Variations of leaf eco-physiological traits in relation to environmental factors during forest succession

Taotao Han[a,b], Hai Ren[a,*], Jun Wang[a], Hongfang Lu[a], Guangman Song[a,c], Robin L. Chazdon[d]

[a] Guangdong Provincial Key Laboratory of Applied Botany, CAS Engineering Laboratory for Vegetation Ecosystem Restoration on Islands and Costal Zones, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China
[b] State Key Laboratory of Urban and Regional Ecology, Chinese Academy of Sciences, Beijing 100085, China
[c] College of Horticulture and Landscape Architecture, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, China
[d] University of the Sunshine Coast, Tropical Forests and People Research Centre, Locked Bag 4, Maroochydore DC, Queensland 4558, Australia

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ABSTRACT

Changes in leaf physiological trait indicators and shifts in their relationships are expected to reveal plant ecological strategies during succession, and how they interact with the changing environment are thought to be useful in forest restoration and management. In this study, 9 physiological trait indicators, 7 leaf structural and chemical trait indicators of each dominant species across a successional series were measured in southern China; 14 environmental factors were also measured to identify which factors were most associated with leaf physiological trait indicators. Results showed that leaf photosynthesis (A_{mass} and A_{area}), respiration, transpiration (T_{mass} and T_{area}), photosynthetic N-, and P-use efficiency (PNUE and PPUE) decreased during succession, which could be explained by plant life forms. Species at a given A_{mass} had higher PPUE and specific leaf area (SLA) in later- than in early-succession. At a given SLA, leaf N content was higher in early- than in later-succession. Changes in leaf physiological trait indicators were associated with soil pH. The higher N, P assimilation efficiencies of pioneer species support their rapid growth in the relatively low N, P soils in early stage of succession. Leaf nutrient-utilization strategies, interacting with soil pH, played an important role in the succession of subtropical forest. Soil pH could be used as an indicator of the process of forest dynamic succession in south China. This study can also advance the understanding of plant survival and replacement strategies during forest succession.

1. Introduction

As important indicators of plant leaf economic spectrum, leaf physiological trait indicators are associated with plant resource capture, competitive interactions, and plant life history strategies (Raaimakers et al., 1995; Atherton et al., 2017; Fotis and Curtis, 2017). Studies of leaf physiological trait indicators across species and biomes can advance the understanding of the adaptability of plants to environmental change (Boukili and Chazdon, 2017). Differences in the relationship between leaf physiological trait indicators and leaf chemical, structural trait indicators among different environments have been widely used to identify trade-offs and functional constraints underlying key plant ecological strategies (Ackerly, 2004; Wright et al., 2005a; Li et al., 2015a; Buzzard et al., 2016; Boukili and Chazdon, 2017). Identifying what and how indicators of soil resources and conditions (water and nutrients) shape the leaf physiological traits is one of the primary questions for ecology and biogeography (Maire et al., 2015).

Understanding how leaf physiological trait indicators relate to the environmental factors is also useful for informing species selection for forest restoration projects (Arroyo-Rodríguez et al., 2017).

Leaf physiological trait indicators, such as leaf maximum photosynthetic rate, dark respiration, and transpiration, are strongly related to leaf-level CO_2 assimilation, water exchange, and light capture strategies, and are seen as critical importance in plant primary production and plant competitive abilities (Li et al., 2015b; Atherton et al., 2017; Fotis and Curtis, 2017). In general, high values of these physiological trait indicators represent acquisitive strategies for plants while low values represent conservative strategies (Wright et al., 2001; Wright et al., 2005a). According to Buzzard et al. (2016), changes in plant productivity are hypothesized to be associated with key leaf traits that are deeply embedded in the core physiology of plants. Therefore, the changes of leaf physiological trait indicators can be predicted along a productivity gradient. Previous studies have found plant ecological strategies tend to transform from resource acquisition (high

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* Corresponding author.
  E-mail address: renhai@scbg.ac.cn (H. Ren).

productivity) to resource conservation (low productivity) during secondary succession (Marino et al., 2010; Ali and Yan, 2017; Boukili and Chazdon, 2017). Thus, viewing secondary succession as a productivity gradient, the states of leaf physiological trait indicators of plants might change from acquisitive strategy to conservative strategy during secondary succession.

According to Lechowicz and Blais (1988), both resource availability in local environments and allocation strategy of plants affect the availability of resources to plants. That is, plants must trade-off between leaf physiological trait indicators and other structural and chemical trait indicators to maximize fitness during succession (Lohbeck et al., 2013). For example, from dry to wet habitats, plants maintain their comparable growth by increasing water use while reducing investments in photosynthetic proteins resulting in low leaf N (Wright et al., 2001). When assessing the physiological and structural trade-offs underlying leaf economics spectrum at a global scale, Onoda et al. (2017) showed that long leaf lifespans were achieved by higher leaf mass per area (LMA) and higher cell wall mass fraction, and as a result, the efficiency of photosynthesis decreases. Although many positive and negative relationships among leaf physiological trait indicators and other structural and chemical trait indicators have been studied on a global or regional scale (Reich et al., 1999; Wright et al., 2005b; Rozendaal et al., 2006), field experiments are needed to further explore how these relationships vary during succession at finer scales.

