in plant ecological strategies, the scaling of twig size to leaf size is fundamentally important for biomass allocation between twig and leaves, a property that affects the architecture and functioning of plants (White 1983*a*, 1983*b*; Niklas 1992; Niklas and Enquist 2002; Pickup et al. 2005; Gotsch et al. 2010). For mechanical and hydraulic reasons, the twig–leaf size relationship can greatly influence plant carbon gain and allocation, as well as plant hydraulic transport efficiency (Shinozaki et al. 1964; Tyree and Ewers 1991; Niklas 1994; Westoby et al. 2002; Cochard et al. 2005; Ishida et al. 2008). Since the twig–leaf size relationship links closely with the plant's water and carbon economics, understanding scaling relationships between twig size and leaf size is important for revealing strategies of plant biomass allocation with changes of environmental stresses (Hacke et al. 2006; Wright et al. 2006; Hao et al. 2008; Meinzer et al. 2008; Sperry et al. 2008; Olson et al. 2009; Johnson et al. 2011; Bucci et al. 2012).

The log–log slope in the twig–leaf size relationship describes the way two variables scale with each other (see Niklas 1994—plant allometry, among many possibilities). Most empirical studies have demonstrated that the twig–leaf size relationship follows a positive allometric relationship (Ackerly and Donoghue 1998; Preston and Ackerly 2003; Westoby and Wright 2003; Sun et al. 2006; Normand et al. 2008; Yang et al. 2009), although isometric relationships have also been observed (e.g., Brouat et al. 1998; see Table 1). In addition, these studies have shown homogeneous (constant) slopes but regular variation in the *y*-intercept in the twig–leaf size relationship, relating to water or nutrient availability (Westoby and Wright 2003; Sun et al. 2006), plant ontogeny (Ackerly and Donoghue 1998; Preston and Ackerly 2003) or taxonomic groups (Brouat et al. 1998; Normand et al. 2008).

Linking twig–leaf size relationships with changes in environmental conditions along forest successional series is important for understanding plant adaptive strategies. In general, plant growth strategies (Yan et al. 2006; Selaya and Anten 2010) and tree architecture (Ackerly and Donoghue 1998; Poorter et al. 2006; Wright et al. 2006; McCulloh et al. 2011) vary with changes in environmental properties. For hydraulic and mechanical reasons, plants may carry more total leaf area per twig in relatively shady and wet habitats, but less total leaf area in windy and dry habitats (Westoby and Wright 2003; Sun et al. 2006; Wright et al. 2006; Broeckx et al. 2012). In a forest successional series, early stages are usually more irradiation- and wind-exposed, thus are drier and have larger evaporative demand, than late-successional stages (Selaya et al. 2008; Yan et al. 2009). All these factors would suggest that plants in the late-successional stages would support a larger leaf area per twig than those in the early-successional stages (Shukla and Ramakrishnan 1986), because plants require less water (lower evaporative demand) and mechanical support (lower wind speed) in late-successional forests (Selaya et al. 2008). In addition to the larger leaf size, lower leafing intensity (the number of leaves per twig) is also expected for plants in late-successional than in early-successional stages because of the

Table 1. Summary of log–log standardized major axis regression parameters for scaling relationships between twig cross-sectional area and total leaf area per twig from the literature.

Site, species, leaf type or forest type	Slope	95% CI[a]	r^2	Reference
Deciduous species	1.11	1.03–1.19	0.98***	Brouat et al. (1998)[b]
Evergreen species	1.19	0.85–1.72	0.87***	
Gymnosperms	1.04	0.75–1.47	0.82***	
Sydney site	1.38	>1	No data	Westoby and Wright (2003)
Woodland	1.86	>1	No data	
Arctostaphylos bakeri Eastw.	1.60	0.47	0.72***	Preston and Ackerly (2003)
Arctostaphylos glauca Lindl.	1.48	0.75	0.51**	
Arctostaphylos morroensis Wies. & Schreib.	1.47	0.42	0.63***	
Arctostaphylos tomentosa (Pursh) Lindl.	1.47	0.65	0.51***	
Baccharis malibuensis R.M. Beauch. & J. Henrickson	1.35	0.25	0.85***	
Baccharis plummerae A. Gray	1.35	0.37	0.47***	
Baccharis emoryi A. Gray	1.33	0.96	0.38	
Baccharis pilularis DC.	1.49	0.28	0.72***	
Ceanothus integerrimus Hook. & Arn.	1.25	0.45	0.14*	
Ceanothus thyrsiflorus Eschsch.	1.75	0.67	0.30**	
Ceanothus fresnensis Dudley ex Abrams	1.53	0.82	0.42*	
Ceanothus prostratus Benth.	1.67	0.67	0.81***	
Coniferous forest	1.21	>1	No data	Sun et al. (2006)
Birch forest	1.68	>1	No data	
Four mango cultivars	1.22	1.16–1.29	No data	Normand et al. (2008)
Compound leaf species	1.40	1.20–1.63	0.87***	Yang et al. (2009)
Deciduous species	1.32	1.15–1.50	0.86***	
Evergreen species	1.31	1.07–1.61	0.71***	

