Successional leaf traits of monsoon evergreen broad-leaved forest, Southwest China

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Understanding the variation of functional traits of plant species along forest successional gradients may provides useful insights into community assemblages. However, species performance during forest succession is controversial. We explored the variation of leaf traits along a forest succession by examining ten leaf traits in four successional stages in a monsoon evergreen broad-leaved forest in Southwest China. Results showed significant differences in all leaf traits except leaf area and leaf carbon content among the successional stages. Five leaf traits were highly correlated to successional stage, while the first principal component showed no correlation with successional stage. The first principal component accounted for 56.1% of the total variation in all ten leaf traits. Almost 50% of the relationships between leaf traits differed along the examined successional gradient, indicating that leaf traits were affected by the successional stage.

Keywords: Specific Leaf Area, Leaf Chemistry, Maximum Photosynthesis, Successional Stage, Monsoon Evergreen Broadleaved Forest

Introduction

Plant traits are biological characteristics of plant species that respond to the dominant processes in an ecosystem (Lavorel et al. 1997). Plant traits are defined characteristics that relate to plant species' patterns of establishment, growth and resource allocation. This includes plant evolutionary response to abiotic conditions and interactions with other species (Clark et al. 2012, Reich et al. 2003). Therefore, plant traits strongly influence plant fitness and performance (McGill et al. 2006). Plant traits can also be used as indicators of the mechanisms by which species affect ecosystem properties and processes (Suding et al. 2008). For example, specific leaf area (SLA), one of the most widely accepted key traits, has been proven to strongly correlate with relative growth rate (Vendramini et al. 2002) and was one of the major contributors to an axis of resource capture, usage and availability. Leaf nitrogen (N_{mass}) and phosphorus (P_{mass}) concentrations can reflect plant and soil nutrient status, and influence vegetation composition (Güse-

well 2004). Leaf N_{mass} to P_{mass} ratios are determined by the uptake and loss of N and P and are useful in assessing N *versus* P limitation to primary production in terrestrial ecosystems (Han et al. 2005). Plant traits provide important information about ecological processes and improve our understanding of the mechanisms behind those processes (Garnier et al. 2004), especially forest succession (Kahmen & Poschlod 2004).

The advantage of utilizing plant traits is that different vegetation types can be directly compared with specific processes, resulting in the extraction of general trends (Díaz & Cabido 2001). This allows the prediction of vegetation dynamics by using plant trait responses (Díaz & Cabido 2001). Furthermore, plant trait responses provide insight into the mechanisms underlying successional processes and the basis for thorough experimental studies on those mechanisms involved. Trait-based studies have been used successfully to evaluate complex ecosystem responses to human disturbance occurring in grasslands

(Mládek et al. 2010) and forest ecosystems (Aubin et al. 2007).

The aim of this study is to determine how plant traits relate to successional stages and how plant traits change throughout succession. We selected the 20 most abundant plant species in four successional stages of 15, 30, 40-yr-old forest and old-growth forest (older than 80 years) of monsoon evergreen broad-leaved forests in Southwest China. Ten morphological and physiological leaf traits were used to evaluate variations and correlations between successional stages. We asked two main research questions: (1) are different leaf traits related to different successional stages? (2) If so, how do these relationships change with successional stage?

Materials and methods

Study sites

The study was conducted in a monsoon evergreen broad-leaved forest in the Taiyang river provincial nature reserve (22° 32′ – 22° 37.8′ N, 101° 2.6′ – 101° 18′ E) and the forest farm of the Vocational Education center of Pu'er (22° 43.2′ – 22° 45′ N, 100° 55.8′ – 100° 57′ E) in the Pu'er region, Yunnan Province, China. The elevation of Taiyang river provincial nature reserve ranges from 585 to 1707 m a.s.l., with a mean annual temperature (from 2000 to 2010) of 17.7 °C and mean annual rainfall (from 2000 to 2010) of 1535 mm, which occurs mainly between May and October. The elevation of the forest farm of the Vocational Education center of Pu'er ranges from 1142 to 1468 m a.s.l., with a mean annual temperature (from 2000 to 2010) of 17.7 °C and mean annual rainfall

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Tab. 1 - Environmental factors of the four successional stages. (N): soil nitrogen contents (g kg^{-1}); (P): soil phosphorus contents (g kg^{-1}); (C): soil carbon contents (g kg^{-1}). Different letters in the same row indicate significant differences after Bonferroni's *post-hoc* test (p<0.05).