At finer scales, soil nutrients and microclimatic conditions (e.g. the understory light environment, soil water content and litter fall in forests) are the primary factors in determining which dominant species arrive and establish in the regenerating forests (Reddy and Singh, 1993; Meiners et al., 2015). Secondary succession on fertile soils typically promotes the early dominance of pioneer species (i.e., high resource demanding grasses with high photosynthetic and respiratory rates). On nutrient-deficient soils, C_4 bunchgrasses dominate early but with patchy colonization (Meiners et al., 2015). Along a gradient from nutrient-poor to nutrient-rich habitats, Wright et al. (2001) found that plants increasingly rely on high leaf N while reducing water use through lower transpiration rate. Soil fertility, sometimes defined by exchangeable base cations, total N and P, and soil pH (Quesada et al., 2010), exhibits strong geographic heterogeneity and can greatly affect local species compositions and the related physiological traits among different successional stages (Achat et al., 2015; Villa et al., 2018).

Monsoon evergreen broadleaved forest, the zonal vegetation of subtropical region in southern China, has faced various degree of degradation owing to long-term disturbance (Yan et al. 2006a; Yan et al. 2006b). Dinghushan National Nature Reserve of the lower subtropical region, is now composed of a mosaic of series, including typical degraded grassland, coniferous forest, mixed coniferous and broadleaved forest and monsoon evergreen broadleaved forest (Brown et al., 1995; Yan et al., 2006b; Zhao et al., 2011). To demonstrate the plant ecological strategies during succession, 9 leaf physiological trait indicators, 7 leaf structural and chemical trait indicators of each dominant species were measured in each successional stage of Dinghushan. About 14 environmental factors were also measured to illustrate the main influencing factors of these plant strategies. The hypotheses of this study were that: (i) the values of leaf physiological trait indicators related to plant light capture and competitive strategies would decrease during succession, (ii) pioneer species would have higher nutrient utilization efficiency to support their rapid growth than later successional species; and (iii) soil nutrients and soil pH will influence plant leaf physiological trait indicators during subtropical forest succession in Dinghushan.

2. Material and methods

2.1. Study site

This study was conducted at Dinghushan National Nature Reserve (Fig. 1), southern China (E 112°32′57″, N 23°9′51″), which has a monsoon climate and is located in the subtropical moist forest life zone (Holdridge, 1967). The mean annual precipitation of 1748 mm has strong rainfall seasonality, with approximately 80% precipitation falling from April to September (Lu et al., 2018). The annual mean relative humidity is about 80%. The mean annual temperature is 21.9 °C, with an average lowest temperature of 12.6 °C in January and an average highest temperature of 28.0 °C in July (Lu et al., 2013).

This nature reserve contains four successional communities, including a degraded grassland (SUC-1, about 4 years old), a coniferous forest (SUC-2, about 60 years old), a mixed coniferous and broadleaved forest (SUC-3, about 100 years old) and an old-growth monsoon evergreen broadleaved forest (SUC-4, about 400 years old), which have been the subject of long-term space-for-time succession studies (Yan et al., 2006b; Sun et al., 2013). According to the survey of the four communities in 2015, the dominant species of each community were determined as those species for which the sum of their "importance values" exceeded 75% (Peng, 1996). The dominant species in SUC-1 were mainly grasses or herbs, while in other forest stages were mainly trees and shrubs (i.e. SUC-2 to −4). (The dominant species were shown in Tables S1, S2).

2.2. Leaf sampling and analysis

Five 30 m × 30 m plots in each successional stage were surveyed during June to August each year in 2016 and 2017, respectively (the selected plots in all successional stages share similar altitude, slope, aspect.). In each plot, 50–100 fully expanded outer canopy leaves were sampled from at least two individuals per species. Individuals of some species were sampled from areas adjacent to established plots when they were absent in the plot. The sampled leaves were sealed in large polyethylene bags to maintain moisture and were transported to laboratory within 3 h. About 15–20 leaves were separated to measure leaf thickness (Lth) with a thousandth digital thickness gauge (EXPLOIT, China) and chlorophyll content per unit area (CHl) with a SPAD-502Plus chlorophyll meter (Konica Minolta, China) (Rozendaal et al., 2006). Additional leaves were used for calculating SLA (leaf area/leaf dry weight) and leaf mass per area (LMA) (leaf dry weight/ leaf area). Leaf area was determined using a LI-3000C (LI-COR), and the fresh weight was recorded (the leaf petiole was removed before measuring). These leaves were then oven-dried at 60 °C for 72 h for the determination of leaf dry weight, and were ground for determination of LN and LP. Leaf dry matter content (LDMC) was measured as the ratio of leaf dry weight to leaf fresh weight. LN and LP were measured by modified Kjejdahl method, and molybdenum anti-colorimetric method, respectively (Dong, 1997).