[a] Confidence interval (CI) > 1 means that the CI was significantly greater than 1, in this case, the 95% CI in the original literature was not given.
[b] In all other references listed, slopes were calculated by fitting a line through total leaf area (*Y*) vs. twig size (*X*). In Brouat et al. (1998), the opposite was done (i.e., *X* and *Y* were reversed). To compare the results of the Brouat et al. (1998) study with the other results, the reported slopes were adjusted by taking the inverse of the slopes reported (i.e., 1/B).
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

strong negative relationship of leaf size to leaf number at a given twig size (Kleiman and Aarssen 2007; Yang et al. 2008; Milla 2009). Based on those analyses, we hypothesize that plants in late-successional stages should have larger but fewer leaves than those in early-successional stages as wind and drought stress are mitigated, and shade stress is exacerbated in late successional stages (Selaya et al. 2008; Yan et al. 2009). In this context, plants would respond to forest succession by changing the scaling slope or the *y*-intercept in the twig–leaf size relationship.

Even though the bulk of evidence suggests that the scaling slope in the twig–leaf size relationship is largely invariant across habitats (Ackerly and Donoghue 1998; Preston and Ackerly 2003; Westoby and Wright 2003; Sun et al. 2006; Yang et al. 2009), this relationship has not been studied with successional changes. So far, most research in twig–leaf size relationship has been done among species (Westoby and Wright 2003; Sun et al. 2006; Yang et al. 2009). Moreover, most research related to twig–leaf size relationship has focused on the cross-species patterns in different types of forests with different species groups, without considering the effects of species recurrence and substitution on the twig–leaf size relationship (Westoby and Wright 2003; Sun et al. 2006; Yang et al. 2009). Currently, little is understood as to how the twig–leaf size relationship differs between recurrent and non-recurrent species along a successional sequence. Recurrent species are defined here as species that appear in more than one successional stage. Species that appear in only one stage are defined as non-recurrent species. It is commonly assumed that some species recur but others disappear in the course of succession. Therefore, the natural variability in plants' twig–leaf size relationship along a successional sequence is structured mainly by the composition of recurrent and non-recurrent species. The comparison of recurrent and non-recurrent species in their twig–leaf size relationship along successional sequences is crucial for understanding its variations while controlling phylogenetic inertia. To cope with changing environmental conditions through forest succession, recurrent species are expected to have greater plasticity (genetic or phenotypic) than non-recurrent species. Therefore, we hypothesize that recurrent species would behave similarly in their twig–leaf size relationship to non-recurrent species between pairs of succession stages, because recurrent species would adapt to environmental stresses in a given successional stage by adjusting their twig and leaf deployment pattern to similar to what the non-recurrent species display (Shukla and Ramakrishnan 1986; Preston and Ackerly 2003).

In this study, we investigated the variation in the twig–leaf size scaling relationship for 78 woody species along a successional series in subtropical forests in eastern China. Specifically, we were interested in understanding (i) how the slopes and intercepts of the twig–leaf size relationship change with forest succession and (ii) how the twig–leaf size relationship differs between recurrent and non-recurrent species in a particular pair of successive stages.

Materials and methods

Study sites and forest successional stages

This study was conducted in the lower eastern extension of the Siming Mountain (29°41–50′N, 121°36–52′E), located on the Ningbo coastal area, Zhejiang province, in eastern China. The area has the highest peak at 653 m above sea level, with most other relief in a range of 70–300 m. The region has a typical monsoon climate with a hot, humid summer and a drier cold winter. Details of climatic and edaphic conditions were described in Yan et al. (2006).

