

Experimental evidence for weakened tree nutrient use and resorption efficiencies under severe drought in a subtropical monsoon forest

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Abstract

Aims The functions of global forests are threatened by the increasing frequency of severe drought. Due to drought inducing reductions in soil nutrient availability, efficiencies of nutrient use and resorption of trees become crucial for forest functions and biogeochemical cycles. However, understanding the dynamics of responses of foliar nutrient use and resorption efficiencies to drought, especially in tropical or subtropical forests, is still limited. Our goal was to detect whether and how the importance of leaf nutrient use and resorption changes across different species in the hot and wet forests when suffering drought stress in different months.

Methods Based on a 70% throughfall exclusion experiment in a subtropical forest, we collected green and senesced leaves of *Schima superba* and *Lithocarpus glaber* in different months from October 2016 to May 2019, to estimate the effects of drought on leaf nitrogen (N) and phosphorus (P) use and resorption efficiencies (i.e. NUE and PUE, NRE and PRE).

Important Findings The effects of drought on nutrient use and resorption efficiencies varied between species and months. Based on a 2-year observation, drought had no effect on *S. superba*, but significantly decreased NUE, NRE and PRE of *L. glaber* by 3.4%, 20.2% and 7.1%, respectively. Furthermore, the negative drought effects were aggravated by the natural summer drying in 2017. As a result, NUE and PUE of *L. glaber* were significantly depressed by 17.2% and 58.1%, while NRE and PRE were significantly reduced by 56.5% and 53.8% in August 2017. Moreover, the responses of NRE, PRE and NUE to drought were related with soil moisture (SM) for *L. glaber*, and when SM decreased to a threshold near 9 v/v%, drought effects were shifted from unresponsive to negative. Our results highlight a species-specific threshold response of nutrient use under drought in a subtropical forest.

Keywords: drought, nitrogen, nutrient resorption efficiency, nutrient use efficiency, phosphorus, subtropical forest

摘要: 日益频发的干旱严重威胁着全球森林生态系统的功能。由于干旱胁迫抑制了土壤养分可利用性,因此植物的养分利用和重吸收效率对森林生态系统的功能以及生物地球化学循环至关重要。然而,目前对于植物养分利用和重吸收效率在干旱条件下的动态响应规律的理解十分有限,且在(亚)热带区域尤为明显。因此,本研究主要探讨了在湿热森林中不同植物物种在不同月份遭受干旱胁迫时,其养分利用和重吸收效率的重要性是否会发生变化以及如何变化。本研究在2016年10月–2019年5月期间,依托一处位于亚热带常绿阔叶林的隔离70%自然穿透雨的干旱实验平台,采集了木荷(*Schima superba*)和石栎(*Lithocarpus glaber*)两个物种在不同月份的鲜叶及凋落叶,用于分析干旱对两种植物叶片氮磷利用和重吸收效率的影响(NUE和PUE, NRE和PRE)。研究结果表明,干旱对氮磷利用和重吸收效率的作用在不同植物物种和月份之间有差异。基于两年的观测结果显示,干旱对木荷的养分利用和重吸收效率无显著影响,却使石栎的NUE、NRE和PRE分别降低了3.4%、20.2%和7.1%。另外,2017年夏季发生的自然干旱进一步加剧了干旱对石栎养分重吸收的负作用。在2017年8月,石栎的NUE和PUE在干旱处理下分别降低了17.2%和58.1%,而NRE和PRE分别下降了56.5%和53.8%。此外,石栎的NRE, PRE和NUE对干旱的响应显著依赖于土壤水

分条件, 即当土壤湿度降至约9 v/v%时, 存在一个阈值使干旱处理的效果从无影响转变为负作用。我们的结果表明, 在干旱条件下亚热带常绿阔叶林树木的养分利用呈现出了物种特定的阈值响应。

关键词: 干旱, 氮, 养分重吸收效率, 养分利用效率, 磷, 亚热带森林

INTRODUCTION

It is predicted that global climate changes will increase the risk of drought through decreasing precipitation or enhancing evaporation under warming (Dai 2013; IPCC 2013). By limiting water availability and water functions on the physiological processes of plants, drought may influence plant growth and ecosystem functions, e.g. primary production (da Costa *et al.* 2010; Huang and Xia 2019) and nutrient cycling (Schlesinger *et al.* 2016). Besides, the reduced supply of nutrients under drought could conversely aggravate the drought effects on leaf photosynthesis and stomatal closure, as nutrients are major limiting factors on forest productivity (Gessler *et al.* 2017). Thus, understanding how plant nutrient use responds to drought will improve our predictions of ecosystem functions in the changing future.