Leaf physiological trait indicators per unit leaf area were measured with mature leaves using a LI-COR 6400 photosynthesis system (LI-COR, USA) in the morning between 8:30–12:00 am during June to August in 2016 and 2017. The physiological trait indicators included photosynthetic rate, dark respiration rate, water use efficiency, and other traits indicated below. At least three light response curves for each dominant species of each successional stage were measured each year, respectively. The light intensity gradient was set as 1600, 1200, 1000, 800, 600, 400, 200, 100, 50, 20, or 0 umol m^{-2} s^{-1} by using a red-blue LED light source; the chamber temperature was set as 25 °C and reference CO_2 concentration was set as 400 umol mol^{-1}, which was similar to the ambient environment. Maximum net photosynthetic rate per unit of leaf area (A_{area}), maximum transpiration rate per unit of leaf area (T_{area}) and leaf dark respiration rate per unit of leaf area (R_{area}) were calculated with a light curve fitting SAS program (see more in Dubois et al., 2007). Leaf water use efficiency (WUE) was measured as the ratio of A_{area}/T_{area} (Gago et al., 2014). The physiological trait indicators per unit of leaf mass were expressed by dividing by LMA (i.e., $A_{mass} = A_{area}/LMA$; $T_{mass} = T_{area}/LMA$; $R_{mass} = R_{area}/LMA$) (Osnas et al., 2013). Photosynthetic N-use efficiency (PNUE) and photosynthetic P-use efficiency (PPUE) were determined as the ratio of $A_{mass}/$
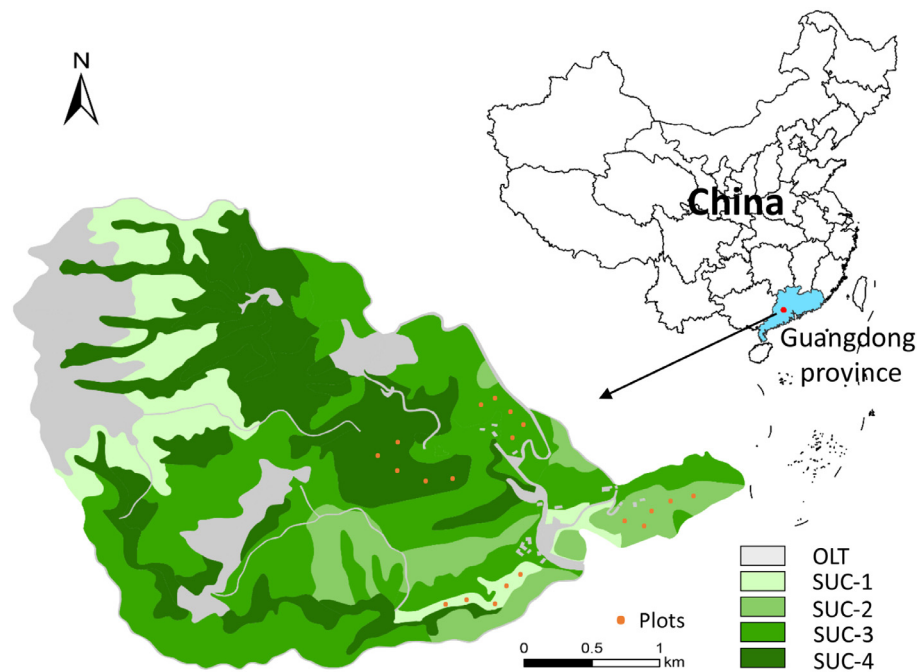
Fig. 1. Location of the Dinghushan National Nature Reserve. SUC-1, SUC-2, SUC-3, SUC-4, and OLT refer to grassland, coniferous forest, mixed coniferous broadleaved forest, monsoon evergreen broadleaved forest, and other land-use types, respectively.

LN and A_{mass}/LP, respectively (Wright et al., 2005b).

2.3. Sampling and analysis of environmental factors

Six soil cores (5 cm diameter × 20 cm deep) were randomly collected from each plot each year during June to August in 2016 and 2017, respectively. Soil samples from within each plot were mixed and sealed in polyethylene bags and transported to laboratory. The samples were passed through 2-mm sieve to remove roots and stones (Lu et al., 2018). Nitrate nitrogen (NO_3^--N) and ammoniacal nitrogen content (NH_4^+-N) of fresh soil samples were measured by ultraviolet spectrophotometry and indophenol blue method, respectively (Liu, 1996; Wang et al., 2018). The organic carbon content (OC), total nitrogen content (TN) and total phosphorus content (TP) of air-dried soil samples were measured with potassium dichromate method, modified Kjejdahl method, and molybdenum anti-colorimetric method, respectively (Liu, 1996). The pH of a 1:2.5 mixture of soil:water was measured with a glass electrode (Lu et al., 2018). In addition, the soil samples were extracted with 0.1 M $BaCl_2$, for the determination of exchangeable K^+, Na^+, Ca^{2+}, and Mg^{2+} (Ek, Ena, Eca, and Emg, respectively) with an ICP optical emission spectrometer (Perkin Elmer, USA) (Wright et al., 2001). Soil bulk density (SBD) was measured by cutting-ring method. The soil field capacity (SFC) was defined as the ratio of increased soil weight after soaking 48 h to the dry soil weight (Liu, 1996).

Litter fall was collected from five randomly selected locations in each plot with a 0.25 m × 0.25 m sample box in June-August each year, in 2016 and 2017 respectively. The litter fall included leaves, small woody material (branches and bark), and miscellaneous parts (mainly reproductive parts) (Lu et al., 2018). Litter standing crop was defined as the weight of average dry litter fall per square meter in each plot. In the plots of SUC-2 to SUC-4, leaf area index (LAI, and was considered as the indicators of understory light environment of forests) was measured on a cloudless morning (6:30–7:30am when the sun had not completely risen) with a LAI-2200C plant canopy analyzer (LI-COR, USA). Each LAI value was calculated by recording 30 points, which were distributed evenly in the 30 m × 30 m plot under the forest canopy. In each forest, 4–5 LAI measurements were taken each year, in 2016 and 2017 respectively.