The zonal vegetation in this region is subtropical evergreen broad-leaved forests (EBLFs). The vegetation of the region has been severely disturbed in the past with only small tracks of intact or semi-intact EBLFs left around the Tiantong Buddhist temple. Outside of this site, virtually all vegetations are secondary. Our study sites are located around the temple area. The secondary shrubs are mostly formed after the cessation of repeated harvesting. After ~20 years in the shrub stage due to very dense stem density, the succession proceeds to young evergreen broadleaved forests that can reach tree canopy closure. As the succession further progresses, the young evergreen broadleaved forests are replaced by sub-climax EBLFs. At the mature stage of succession, the climax EBLFs form ~120 years after the cessation of forest harvest (Yan et al. 2009). In this study, we chose secondary shrub (S1), young EBLF (S2), sub-climax EBLF (S3) and climax EBLF (S4) as the four representative successional stages, based on both forest age and species composition. The four vegetation types used for this study were located on similar slope positions, had the same historical vegetation and the soils were developed from the same quartzitic parent material (Yan et al. 2009). We established 3 plots for S4, 7 plots for S3, 6 plots for S2, and 12 plots for the S1 across the studied region, with each plot (20 m × 20 m) located at least 100 m from the forest edge. The replicated plots were established by selecting stands with similar species composition and community structure for each successional stage. Generally, the forest structure in the S1 was made up of two vertical layers: 4–8 m and 2–4 m. In the S2, S3 and S4, there were three vertical layers: >8 m, 4–8 m and 2–4 m.

Collection and measurement of twigs and leaves

A total of 33, 19, 32 and 31 species were sampled from S1, S2, S3 and S4, respectively. The number of recurrent species between successional stages was 12, 8, 2, 12, 4 and 15, for each of the S1–S2, S1–S3, S1–S4, S2–S3, S2–S4 and S3–S4 pairs, respectively. The total number of species sampled was

78, belonging to 30 families and 58 genera, including ~1129 individual trees and shrubs (see Table S1 available as Supplementary Data at *Tree Physiology* Online).

In each plot, at least three plants per species were randomly selected, and twig and leaf samples were collected in July and August 2008. For each plant, leaf size may differ between sun and shade leaves as sunlight intensity varies with plant crown position. To reduce effects of this micro-environment on leaf size within a tree, five branches from the sun-lit part of the canopies only were chosen. In the field, the first-year twigs (i.e., twigs of the current year) were separated from the branch according to the terminal set of internodes. The first-year shoots were defined here as the twigs. From each branch, one twig without apparent leaf loss and/or damage was chosen, stored in a plastic zip-lock bag and kept cool until brought back to the laboratory for measurement, which was usually performed within 12 h of sample collection. In the laboratory, the leaf and stem were separated for each twig and the number of leaves was counted. Then, twig length and twig diameter at the mid-point along the length were measured. Twig diameter was measured using an electronic vernier caliper, accurate to 0.1 mm. Twig cross-sectional area was calculated from diameter by treating the twig cross-sectional area as a perfect circular shape. At the same time, all leaves from each twig were scanned using a leaf area meter (LI-3100C, Li-Cor, Lincoln, NE, USA) to determine the leaf area. Volume-based leafing intensity was calculated as the number of leaves supported on a twig divided by the twig stem volume, which can be used to reflect functional trade-offs between leaf size and leaf number per unit twig size (Kleiman and Aarssen 2007). Stem volume was assumed to approximate a cylinder shape, with mid-point stem diameter as the cylinder width and stem length as the cylinder height.

Data analysis

We used a linear mixed model to decompose trait variation across successional stages, plots within a stage, species within a plot, trees within a species and twigs within a tree, to test how the distribution of trait variance differs among five ecological scales. The linear mixed model used the five ecological scales in increasing order of twig, individual tree, species, plot and successional stage. In this procedure, the proportion of variance in the lowest level of the scale (twig) is the combination of variance partitioning in this scale and variances resulting from measurement error. Given that the mixed model assumes that the observations within each subgroup are normally distributed and have equal variances, we $\log_{10}$-transformed the data for each of the twig cross-sectional area, total leaf area per twig, individual leaf area and leafing intensity to achieve the normality of both residuals and random effects in the calibrated linear model. This analysis was conducted using a restricted maximum likelihood method in the 'lme' function of R (version 2.11.1). In this procedure, we extracted variance components with the 'varcomp' function in the R package 'ape' (R-Development CT 2009).