Leaf traits	Successional stages			
	15-yr-old	30-yr-old	40-yr-old	Old-growth
Elevation (m)	1612 ± 2 [a]	1369 ± 5 [b]	1349 ± 5 [b]	1383 ± 123 [ab]
Slope (deg)	20.0 ± 1.2 [a]	19.7 ± 3.9 [a]	18.3 ± 4.4 [a]	18.3 ± 6.0 [a]
N (g kg^{-1})	2.07 ± 0.14 [a]	1.03 ± 0.13 [b]	1.33 ± 0.11 [bc]	1.99 ± 0.37 [ac]
P (g kg^{-1})	0.35 ± 0.00 [ab]	0.23 ± 0.02 [a]	0.30 ± 0.03 [a]	0.42 ± 0.06 [b]
C (g kg^{-1})	49.8 ± 3.9 [a]	31.2 ± 3.2 [b]	30.1 ± 3.4 [b]	45.8 ± 8.3 [ab]
pH	4.54 ± 0.06 [a]	4.82 ± 0.08 [a]	4.80 ± 0.10 [a]	5.09 ± 0.25 [a]
Soil water content (%)	24.4 ± 4.18 [a]	27.6 ± 2.94 [a]	27.2 ± 2.18 [a]	32.2 ± 0.89 [b]

(from 2000 to 2010) of 1535 mm which occurs mainly between May and October. The monsoon evergreen broad-leaved forest is the dominant vegetation type in the two study sites. However, parts of the forest in the forest farm of the Vocational Education center of Pu'er were logged in the 20th century, resulting in four successional stages: 15, 30, 40-yr-old forest and old-growth forest (>80 years). The 15-yr-old forest has a high stand density of relatively small trees. *Lithocarpus fenestratus, Helicia nilagirica, Litsea cubeba, Castanopsis echinocarpa* and *Schimawallichii* were the main canopy trees. The dominant understory species were *Canthium horridum, Dalbergia rimosa, Scleria herbecarpa* and Pteridophyta. In the 30 and 40-yr-old forest, stand density was lower compared with the 15-yr-old forest. The mean plant height was higher with numerous trees diameter at breast height (DBH) > 20 cm. The main canopy trees included *C. echinocarpa, L. fenestratus, Aporusa villosa, Machilus robusta* and *C. hystrix*. The dominant understory species were *Canthium horridum, Ficus hirta* and Pteridophyta. In the old-growth forest, the dominant tree species were *C. echinocarpa* and *C.hystrix*, with the understory containing *Ardisia maculosa, Fordia*

microphylla, S. herbecarpa and Pteridophyta. Pteridophyta and Orchidaceae grow epiphytically on stems or the forest canopy occurring frequently in all the surveyed forest types. In addition, rich buttressed trees and lianas formed an important characteristic of monsoon evergreen broad-leaved forests in the Pu'er region.

Data collection

Three 30 × 30 m plots were established in each of four successional stages, for a total of twelve plots. Each plot was located in a representative area of each successional stage with a minimum distance of 100 m from any major trail. The minimum distance between any two plots was 200 m. All plots were established on similar terrain. Each plot was divided into thirty-six 5 × 5 m subplots. All living stems >1 cm diameter (DBH) in the subplots were tagged, identified to species, numbered, and their DBH and height were recorded. In each plot we sampled ten individual trees per species taking two leaves from each individual tree. The 20 most abundant species were sampled. This resulted in more than 70% of the total individuals in each successional stage (15-yr-old forest: 72.4%; 30-yr-old forest: 83.6%; 40-yr-old forest: 83.5%;

old-growth forest: 74.2%). Five soil samples were collected from each plot and used to analyze the physical and chemical soil properties (Tab. 1). Elevation and slope of each plot were also recorded (Tab. 1).