2.4. Data analysis

Because most traits and environmental factors had a right skewed distribution, all data were log_{10} transformed before analysis. The data of two years for each trait indicator and each environmental factor was averaged to reduce the effect of year. One-way analysis of variance (ANOVA) with Tukey's honest significant difference (Tukey's HSD) test was performed to determine whether leaf physiological trait indicators or environmental factors changed at different successional stages. Two-way ANOVA analysis was used to distinguish the effects and contributions of plant life forms (i.e. grass, shrub and tree) and successional stages (different ages) on the variances of leaf physiological trait indicators during succession (Plourde et al., 2015; Boukili and Chazdon, 2017; Derroire et al., 2018). Because standardized major axis (SMA) regression has greater precision than major axis regression, SMA regression was used to describe and compare the relationships between leaf physiological trait indicators and leaf structure and chemical trait indicators in different successional stages (Leishman et al., 2007; Crous et al., 2017). SMA regression were performed at the species level (average value of 5 individual observations), and the significances for the slope heterogeneity and elevation heterogeneity were set at $P < 0.05$.

Multiple linear regression (stepwise) with partial regression coefficients was used to determine which environmental factors were most associated with the variances of leaf physiological trait indicators (Wright et al., 2005a; Wright et al., 2006). Statistical significance was set at $P < 0.05$. Unless otherwise stated, all data used in this study met the assumptions of statistical tests. Packages "psych", "corrgram", "multcomp", "smatr" and "ade4" in R3.4.4 were used for all statistical analyses (Torsten et al., 2008; Bougeard and Dray, 2018).

3. Results

3.1. Variation in leaf physiological trait indicators during succession

Grasses or herbs dominated in SUC-1, whereas trees and shrubs dominated in other forest stages (i.e. SUC-2 to -4) (Tables S1, S2). Most leaf physiological trait indicators of dominant species were highest in
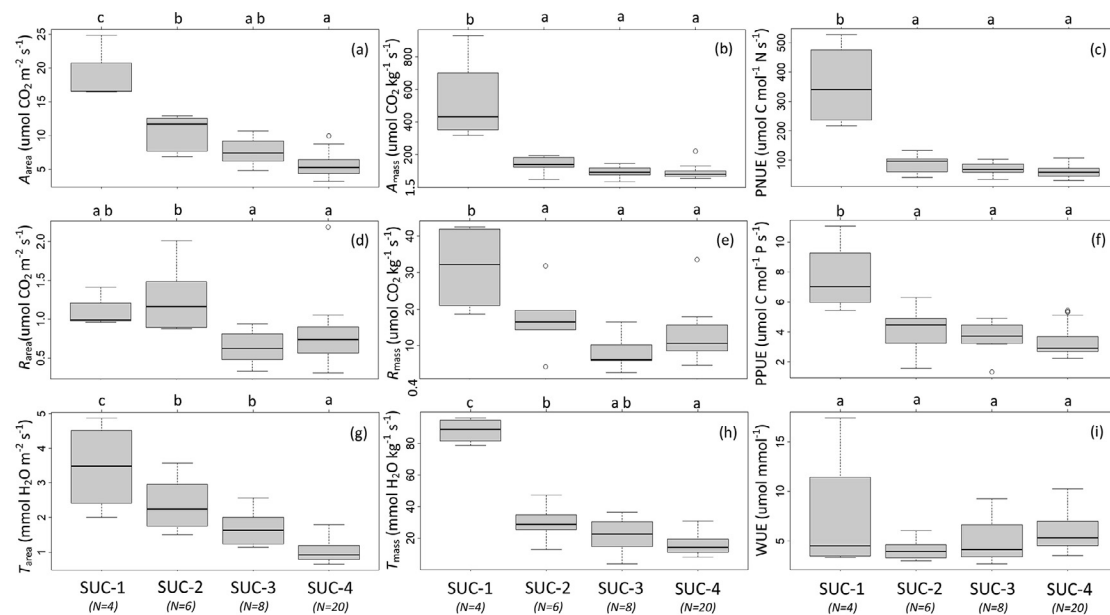
Fig. 2. Variations in different leaf physiological trait indicators during succession. The central box in each box plot shows the interquartile range and median; whiskers indicate the 10th and 90th percentiles. Different lowercase letters in each panel represent the differences among four successional stages. A_{area} and A_{mass} refer to area-based and mass-based leaf light-saturated photosynthesis respectively; R_{area} and R_{mass} refer to area-based and mass-based leaf respiration rate respectively; T_{area} and T_{mass} refer to area-based and mass-based leaf transpiration rate respectively; PNUE and PPUE refer to leaf photosynthetic phosphorus-use and nitrogen-use efficiency respectively; WUE refers to leaf water-use efficiency. SUC-1, SUC-2, SUC-3, and SUC-4 refer to grassland, coniferous forest, mixed coniferous broadleaved forest, and monsoon evergreen broadleaved forest respectively, in Dinghushan.

early succession and decreased during succession (Fig. 2). The effects of life forms and successional stages were significant for almost physiological trait indicators (Table S3). A_{mass} and A_{area} decreased during succession, with greater effects of life form than successional stages. R_{area} and R_{mass} tended to decrease with succession. T_{area} and T_{mass} decreased with succession, and the effects of life forms and successional stages were both significant. Additionally, A_{area}, A_{mass}, T_{area}, and T_{mass} were relatively higher for the grasses and herbs in SUC-1 than that for trees and shrubs in other stages (i.e. SUC-2 to -4). PNUE and PPUE tended to decrease with succession, the effect of successional stages, however, was not significant. WUE did not significantly change with succession.