Since variance between species was consistently the largest component (Table 2), the twig and leaf data were averaged arithmetically for each individual plant and then by species to determine the twig–leaf size relationship at the species level. We realize and caution the reader that mathematically averaging these values may ignore some intraspecific variability of the twig–leaf size relationship. In addition, owing to the lack of variance partitioning at the plot scale (Table 2), data were pooled for each succession stage.

The relationships between twig size and each of leaf size and leafing intensity were described by a mathematical equation of the type $y = bx^a$, linearized under the form $\log(y) = \log(b) + a\log(x)$, x and y being the dimensions of the two parts considered. The value of the slope determines whether the relationship is isometric ($a = 1$, no change of form among species) or allometric. The term b is the y-intercept of the relationship. Its value does not determine the form of the relationship and, if two lines of the same slope are compared, the difference between their respective values of b indicates the differences independent of size.

Model type II regression analysis was used to estimate the parameters of the allometric equations. Slopes of the allometric relationship were calculated as standardized major axis (Falster et al. 2006), which is also known as reduced major axis. Type II regression procedures were carried out using (S)MATR (Version 2.0, Falster et al. 2006, http://www.bio.mq.edu.au/ecology/SMATR/). In this software, the values for a and b were computed using the formulae $a = a_{OLS}/r$ and $\log(b) = \log$

Table 2. Variance partitioning of the full nested linear models on four plant functional traits in leaf and twig tissues across five ecological scales.

Scale	Variance of plant functional traits (%)			
	Twig cross-sectional area	Total leaf area	Individual leaf area	Volume-based leafing intensity
Twig and error	19 (6–21)	19 (14–17)	13 (12–14)	10 (6–19)
Individual tree	17 (3–23)	13 (14–19)	7 (6–9)	8 (6–12)
Species	59 (53–67)	60 (56–63)	72 (70–73)	78 (66–81)
Plot	5 (1–5)	3 (1–5)	5 (4–6)	2 (0–1)
Successional stage	0 (0–1)	5 (4–7)	3 (2–4)	2 (2–3)

All data were $\log_{10}$-transformed prior to analysis. Values in parenthesis represent the 95% CI, which was calculated by bootstrapping with 500 runs with randomly sampled data points with replacement.

(Y) – a log (X), where a_{OLS} is the ordinary least square (OLS) scaling exponent (slope), r is the OLS correlation coefficient and X and Y denote the mean values of x and y, respectively. Confidence intervals (CI) for individual regression slopes were calculated following Pitman (1939). Tests for heterogeneity of regression slopes and calculation of common slopes where homogeneity of slopes was demonstrated were carried out through re-sampling and permutation procedures (Warton and Weber 2002; Warton et al. 2006). When slopes did not differ among successional stages, differences in the y-intercept and the shift along the common slope were tested by analysis of variance (and post hoc Tukey's tests where appropriate) available in (S)MATR.

In the above procedure, cross-species regressions were fitted separately for each successional stage. In addition to cross-species regressions, we re-conducted the above regression procedure to detect whether there is a similar scaling relationship between recurrent and non-recurrent species between a particular pair of successive stages. In this case, we only analyzed S1–S2, S1–S3, S2–S3 and S3–S4 transitions due to the small number of recurrent species in the S1–S4 and S2–S4 pairs.

Results

Variance partitioning of plant traits across ecological scales

The distribution of variance of the twig cross-sectional area, total leaf area per twig, individual leaf area and leafing intensity was unbalanced across the five ecological scales. The majority of variance was among species, with very little at the successional stage and plot levels. Variance at the individual and twig levels was comparatively smaller (Table 2).