Ten easy-to-measure leaf traits were selected to reflect the processes of forest dynamics. Three traits were related to energy (LA: leaf area; A_{mass}: maximum photosynthesis based on mass; A_{area}: maximum photosynthesis based on area), five traits were related to nutrient (N_{mass}: leaf N content based on mass; P_{mass}: leaf P content based on mass; N_{mass}: P_{mass}; N_{area}: leaf N content based on area; P_{area}: leaf P content based on area), and two traits were linked to plant growth (SLA; LCC: leaf C content). LA was measured with a portable area meter (Li-Cor 3000A®, Lincoln, NE, USA). Leaves were dried at 70 °C to a constant weight (Albayrak & Çamas 2007) and weighed (dry leaf weight, DLW) for SLA determination (SLA = LA/DLW). All leaves from the same species in the same plot were pooled for chemical analysis. N_{mass} was measured using an elemental analyzer (NCS2500®, Carlo Erba Instruments, Milan, Italy). P_{mass} was determined using inductively coupled plasma emission spectroscopy (Southwest Forestry University, China). The ratio of N_{mass}: P_{mass} was also calculated. LCC was determined using an elemental analyzer (model EA 1108®, Carlo Erba Instruments, Milan, Italy). Area- and mass-based traits can be inter-converted *via* SLA (for example, $N_{area} = N_{mass}$/SLA, $P_{area} = P_{mass}$/ SLA – Wright et al. 2004), which can be used to estimate A_{mass} and A_{area}. A_{mass} was estimated from SLA and N_{mass}, while A_{area} was estimated using SLA and N_{area}, using the multiple regression model proposed by Wright et al. (2004).

Data analysis

We first calculated the means of LA and SLA for each species in each successional stage and then analyzed these mean values using one-way analyses of variance (ANOVA). Differences in leaf traits and the physical and chemical properties of soil for each successional stage were tested with ANOVA. Further analyses using Bonferroni's *post-hoc* tests (Student-Newmans-Keuls) were performed where required. Dependent variables were log$_{10}$ transformed where necessary to conform to homoscedasticity assumptions.
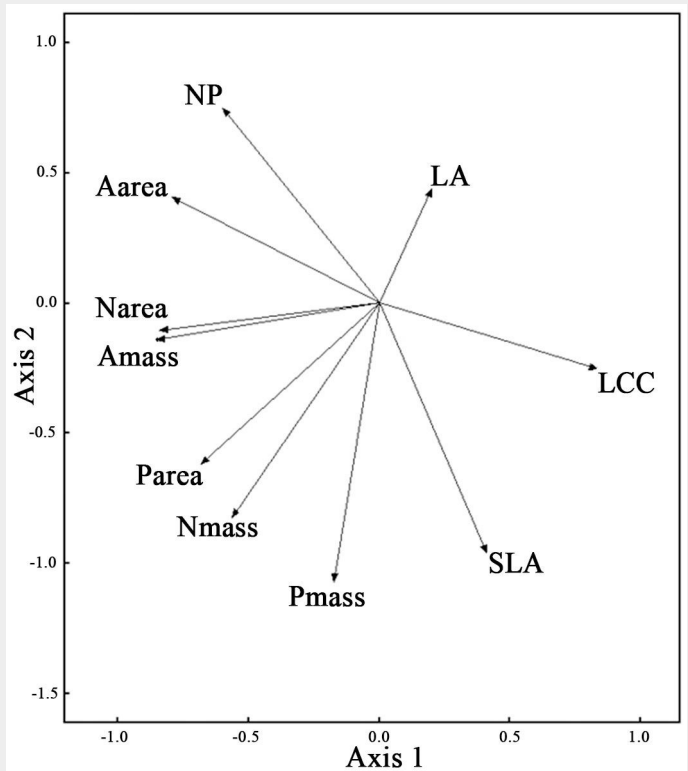
For multivariate analyses we used the indirect ordination method. Principal Components Analysis (PCA) was conducted on the leaf traits using the software PC Ord® ver. 5.02 (MJM Software Design, Gleneden Beach, OR, USA). To detect relationships between successional stage and leaf traits we applied the Spearman's correlation analysis with the leaf traits and PC1 and PC2. Regression analysis was conducted when a significant correlation occurred.

Standardized major axis (SMA) analysis was used to describe the relationship between each possible pairwise combination

Tab. 2 - Leaf traits at the four successional stages. (LA): leaf area (cm^2); (SLA): specific leaf area (m^2 kg^{-1}); (N_{mass}): leaf nitrogen contents (mass basis, g kg^{-1}); (P_{mass}): leaf phosphorus contents (mass basis, g kg^{-1}); (N_{mass}:P_{mass}): the ratio of leaf nitrogen contents (mass basis) to leaf phosphorus contents (mass basis); (LCC): leaf carbon contents (g kg^{-1}); (N_{area}): leaf nitrogen contents (area basis, g m^{-2}); (P_{area}): leaf phosphorus contents (area basis, g m^{-2}); (A_{mass}): maximum photosynthesis (mass basis, nmol g^{-1} s^{-1}); (A_{area}): maximum photosynthesis (area basis, µmol m^{-2} s^{-1}). Different letters in the same row indicate significant differences after Bonferroni's *post-hoc* test (p<0.05).