3.2. Leaf trait relationships during succession

At least two successional stages, the following traits were positively related: PNUE, PPUE, and T_{mass} with A_{mass}; WUE with A_{area}; and PNUE with R_{mass} (Fig. 3; Table S4). For the PPUE-A_{mass}, the slopes of fitted lines were not significantly different, but the elevations were. At a given A_{mass}, PPUE was significantly lower in SUC-2 than in SUC-3 or SUC-4 (SUC-2 < SUC-3 = SUC-4) (Table S4). Thus, the leaves of dominant species had a higher PPUE at a given A_{mass} in later than in early succession. For the WUE-A_{area}, SMA analysis indicated that their slopes were steeper for SUC-1 and SUC-3 than for SUC-4.

A_{mass} was negatively related to LMA, but positively related to SLA across all four successional stages (Fig. 4a, e), and was negatively related to Lth in three stages (Fig. 4i). Succession did not affect the slopes of their fitted lines but affected their elevations, i.e., at a given A_{mass}, LMA was highest for SUC-2, followed by SUC-3, SUC-4, and SUC-1 (Table S4).

R_{mass} was negatively related to LMA and Lth in SUC-2 and SUC-3 (Fig. 4b, j). The elevations of fitted lines significantly differed among the two stages (i.e. LMA and Lth were higher in SUC-2 than in SUC-3 at a given R_{mass}; Table S4). R_{mass} was positively related to SLA in SUC-2 and SUC-3 (Fig. 4f); the fitted lines of them had similar slopes but different elevations (Table S4).

T_{mass} was negatively related to LMA but positively related to SLA in the three later-successional forests (Fig. 4c, g). The slopes of their fitted lines did not significantly differ but their elevations did during succession. For example, at a given T_{mass}, the LMA was higher in SUC-2 and SUC-3 than in SUC-4 (Table S4).

PPUE was negatively related to LMA but positively related to SLA in SUC-2 and SUC-3(Fig. 4d, h). There were no significant differences of slopes and elevations of their fitted lines in SUC-2 and SUC-3 (Table S4).

3.3. Major environmental factors associated with variation of physiological trait indicators

Soil NO_3^--N was negatively related to A_{area} and explained 68.5% of its total variation (Table 1). Soil pH was positively related to A_{mass}, PNUE, and PPUE, and explained 64.5%, 66.0%, and 33.3% of their variations, respectively. In addition, some of variations in R_{mass}, T_{area}, and T_{mass} were explained by more than one environmental factor (Table 1). R_{mass}, T_{area} and T_{mass} were all positively related to soil pH, which explained more variation than other environmental factors (Table 1).