Scaling relationship of twig cross-sectional area, leaf area and leafing intensity

Twig cross-sectional area was positively correlated with both total leaf area per twig and individual leaf area in all four successional stages (Figure 1a and b). There was no difference in the regression slope between any two successional stages for both total and individual leaf areas. Since the slopes were homogeneous (not statistically different, $P = 0.26$), the common regression slope was calculated to be 0.79 (95% CIs were between 0.70 and 0.90) for total leaf area per twig. In contrast, for individual leaf area the common slope was 1.02 (with CIs between 0.91 and 1.15), because slopes were not significantly different ($P = 0.33$). The intercept for total leaf area per twig was the same (Figure 1a), while significant shifts ($P < 0.001$) in the intercept were found for individual leaf area between successional stages: S4 > S3 > S2 = S1 (Figure 1b).

Twig cross-sectional area was negatively correlated with volume-based leafing intensity. The regression slopes did not differ from –1.0 in all successional stages (Figure 1c). No difference was found in regression slope between any two successional stages. The common regression slope was –1.18 (CIs were between –1.05 and –1.34; $P = 0.95$ for slope difference test). Significant shifts of the intercept were found between successional stages ($P < 0.001$). The intercept was greatest in S1 and S2, least in S3 and S4, with no significant difference between S1 and S2, S2 and S3 or S3 and S4 (Figure 1c).

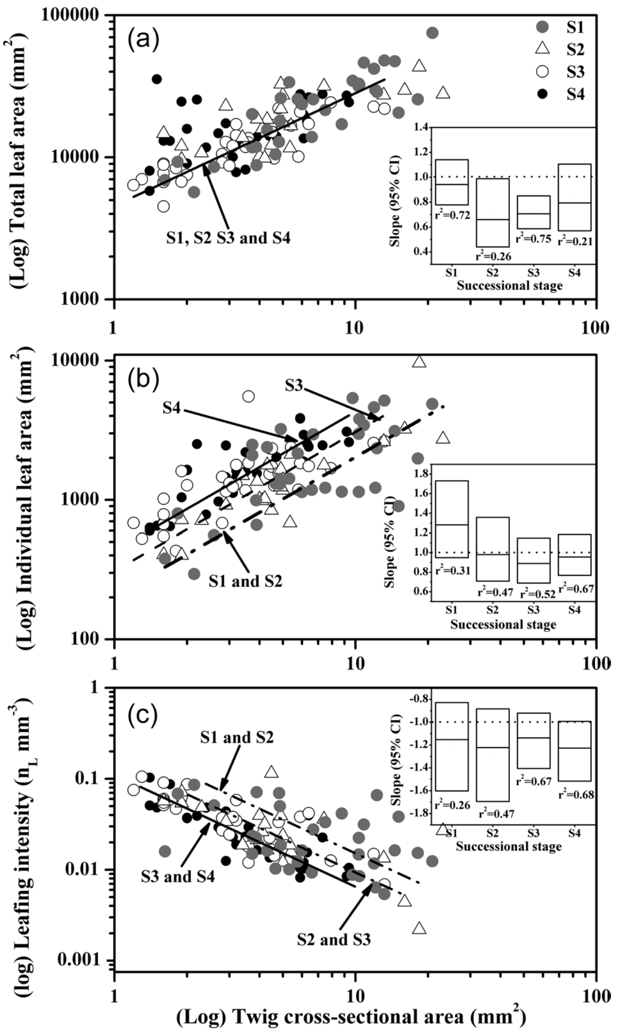

Figure 1. (a) The relationship between twig cross-sectional area and total leaf area. (b) The relationship between twig cross-sectional area and individual leaf area. (c) The relationship between twig cross-sectional area and volume-based leafing intensity. The lines are the reduced major axis regression lines. The inserted box plot in each panel shows regression slopes, 95% CIs and coefficients of determination (r^2) for each of the four relationships. The dotted line in each box plot indicates isometry position (slope = 1 or –1). The symbols in panel (a) represented the successional stages: S1, secondary shrubs; S2, young EBLF; S3, sub-climax EBLF; and S4, climax EBLF.