Leaf traits	Successional stages			
	15-yr-old	30-yr-old	40-yr-old	Old-growth
LA (cm^2)	40.6 ± 5.2 [a]	48.6 ± 6.3 [a]	41.5 ± 4.8 [a]	44.8 ± 4.9 [a]
SLA (m^2 kg^{-1})	21.5 ± 2.0 [a]	17.6 ± 0.9 [ab]	15.7 ± 0.6 [ab]	15.1 ± 0.7 [b]
N_{mass} (g kg^{-1})	20.5 ± 1.5 [a]	14.8 ± 0.6 [b]	14.3 ± 0.6 [b]	19.2 ± 1.1 [a]
P_{mass} (g kg^{-1})	1.64 ± 0.22 [a]	1.04 ± 0.08 [ab]	0.85 ± 0.04 [b]	1.10 ± 0.04 [b]
N_{mass}:P_{mass}	14.10 ± 0.80 [a]	15.10 ± 0.40 [a]	17.60 ± 0.60 [b]	17.80 ± 0.80 [b]
LCC (g kg^{-1})	465.10 ± 28.2 [a]	472.70 ± 37.6 [a]	465.80 ± 37.2 [a]	468.90 ± 34.3 [a]
N_{area} (g m^{-2})	1.02 ± 0.29 [ac]	0.79 ± 0.23 [b]	0.93 ± 0.17 [ab]	1.41 ± 0.67 [c]
P_{area} (g m^{-2})	0.07 ± 0.02 [ac]	0.06 ± 0.02 [ab]	0.05 ± 0.01 [b]	0.08 ± 0.03 [c]
A_{mass} (nmolg^{-1} s^{-1})	4.70 ± 0.04 [a]	4.55 ± 0.02 [bc]	4.49 ± 0.02 [b]	4.61 ± 0.11 [ac]
A_{area} (µmol m^{-2} s^{-1})	1.77 ± 0.02 [a]	1.68 ± 0.01 [b]	1.71 ± 0.01 [b]	1.81 ± 0.02 [a]

Fig. 1 - Principal Components Analysis (PCA) of the ten leaf traits. (LA): leaf area (cm²); (SLA): specific leaf area (m² kg⁻¹); (NP): the ratio of leaf nitrogen contents (mass basis) to leaf phosphorus contents (mass basis); (N_{mass}): leaf nitrogen contents (mass basis, g kg⁻¹); (P_{mass}): leaf phosphorus contents (mass basis, g kg⁻¹); (LCC): leaf carbon contents (g kg⁻¹); (N_{area}): leaf nitrogen contents (area basis, g m⁻²); (P_{area}): leaf phosphorus contents (area basis, g m⁻²); (A_{mass}): maximum photosynthesis (mass basis, nmol g⁻¹ s⁻¹); (A_{area}): maximum photosynthesis (area basis, μmol m⁻² s⁻¹).

of leaf traits. SMA has become a standard procedure in leaf trait studies where bivariate relationships are quantified between variables that are measured with error (Warton et al. 2006). Our aim was to estimate the line of best-fit describing the bivariate scatter of two traits. SMA regression is used to estimate lines with greater precision than major axis regression (Warton et al. 2006). On log-log axes, SMA regression describes the best-fit scaling relationship between pairs of traits. SMA regression analyses were performed using the free software package SMATR ver. 2.0 (Falster et al. 2006). Other statistical computations were carried out with SPSS® ver. 17.0 (SPSS Inc., Chicago, IL, USA). All tests were carried out at a significance level of α = 0.05.

Results

Leaf traits variations with successional stages

All leaf traits except two (LA and LCC) were significantly different among successional stages. As vegetation recovered, SLA decreased (Tab. 2). Conversely, N_{mass}: P_{mass} was positively associated with forest succession (Tab. 2). The 15-yr-old forest had the highest N_{mass}, A_{mass} and P_{mass}. The 40-yr-old forest had the lowest N_{mass} and P_{mass}. The N_{area}, P_{area} and A_{area} were the highest in the old-growth forest and lowest in 30-yr-old and 40-yr-old forests (Tab. 2).

Correlations between leaf traits and successional stage

The PCA of the 10 measured traits captured 90.6% of the variance in two principal

components, with the first axis accounting for 56.1% (eigenvalue = 2.929 – Fig. 1). The first axis mainly described resource use and was positively associated with LA, SLA and LCC and negatively associated with N_{area}, N_{mass}, P_{area}, A_{mass} and A_{area}. PC1 was unrelated to successional stage (Tab. 3). The second axis (accounting for 34.5% of the total variance) mainly described resource availability and was positively associated with LA, N_{mass}: P_{mass} and A_{area} and negatively associated with SLA, N_{mass}, P_{mass} and P_{area}. PC2 was unrelated to successional stage (Tab. 3).