4. Discussion

4.1. Changes in leaf physiological trait indicators during succession

Results showed that most leaf physiological trait indicators of the dominant species varied between successional stages in South China. Their variances can be largely explained by plant life forms. Leaf photosynthesis and respiration of dominant species were relatively higher for the grasses and herbs in early-successional stage than that for trees and shrubs in some later-successional stages. Which was consistent with the study of Zhu et al. (2013) in south China. In assessing the generality of global leaf trait indictator relationships, Wright et al. (2004) also reported that grasses and herbs had higher A_{mass}, A_{area}, and R_{mass} than trees and shrubs. This is reasonable because the high
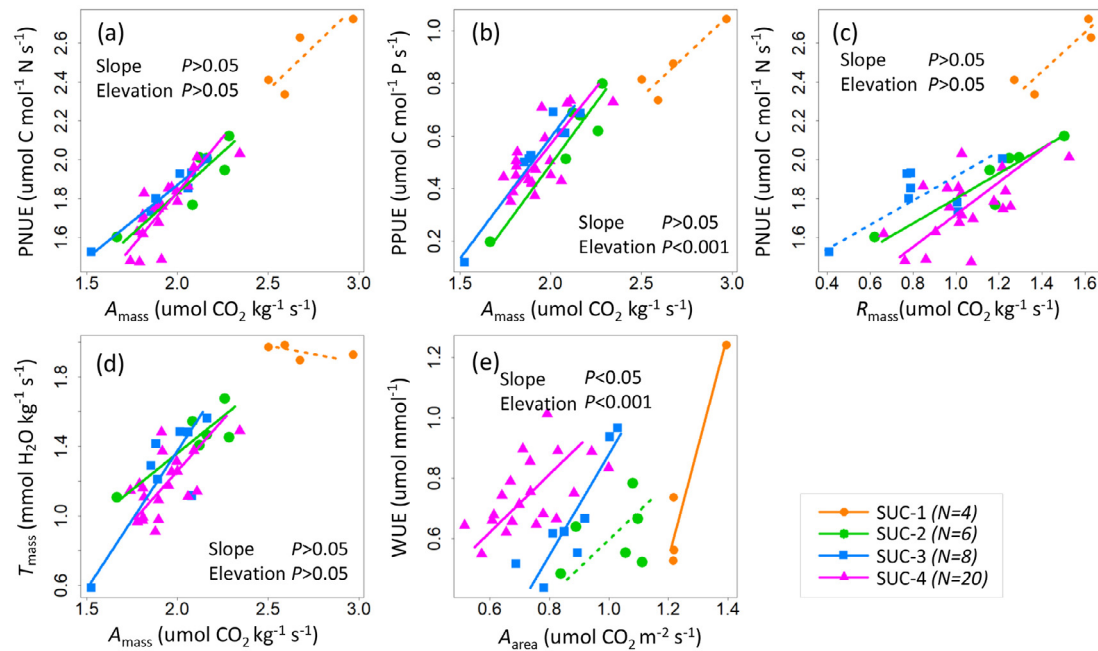
Fig. 3. Variations in relationships among leaf physiological trait indicators during succession. Solid lines and broken lines indicate the significant and non-significant fitting curves, respectively. A_{area} and A_{mass} refer to area-based and mass-based leaf light-saturated photosynthesis respectively; PNUE and PPUE refer to leaf photosynthetic nitrogen-use and phosphorus-use efficiency respectively; T_{mass} refer to mass-based leaf transpiration rate; WUE refers to leaf water-use efficiency; R_{mass} refers to mass-based leaf respiration rate. SUC-1, SUC-2, SUC-3, and SUC-4 refer to grassland, coniferous forest, mixed coniferous-broadleaved forest, and monsoon evergreen broadleaved forest, respectively.
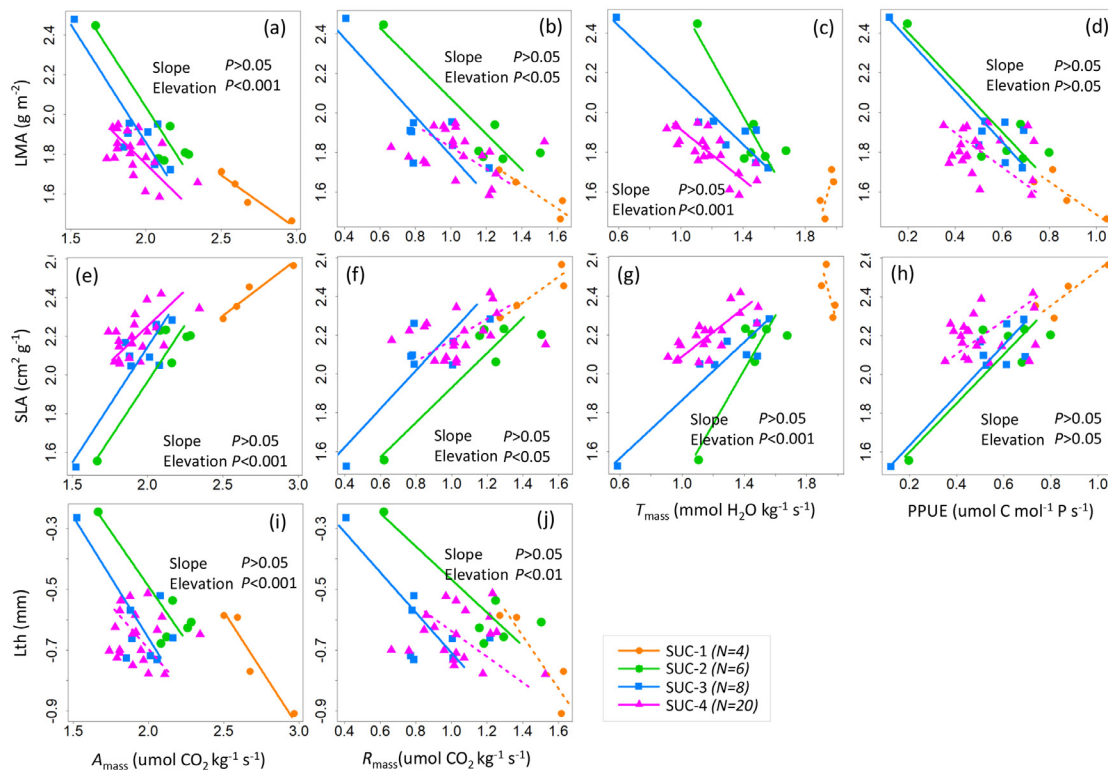

Fig. 4. Variations in relationships between leaf physiological and structural trait indicators during succession. Solid lines and broken lines indicate the significant and non-significant fitting curves, respectively. LMA, SLA, and Lth refer to leaf mass per area, specific leaf area, and leaf thickness, respectively; A_{mass}, R_{mass}, and T_{mass} refer to mass-based leaf light-saturated photosynthesis, respiration rate, and transpiration rate, respectively; PPUE refers to leaf photosynthetic phosphorus-use efficiency. SUC-1, SUC-2, SUC-3, and SUC-4 refer to grassland, coniferous forest, mixed coniferous-broadleaved forest, and monsoon evergreen broadleaved forest, respectively.

Table 1
Equations of multiple regression analysis (stepwise regression) expressing leaf physiological trait indicators as a function of environment factors across all the dominant species in four successions.