Differences in twig–leaf size scaling between recurrent and non-recurrent species

As twigs usually hold more than one leaf, the allometric question describes how much twig area is needed to support the leaf area in a mechanical or hydraulic context. Here, we report only the relationship between twig cross-sectional area and total leaf area. First, we compared recurrent and non-recurrent species, respectively, by splitting the data between the two stages of a pair in which they appear. In this case, we asked (i) whether the scaling relationship changes for recurrent and non-recurrent species themselves across stages and (ii) whether the change patterns of the scaling relationship across stages are consistent between recurrent and non-recurrent species. There was no difference in the regression slope within recurrent and non-recurrent species themselves and between recurrent and non-recurrent species for each of the S1–S2, S2–S3 and S3–S4 pairs. Since the slopes were homogeneous, we calculated their common regression slope (Figure 2a, c and d). In both the S1–S2 and S3–S4 pairs, no significant difference in intercept was found between recurrent and non-recurrent species and within recurrent and non-recurrent species themselves (Figure 2a and d). However, in the S2–S3 pair the intercept was greatest for non-recurrent species in S2, and least for non-recurrent species in S3 and recurrent species in S2 and S3 (Figure 2c). In contrast, for the S1–S3 pair, the regression slope was heterogeneous between non-recurrent species (1.02 and 0.68 for S1 and S3, respectively) (Figure 2b). Further, the values of the recurrent species were averaged across the pairs of successional stages, and then compared against the values of the pooled non-recurrent species in either of the two stages. Interestingly, across pairs of successional stages, patterns of the twig–leaf size scaling for recurrent and non-recurrent species were similar to the patterns calculated from the first case (see Figure S1 available as Supplementary Data at *Tree Physiology* Online).

Discussion

Variability of twig–leaf size scaling relationship among successional stages

The scaling slopes in the relationship of twig cross-sectional area to each of total leaf area, individual leaf area and leafing intensity were not different among the four successional stages. The invariant scaling slopes for the twig–leaf size and twig size-leafing intensity relationships among successional stages indicate that twig size increases with leaf size, but decreases with leafing intensity, regardless of the successional status of the forest. This is consistent with several other studies that have shown homogeneous slopes in twig–leaf size relationship among forest types along rainfall and soil nutrient availability (Westoby and Wright 2003), altitude (Sun et al. 2006; Yang et al. 2009) and moisture gradients (Ackerly and Donoghue 1998; Preston and Ackerly 2003).