Significant correlations were found between most leaf traits and successional stage. SLA, P_{mass} and A_{mass} were strongly

negatively correlated to successional stage (Tab. 3). In contrast, N_{mass}: P_{mass} and N_{area} were positively correlated to successional stage (Tab. 3). The best-fitting regression models of the relationships between leaf traits and the successional stage are shown in Fig. 2. However, N_{mass}, P_{area} and A_{area} were unrelated to successional stage, although they showed significant differences between different stages. Likewise, LA and LCC were uncorrelated to successional stage.

Relationship between plant traits in different successional stages

The relationship between SLA and LA,

Tab. 3 - Correlations between leaf traits and successional stage. (LA): leaf area (cm²); (SLA): specific leaf area (m² kg⁻¹); (N_{mass}): leaf nitrogen contents (mass basis, g kg⁻¹); (P_{mass}): leaf phosphorus contents (mass basis, g kg⁻¹); (N_{mass}: P_{mass}): the ratio of leaf nitrogen contents (mass basis) to leaf phosphorus contents (mass basis); (LCC): leaf carbon contents (g kg⁻¹); (N_{area}): leaf nitrogen contents (area basis, g m⁻²); (P_{area}): leaf phosphorus contents (area basis, g m⁻²); (A_{mass}): maximum photosynthesis (mass basis, nmol g⁻¹ s⁻¹); (A_{area}): maximum photosynthesis (area basis, μmol m⁻² s⁻¹).

Leaf traits	Pearson's correlation	P
LA (cm²)	0.063	0.580
SLA (m² kg⁻¹)	-0.373**	0.001
N_{mass} (g kg⁻¹)	-0.099	0.381
P_{mass} (g kg⁻¹)	-0.338**	0.002
N_{mass}: P_{mass}	0.460**	0.000
LCC (g kg⁻¹)	0.015	0.896
N_{area} (g m⁻²)	0.325**	0.003
P_{area} (g m⁻²)	0.086	0.446
A_{mass} (nmol g⁻¹ s⁻¹)	-0.244*	0.029
A_{area} (μmol m⁻² s⁻¹)	0.202	0.072
PC1	-0.673	0.327
PC2	0.704	0.248

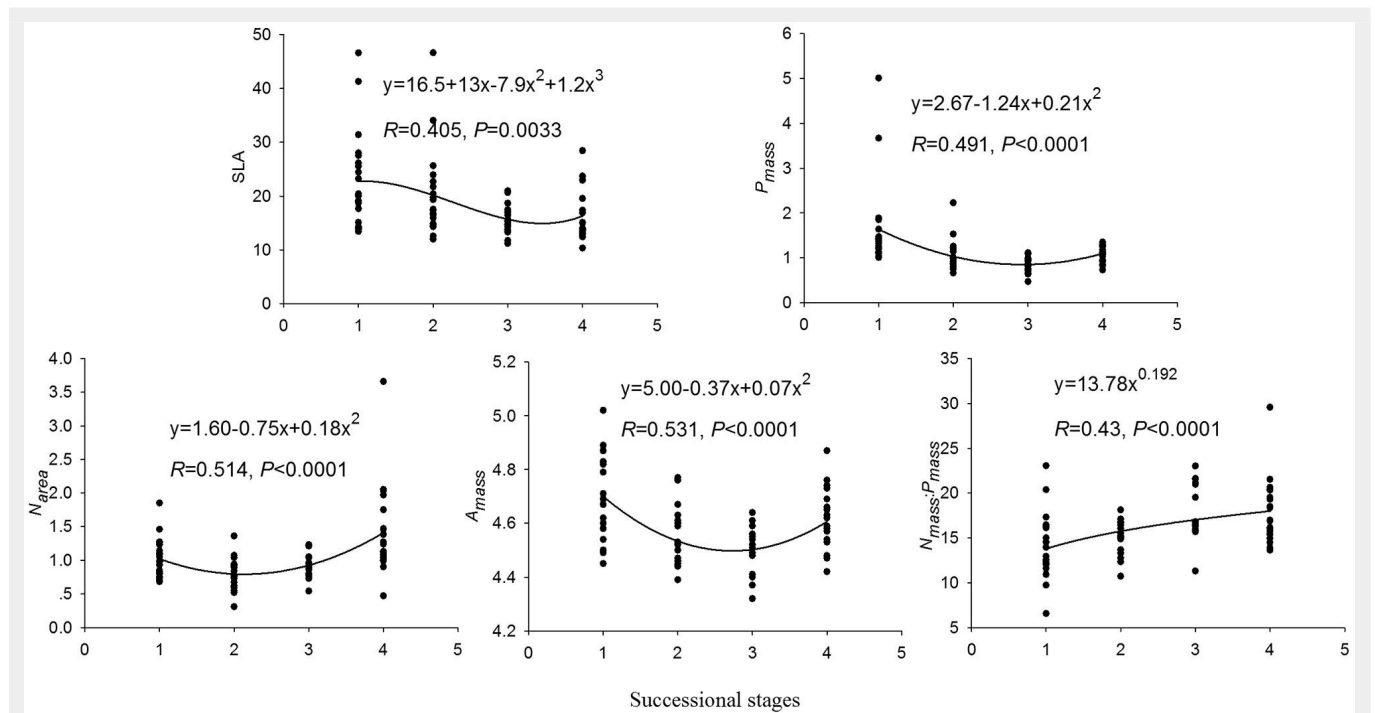**Fig. 2** - Regression analysis between successional stage and leaf traits. (SLA): specific leaf area (m² kg⁻¹); (N_{mass}): leaf nitrogen contents (mass basis, g kg⁻¹); (P_{mass}): leaf phosphorus contents (mass basis, g kg⁻¹); (N_{area}): leaf nitrogen contents (area basis, g m⁻²); (A_{mass}): maximum photosynthesis (mass basis, nmol g⁻¹ s⁻¹); successional stages (x-axis): (1) 15-yr-old; (2) 30-yr-old; (3) 40-yr-old; (4) old-growth forests (>80 years).