Equation ($N = 38$)	R^2 (adj)	β_1	β_2	β_3
Log A_{area} = -0.60 log (NO_3^--N) + 1.13	0.685			
Log A_{mass} = 6.34 log pH – 1.75	0.645			
Log R_{area} = -1.28 log SFC + 1.72	0.250			
Log R_{mass} = 8.85 log pH – 3.07 log TP + 1.13 log Emg – 4 0.61	0.350	1.19	-1.23	0.85
Log T_{area} = 5.49 log pH – 0.68 log Emg – 3.89	0.691	0.88	-0.61	
Log T_{mass} = 7.03 log pH – 0.34 log Emg – 3.26	0.572	0.84	-0.23	
Log PNUE = 6.29 log pH – 1.88	0.660			
Log PPUE = 2.95 log pH – 1.19	0.333			
Log WUE = 0.27 log Emg + 1.00	0.082			

Notes: All equations shown here were significant ($P < 0.05$). Relative contributions of more than two environment variables can be ascertained from standardized partial regression coefficients (β_1, β_2 and β_3). Units: area-based leaf light-saturated photosynthesis (A_{area}; umol m^{-2} s^{-1}); mass-based leaf light-saturated photosynthesis (A_{mass}; umol kg^{-1} s^{-1}); area-based leaf respiration rate (R_{area}; umol m^{-2} s^{-1}); mass-based leaf respiration rate (R_{mass}; umol kg^{-1} s^{-1}); area-based leaf transpiration rate (T_{area}; mmol m^{-2} s^{-1}) and mass-based leaf transpiration rate (T_{mass}; mmol kg^{-1} s^{-1}); leaf photosynthetic nitrogen use efficiency (PNUE; umol C mol^{-1}N s^{-1}); leaf photosynthetic phosphorus use efficiency (PPUE; umol C mol^{-1}P s^{-1}); leaf water use efficiency (WUE; umol mmol^{-1}); soil nitrate nitrogen content (NO_3^--N; mg kg^{-1}); soil pH (pH); Soil field capacity of 0–20 cm (SFC; %); soil total P content (TP; g kg^{-1}); soil exchangeable Mg content (Emg; cmol kg^{-1}).

productivity and fertility in early-successional stages are often associated with high photosynthetic, respiration rates, etc. (Swanson et al., 2011). Consistent with other studies, this result also indicated that species leaf trait indicators gradually shifted from favoring resource acquisition to favoring resource conservation (Table 2) (Guariguata and Ostertag, 2001).

The photosynthetic nutrient-use efficiencies (PNUE and PPUE) were also higher in early- than in later- successional stages. Similar results were also reported by Wright et al. (2004). TN was lower in early- than in later- succession (Fig. S1), which was also confirmed in Dinghushan by Liu et al. (2010). Given the finding that PNUE was higher in the grass community and that T_{mass} was higher in pioneer forest at a given LN (Fig. S2) in this study, pioneer species (e.g., grasses and pine species) might have higher N assimilation efficiency and allocate more N to photosynthetic tissues than non-photosynthetic tissues to ensure their productivity in the relative low N environment of early succession. In fact, the trade-off of N partitioning between photosynthetic and non-photosynthetic structures in different environments has been reported by many studies (Hikosaka and Hirose, 2000; Hikosaka, 2004). This result might be one indirectly evidence for such hypothesis (Table 2).

4.2. Variation in leaf trait relationships during succession

Adult leaf photosynthesis is highly associated with other physiological trait indicators including leaf dark respiration, transpiration, and photosynthetic nutrient-use efficiency (Reich et al., 1998; Wright et al., 2005a). However, it is not clear whether their relationships change during succession. In current study, A_{mass} was positively related to PNUE, PPUE, and T_{mass} in the forests (SUC-2 to -4); although these relationships were not significant in the grassland (SUC-1), they exhibited similar trends. One interesting finding was that significant differences of the A_{mass}-PNUE and A_{mass}-T_{mass} were not found among the three successional forests (SUC-2 to –4), but the A_{mass}-PPUE differed between SUC-2 and SUC-3 to SUC-4. This indicated that, at a given A_{mass}, PPUE of the dominant species was higher in mature forests than in pioneer forest. Because TP was higher in SUC-4 than in SUC-2, while PPUE did not differ between them (Fig. 2, S1), these results were inconsistent with the hypothesis that PPUE increases following the decrease of total soil P (Hidaka and Kitayama, 2009). If the increased P was mostly stored in vacuoles or was largely allocated to lipids, PPUE will decrease with increasing LP. Conversely, in P-poor soils, a higher PPUE could be achieved by increasing that fraction of LP allocated to photosynthetic P rather than to structural and storage P. In this study, however, LP was not different between SUC-2 and SUC-4, while PPUE was higher in SUC-4 than SUC-2 at a given A_{mass} (Fig. 2, S1). Which indicated that the pioneer species might have higher P assimilation efficiency and allocate more P to non-photosynthetic P tissues to ensure their higher productivity (Table 2). Additional research is needed on the partitioning of P during succession.

In this study, most physiological trait indicators (i.e. A_{mass}, T_{mass}, R_{mass} and PPUE) showed negative relationships with LMA and positive relationships with SLA (although some were not statistically significant). Such patterns were consistent with many previous studies (e.g. Wright et al., 2005b; Bahar et al., 2017; Crous et al., 2017). The elevation of the fitted lines of A_{mass}-LMA was found decreased with succession, i.e., at a given A_{mass}, the LMA was lowest in SUC-1 but higher in other forest stages. Wright et al. (2004) found that LMA was lower in grasses than shrubs or trees at a given rate of photosynthesis. Onoda et al. (2017) also showed that plant species of lower LMA tend to have high PNUE and A_{mass} to support their fast growth. The results of current study might indicate that the pioneer species allocate more in non-structural tissues to support their fast growth than later-successional species. A possible explanation was related to the trade-off between size-number of living leaf cells and cell wall thickness (Shipley et al., 2006). Onoda et al. (2017) also showed that larger fraction of leaf mass in cell walls was associated with lower fraction of LN in photosynthetic tissues. This trade-off could generate the broad interspecific coordination between A_{mass} and LMA or LN (Table 2).