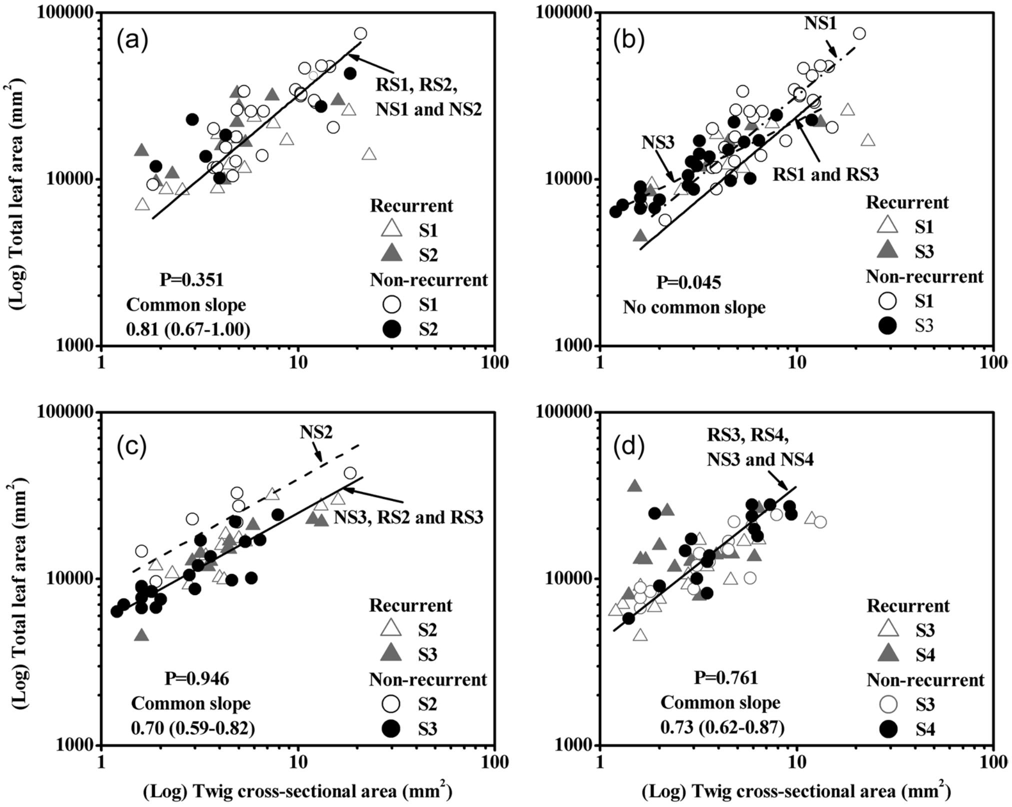

Figure 2. Scaling relationships between twig cross-sectional area and total leaf area for recurrent and non-recurrent species between pairs of successional stages. The lines are the reduced major axis regression lines. The common slope and test significance are given in each panel. See caption of Figure 1 for explanation of symbols.

The invariant scaling slopes for the twig–leaf size and twig size–leafing intensity relationships may be related to the lack of variability of twig and leaf traits at the level of successional stage. In this study, twig cross-sectional area, total and individual leaf area and leafing intensity decomposed a much greater proportion of the variance at the species level, but very little at the level of successional stage (Table 2). This means that the variability of these four traits was largely driven by species differences, instead of forest successional status. Consequently, the scaling slope for twig–leaf size and twig size-leafing intensity relationships remain constant across successional stages, even though species composition varies considerably among succession stages.

It has been shown that species with larger leaves tend to support more total leaf area per twig cross-sectional area (Preston and Ackerly 2003; Westoby and Wright 2003; Sun et al. 2006; Normand et al. 2008; Yang et al. 2009). This is represented as a scaling relationship between twig cross-sectional area and total leaf area with slope larger than 1 (see Table 1). However, this study showed that the scaling slope between twig cross-sectional area and total leaf area was a negative allometry (common slope is 0.79, the 95% CI is between 0.7 and 0.9) across successional stages. The negative allometry in twig–leaf size relationship demonstrates that the increase in total leaf area fails to keep pace with the increase in twig size. The mechanisms underlying this pattern are probably related to biomass allocation strategies of plants in the studied region. The studied region is located in the coastal area, 20 km from the East China Sea and is frequently affected by typhoons (usually 3–4 times a year). In this typhoon-prone area, plants that lose their leaves or deploy small leaves might be more likely to survive than those that strongly hold to the leaves or configure large leaves, as leaf loss or small leaves strongly reduce wind drag and breakage of branches and stems (Olson et al. 2009). Moreover, the mechanical stability of tree organs depends not only on their geometric allometry but also on wood density and associated wood strength (King et al. 2006). For example, Anten and Schieving (2010) demonstrated that, in order to achieve a certain degree of stem mechanical stability, trees with low wood density need to produce thicker stems with a lower mass per unit stem length than those with high wood density. Consequently, wood density may be also responsible for twig–leaf size allometry. It should be studied further in the future.

The negative relationship between twig size and leafing intensity results from the triangular trade-off among twig size, leaf size and leaf number. Generally speaking, leaf size is positively correlated with twig size, but is negatively correlated with leaf number (Kleiman and Aarssen 2007; Yang et al. 2008; Milla 2009). When total leaf area attached on a given twig size is made up by more small leaves, leafing intensity would be negatively correlated with twig size.

In addition to the scaling slope, the *y*-intercept in the scaling relationship of twig cross-sectional area to individual leaf area was highest in S4, intermediate in S3 and lowest in S1 and S2. This suggests that the individual leaf size being supported on a given twig size was largest for plants in late-successional, intermediate in mid-successional and smallest in early-successional forests. In contrast, the *y*-intercept in the relationship of twig size to leafing intensity was the highest in early-successional and the lowest in late-successional forests. These contrasting patterns demonstrate that, at a given twig size, the total leaf area deployed by late-successional species is caused by having fewer large leaves rather than more small leaves, while early-successional plants were made up by many small leaves, instead of fewer large leaves. Large leaves of late-successional species may be associated with microclimatic adaptations (Wright and Cannon 2001; Preston and Ackerly 2003; Westoby and Wright 2003). In our study area, soil moisture increases, but light availability decreases with forest succession (Ding and Song 1998; Yan et al. 2009). Since water evaporation demand is lower in late-successional forests, plants in those forests would require less water supply and mechanical support. Hence, plants tend to deploy larger leaves at a given twig size (Preston and Ackerly 2003). Conversely, the overall high irradiation and wind-exposed conditions in early-successional stages favor small leaves.