N_{mass}: P_{mass}, and LCC were non-significant in all successional stages except for N_{mass}: P_{mass} in the 30-yr-old forest (negative association, $P = 0.000$) and LCC in the 15-yr-old forest (negative association, $P = 0.012$). The SMA slopes for the SLA – LA, SLA – N_{mass}: P_{mass} and SLA – LCC relationships were homogeneous among all successional stages (Tab. S3 in Supplementary material). SLA was positively associated with N_{mass} in all successional stages except the 30-yr-old forest ($P = 0.100$) and with P_{mass} in all four successional stages ($P < 0.05$). The SMA slopes for the SLA – N_{mass} and SLA – P_{mass} relationships were homogeneous among successional stages. SLA was negatively associated with N_{area}, A_{mass} and A_{area} in all successional stages ($P < 0.05$). The SMA slopes for the SLA – N_{area}, SLA – A_{mass} and SLA – A_{area} relationships were heterogeneous among successional stages, the greatest SMA slopes were in the 15-yr-old forest for the SLA – N_{area} and the SLA – A_{mass} relationships and in the 40-yr-old forest for the SLA – A_{area} relationships (Tab. S3 in Supplementary material). The SMA slope for the SLA – P_{area} relationship was homogeneous among successional stages (Tab. S3).

The SMA slope for the LA – N_{mass}: P_{mass}, LA – N_{mass}, LA – P_{mass}, LA – LCC, LA – N_{area}, LA – P_{area} and LA – A_{mass} relationships were homogeneous among successional stages ($P > 0.05$), but the SMA slope for the LA – A_{area} was heterogeneous among successional stages with the highest slope in the 40-yr-old forest.

The SMA slope for the N_{mass}: P_{mass} – N_{mass} and N_{mass}: P_{mass} – P_{mass} relationships were ho-

mogeneous among successional stages ($P > 0.05$), but the SMA slope for the N_{mass}: P_{mass} – LCC, N_{mass}: P_{mass} – N_{area}, N_{mass}: P_{mass} – P_{area}, N_{mass}: P_{mass} – A_{mass} and N_{mass}: P_{mass} – A_{area} were heterogeneous among successional stages. The 15-yr-old forest had the highest SMA slope in the SLA – N_{area}. In the N_{mass}: P_{mass} – LCC, N_{mass}: P_{mass} – N_{area}, N_{mass}: P_{mass} – P_{area} and N_{mass}: P_{mass} – A_{mass} relationships, the 40-yr-old forest had the highest SMA slope in the N_{mass}: P_{mass} - A_{area} relationship (Tab. S3).