Table 2
Summary results of this study and the related hypotheses.

Early succession	Later succession	Results indicated	Hypotheses related/matched
High value of most leaf physiological trait indicators	Low value of most leaf physiological trait indicators	The values of leaf physiological trait indicators gradually decrease during succession, which can be explained by plant life forms.	Plant species gradually transform form resource acquisition to resource conservation (Guariguata and Ostertag, 2001).
High PNUE and low PPUE, while low soil total N and P	Low PNUE and high PPUE, while high soil total N and P	Pioneers might have high N, P assimilation efficiency than later-successional species.	There are trade-offs of N, P partitioning between plant photosynthetic and non-photosynthetic tissue in resource-limited environments (Hikosaka, 2004; Hidaka and Kitayama, 2009; Onoda et al., 2017).
High SLA and low LMA at a given A_{mass}	Low SLA and high LMA at a given A_{mass}	Pioneers might allocate more in non-structural tissues to support their fast growth than later-successional species.	Plant have the trade-off between size-number of living leaf cells and cell wall thickness (Shipley et al., 2006).
High leaf physiological trait indicator s and high soil pH	Low leaf physiological trait indicators and low soil pH	Soil pH could explain much of the variations of most leaf physiological trait indicators.	Plant allelopathy play a role in forest succession (Zhao et al., 2011).

Notes: PNUE and PPUE refer to leaf photosynthetic nitrogen and phosphorus use efficiency, respectively; SLA and LMA refer to leaf specific area and leaf mass per area, respectively; A_{mass} refers to mass-based leaf light-saturated photosynthesis.

4.3. Environmental factors associated with variations in physiological trait indicators

Soil NO_3^--N and pH have larger contributions on the leaf photosynthesis than other environmental factors according to the regression equations (Table 1). The leaves of dominant species had a low photosynthetic rate in the high NO_3^--N successional stage (SUC-4) but had a high photosynthetic rate in the high pH successional stage (SUC-1). The low pH in SUC-4 might have reduced the absorption efficiency of the roots, leading to reduced production. The soil P has previously been reported important at finer scale (Liu et al., 2010; Liu et al., 2014; Huang et al., 2016). When assessing the relationships among leaf trait indicators, climate, and soil nutrients, P was also found to be very important at a global scale (Ordoñez et al., 2009). Soil pH and Emg explained much of the variation of most physiological trait indicators (i.e. transpiration, nutrient-use efficiency, and water-use efficiency). According to the results that the soil pH gradually decreased during succession in this study (Fig. S3), soil pH might affect most physiological processes by influencing nutrient absorption, thus affecting the species composition in different successional stages. Which is consistent with the hypothesis of Jung and Chang (2013). The effect of soil pH on leaf physiological trait indicators should further be studied in the future. This study, in some extent, shows that soil pH can be an indicator of the process of forest dynamic during forest succession.

Zhao et al. (2011) has reported that the sensitivity of pioneer species to abscisic acid (ABA) reduced their competitive ability with the later-successional species, thus resulting in different species composition during succession in Dinghushan (Table 2). This was consistent with current results that soil pH was one of the main drivers on forest succession in this region. It is well known that multiple deterministic and stochastic processes determine forest succession, the detailed mechanism still remains elusive. For example, soil pH could be one factor affecting species replacement during succession in the subtropical region, how the biotic and abiotic factors interact remains unclear. This study, however, provides important initial research on the important association between soil pH and leaf physiological trait indicators, which may be an indirectly evidence for the importance of plant allelopathy (Zhao et al., 2011) during succession, and also highlights the needs for more precise research on their relationships of this region in the future.

5. Conclusions

This study indicated that most leaf physiological trait indicators (i.e., plant photosynthesis, transpiration, respiration and photosynthetic nutrient-use efficiencies) of dominant species decreased during succession and they can be largely explained by plant life forms, indicating that the ecological strategies of dominant species changed from resource acquisition to resource conservation during succession. The relationships among most leaf physiological trait indicators and other structural and chemical trait indicators varied during succession, indicating different trade-off strategies of the dominant species across succession. High N, P assimilation efficiency of the pioneer species could support their rapid growth in the relative low N, P early-successional stages. Among the environmental factors, soil pH was strongly associated with most physiological trait indicators. Leaf nutrient-utilization strategies, interacting with soil pH, played an important role in the succession of subtropical forest. The effect of soil pH on leaf physiological processes can be one indicator for the variation of forest dynamic during succession. Additional studies are needed to clarify the partitioning of leaf N, and P of the dominant species and its interaction with soil pH during succession in the lower subtropical region of China.

CRediT authorship contribution statement

Taotao Han and Hai Ren conceived and designed this experiment.

Taotao Han, Jun Wang, and Guangman Song performed the experiments and collected the data. Taotao Han wrote the paper. Hai Ren, Hongfang Lu, Jun Wang and Robin L. Chazdon reviewed and edited the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106511.

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