Variability of twig–leaf size scaling between recurrent and non-recurrent species

In this study, scaling slope in twig–leaf size relationship did not change within recurrent and non-recurrent species themselves and between recurrent and non-recurrent species for each of three adjacent successional pairs. This is consistent with the result that the scaling slope in the relationship of twig cross-sectional area to total leaf area remains constant among successional stages when recurrent and non-recurrent species are pooled together. For recurrent species, the invariant scaling slope in twig–leaf size relationship between adjacent pairs of successional stages may be related to their phenotypic plasticity. That is to say that, with phenotypic plasticity and a strong adaptability to cope with changing environmental conditions through forest succession, recurrent species also follow the twig–leaf size spectrum strategy. Specifically, the total leaf area supported on a given twig may vary correspondingly with the change of twig size for recurrent species during forest succession. Hence, recurrent species in adjacent successional stages may adjust their leaf and twig deployment patterns simultaneously. On this point, recurrent species may generate similar scaling slopes in their twig–leaf size relationship across pairs of successional stages.

The invariant scaling slope in the twig–leaf size relationship for recurrent species between adjacent pairs of successional stages also suggests a strong intra-specific variability in their twig and leaf configuration. Intraspecific variation in the twig–leaf size relationship may be structured by three main components: (i) *population-level variability*—the difference between populations of a species. Population-level variability can be due to different genotypic compositions of populations but also to the plastic response of genotypes to different environmental conditions in the adjacent successional stages (Preston and Ackerly 2003); (ii) *variability between individuals within a given population*—this can be due to the co-existence of different genotypes and also to different plasticity of these different genotypes to environmental conditions such as resource availability (e.g., water and light) or biotic interactions (e.g., competition); and (iii) *intra-individual variability*—the twig–leaf size relationship varies within individuals. This can be due to spatial heterogeneity (e.g., sun vs. shade leaves and associated twigs) and temporal variability through changes such as phenology and ontogeny of plants. Consequently, all of these factors suggest that plants' twig–leaf size relationship would differ across individuals and populations of a given species.

The comparison of recurrent and non-recurrent species in their twig–leaf size relationship between adjacent succession pairs is crucial for understanding variations in their twig and leaf size deployment while controlling phylogenetic inertia. In this study, recurrent and non-recurrent species generated similar scaling slopes in each of the pairs of successional stages. As discussed above, the mechanisms underlying this pattern are related to the intra-specific variability of recurrent species. Owing to strong phenotypic plasticity with changing environmental conditions through forest succession, recurrent and non-recurrent species in a given stage of succession may display similar patterns in their twig–leaf deployment or resource allocation strategies between twig and leaf tissues. The fact that scaling slope in twig–leaf size relationship is not different between recurrent and non-recurrent species in the adjacent successional stages suggests that the change of twig–leaf deployment pattern is largely independent of plant taxonomic groups. This might explain why scaling slope in twig–leaf size relationship stays invariant across habitats differing in water or nutrient availability (Westoby and Wright 2003; Sun et al. 2006), plant ontogeny (Ackerly and Donoghue 1998; Preston and Ackerly 2003) or taxonomic groups (Brouat et al. 1998; Normand et al. 2008).

Conclusions

The invariant slopes for twig–leaf size-leafing intensity relationships suggest that forest succession did not affect the twig–leaf deployment pattern. The significant shift of the *y*-intercept in the relationship between twig cross-sectional area and individual leaf area was most likely related to changes in environmental conditions along successional stages. For recurrent species, the invariant scaling slope in the twig–leaf size relationship between adjacent pairs of successional stages may be related to their phenotypic plasticity. Recurrent and non-recurrent species in a given stage of succession displayed similar scaling slopes in twig–leaf size relationship, suggesting that the change patterns of the twig–leaf relationship are largely independent of plants' taxonomic groups. We realize that, in this study, much of the analysis on recurrent and non-recurrent species is based on the idea that succession is composed of discrete stages. In reality, succession is continuous and also does not always follow the same trajectory. Therefore, this study provides a snapshot of the potential variability of twig–leaf size relationship in a subtropical successional series.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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