The SMA slope for the LCC – P_{mass}, LCC – N_{area}, LCC – P_{area}, LCC – A_{mass}, N_{area} – P_{area}, P_{area} – A_{mass} and P_{area} – A_{area} relationships were homogeneous among successional stages ($P > 0.05$). However, all the SMA slope for the N_{mass} – P_{mass}, N_{mass} – LCC, N_{mass} – N_{area}, N_{mass} – P_{area}, N_{mass} – A_{mass}, N_{mass} – A_{area}, LCC – A_{area}, P_{mass} – N_{area}, P_{mass} – P_{area}, P_{mass} – A_{mass}, P_{mass} – A_{area}, N_{area} – A_{mass}, N_{area} – A_{area} and A_{mass} – A_{area} relationships were heterogeneous among successional stages (Tab. S3 in Supplementary material).

Discussion

Our analysis of a chronosequence of successional stages in a monsoon evergreen broad-leaved forest showed that species with relatively high rates of photosynthesis and leaf traits related to fast growth (high SLA) were progressively replaced by species with low SLA over time. This pattern is consistent with the shift of species along the leaf economics spectrum, as documented in other primary (Caccianiga et al. 2006) and secondary successional series (Chai et al. 2015, Garnier et al. 2004, Navas et al. 2010, Reich 1995). SLA is one of the

most widely accepted key leaf traits (Hoffmann et al. 2005, Kraft et al. 2008) and is strongly linked to relative growth rate and resource use in plants (Vendramini et al. 2002). It is also a major contributor to resource capture, usage and availability (Funk & McDaniel 2010, Westoby et al. 2002). Used as a proxy for relative growth rate of a species, SLA is positively related to resource richness (Westoby 1998, Wilson & Thompson 1999). After mature trees are felled the priority of retaining resources decreases in forest gaps. Complex litter produced by early successional communities is shown to decompose more rapidly than litter produced by communities from more advanced successional stages (Garnier et al. 2004). Therefore, species with high SLA should increase in abundance as resources availability increases (Kahmen & Poschlod 2004). With vegetation succession, the fast-growing, light-demanding species are progressively replaced by slow-growing, shade-tolerant species, which tend to conserve resources more efficiently as succession proceeds. It has been shown that slow-growing, shade-tolerant species have low SLA (Funk & McDaniel 2010), which may explain why SLA was the lowest in old-growth forests.

Nitrogen and phosphorus are essential nutrients and play an important role in plant growth, development and behavior. The circulation of nitrogen and phosphorus limit most terrestrial ecosystem processes (Aerts & Chapin 2000). In this study N_{mass}, which generally co-varies with SLA (Wright et al. 2004), was higher in the initial and lat-

ter stages of succession likely due to the consequences of the relatively low nutrient availability in the intermediate stages (Liu et al. 2010). This shows that, although N_{mass} and SLA are positively correlated as part of the leaf economics spectrum, they can be partly decoupled, possibly to optimize N_{area} and A_{mass} (Wright et al. 2003). Previous studies have reported variable N patterns with succession including leaf N increases consistently with succession (Douma et al. 2012), non-significant shifts (Navas et al. 2010) and slightly negative shifts during the early stages of succession (Reich 1995). After tree felling operations, branches and leaves are usually left *in situ*. Decomposition of tree litter during the initial phase of succession has shown to result in the high concentrations of soil nutrients and thriving microbial communities (Douma et al. 2012). However, in old-growth forests, decomposition of litter generated by the forests themselves also increases soil nutrients. Unlike N_{mass}, P_{mass} tended to decrease as succession proceeded. Our results are in agreement with previous studies (Chai et al. 2016). These difference in N_{mass} and P_{mass} use demonstrates the differences in life history strategy among plants during succession, and reflects the important fundamental niche axes along which the species are differentiated (Westoby et al. 2002). The high P_{mass} indicates high photosynthetic capacity of the plant leaves and a fast-growing, light-demanding species with a high potential for successful establishment in forest gaps (Grotkopp & Rejmanek 2007).

The N:P ratio drives plant community structure and function, and is negatively related to growth rate (Elser et al. 2003). However, in the present study N_{mass}:P_{mass} increased with succession, which is in agreement with a previous study (Chai et al. 2015). Two theories could explain this phenomenon. First, species differ in their optimal N:P ratios for growth and reproduction, with species-specific homeostasis achieved by nutrient-balancing mechanisms (Kay et al. 2005, He et al. 2008). Although the study sites all are in the Pu'er region, environmental filters only determine the possible species pool within a biogeographic region. Successional stage determines species composition and different species composition alone could lead to local-scale and biome-scale differences in N:P ratios. Over 50% of species in this study were different among four successional stages (Tab. S2 in Supplementary material). Species with different biological properties have separate nutrient use strategies, resulting in different N:P ratios among four successional stages. Second, regardless of species composition, different processes drive N and P availability in different communities. P is mainly derived from weathering and leaching, which is a slow biogeochemical process and its availability in the soil reflects the physical and chemical characteristics of the local parent material. In contrast, many processes drive N availability (Aerts & Chapin 2000), and N mineralization and release is more abundant than elemental P in litter (Güsewell 2004). Large differences exist in the availability and the uptake efficiency between elemental N and P. Changes in environmental conditions and community structure affect the availability and uptake of N and P element in successional stages of evergreen broad-leaved forest (Yan et al. 2008). This likely results in different N:P ratios in different species. Moreover, although plant N and P concentrations co-vary consistently, when N and P sources differ, environmental conditions are extreme and the variability of nutrients storage by plants, a decoupling of leaf N and P appears. Based on numerous studies the threshold of foliar N:P ratios are < 14 and > 16 for N and P limitation, respectively (Güsewell & Gessner 2009, Matzek & Vitousek 2009). In this study, N_{mass}:P_{mass} ratios in the 40-yr-old and the old-growth forest were > 16, clearly indicating that P was the main limiting factor in succession. Wardle et al. (2004) found that in the later stages of forest succession, productivity declines and N:P ratios in the humic matter and fresh litter increase, suggesting that P was the limiting factor in succession.

Significant differences in leaf area and leaf carbon content were not observed, but maximum photosynthesis (mass- and area-basis) was different between the four successional stages. The changes in maximum photosynthesis with forest succession point to the replacement of fast-growing species with slower growing species, which tend to conserve internal resources more efficiently as succession proceeds (Garnier et al. 2004). In early successional stages, the fast-growing species have higher maximum photosynthesis in order to obtain higher relative growth rate and rapid biomass gain. Carbon gain is maximized by the constant renewal of the leaf area in early succession, as new leaves have high levels of photosynthetic capacity (Navas et al. 2010). Low photosynthesis values may reflect shade adaptation (Waite & Sack 2010) and relatively low nutrient availability in the intermediate successional stages (Liu et al. 2010). Conversely, in late successional stages carbon gain is maximized by the production of long-lived leaves, which have low photosynthetic capacity (Navas et al. 2010) and relatively high nutrient content.

The relationships between leaf traits in succession stages indicate that successional stage influences leaf characteristics. For SMA, over half of the regression slopes showed significant differences with forest succession. Although the same negative or positive relationship occurred for some trait pairs in all four successional stages, the SMA slope showed a significant difference with the greatest slope occurring in the old-growth or 15-yr-old forests. Although P_{mass} and A_{mass} co-vary consistently in the world-wide leaf economics spectrum (Wright et al. 2004), P_{mass} was negatively associated to A_{mass} in the 15-yr-old, and was positively associated to A_{mass} in the 30-yr-old, 40-yr-old forests and old-growth forest. Moreover, we found the relationship of 11 trait-pairs where the SMA slope showed a non-significant difference. For example, SLA was negatively associated to N_{mass}: P_{mass} in the 15-yr-old, 30-yr-old and 40-yr-old forests, but not in old-growth forest; LA were positively associated to P_{area} in the 15-yr-old, 30-yr-old and 40-yr-old forests, but not in old-growth forest. The results indicate that covariation in leaf traits is not universal and will be affected by forest succession. As succession proceeds, fast-growing species are progressively replaced by slow-growing species, which results in the differences in resource use and life history strategy and the changes in leaf trait relationships.

Conclusions

Although consistent changes of all leaf traits with succession were not observed, the mean trait values of eight out of ten leaf traits differed between the four successional stages. The SLA decreased as the vegetation recovered and N_{mass}:P_{mass} was positively associated with forest succession. Meanwhile, five leaf traits were highly correlated to successional stage. SLA, P_{mass} and A_{mass} were strongly negatively correlated to successional stage and N_{mass}:P_{mass} and N_{area} were strongly positively correlated. Additionally, the SMA analysis showed that the relationship between leaf traits changes with succession. These results show that leaf traits are affected by forest succession and one or a combination of several leaf traits can determine successional stages in the monsoon evergreen broad-leaved forest.

Acknowledgements

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Supplementary Material

Tab. S1 - Stand factors, species richness and abundance in four successional stages.

Tab. S2 - Species information in this study.

Tab. S3 - Relationships between leaf traits and successional stage.

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