Plant nutrient use is characterized by nutrient use efficiency (the amount of organic matter produced per unit nutrient) and resorption efficiency (the percentage of nutrient pool resorbed from senesced tissues) (Aerts 1996; Chapin 1980). Higher nutrient use efficiencies indicate a greater ability of plants utilizing nutrients in fixing carbon (Vitousek 1982). Greater nutrient resorption efficiencies can make plants less dependent on soil nutrients (Aerts 1996; Killingbeck 1996). On average, more than 45% of foliar nitrogen (N) and phosphorus (P) are resorbed before leaf abscission (Yuan and Chen 2009), which are even up to 88% and 91%, respectively, for individual species (Freschet *et al.* 2010). As an available nutrient pool for plant growth, the resorbed nutrients account for 31% and 40%, respectively, of global annual plant N and P acquisitions (Cleveland *et al.* 2013). Therefore, higher nutrient resorption efficiencies suggest a stronger nutrient conservation strategy of plants, especially under environmental stresses (Aerts 1996; Killingbeck 1996). On the other hand, nutrient resorption can regulate the rates of soil mineralization and decomposition via adjusting chemical compositions in leaf litters, which may subsequently influence soil nutrient supplies for plant growth (Deng *et al.* 2018; Hättenschwiler *et al.* 2011). Through these critical pathways in nutrient cycling, nutrient use plays an important role in regulating ecosystem functions and biogeochemical cycles.

As water availability decreases, uptake of soil nutrients by plant roots is depressed due to the constrained rate of soil mineralization and reduced nutrient diffusion in soil (Chapin 1991; Fierer and Schimel 2002). Meanwhile, nutrients resorption from senesced leaves may also be limited, leading to more nutrient loss compared with normally defoliated leaves (Marchin *et al.* 2010; Pugnaire and Chapin 1992). Then, a trade-off between decreased N resorption and stimulated litter N mineralization is hypothesized in the responses of nutrient cycles to drought (Deng *et al.* 2018). However, drought has shown diverse effects on leaf nutrient use among different species. For example, species characterized by deep roots is less lost in leaf nutrients than shallow-root species under drought stress (Luo *et al.* 2018). Besides, nutrient resorption may be unresponsive when plants possess adaptations to drought or decrease nutrient requirements by slowdown plant growth (Sardans *et al.* 2008). In addition, the impacts of drought on nutrient resorption efficiency have been observed to depend on time (Khasanova *et al.* 2013). Thus, understanding the drought effects on nutrient use between species and time is crucial.

Subtropical evergreen broadleaved forest in East Asia is a large carbon sink region (i.e. total net ecosystem productivity of 0.72 ± 0.08

Pg C yr^{-1} over an area of 197 Mha), which benefits from the monsoon climate of synchronous water and heat (Cui *et al.* 2019; Yu *et al.* 2014). However, this region has not only undergone long-term aridity under warming and decreasing precipitation (Dai 2013; Xu *et al.* 2015), but also experienced seasonal drought for the changed distribution of intra-annual precipitation (Leng *et al.* 2015; Zhang and Zhou 2015), which are threatening the function and nutrient cycling of subtropical ecosystems. However, the knowledge of drought effects on nutrient use is mainly from dry regions (e.g. the Mediterranean, semiarid grassland). It is less known whether and how the importance of nutrient use changes in the hot and wet forests when suffering drought stress. Additionally, natural environments are temporally dynamic. How drought effects on plant nutrient use change in different months is rarely assessed. Here, we examined the impacts of drought on nutrient (N and P) use and resorption efficiencies of two dominant tree species in a subtropical forest (*Schima superba* and *Lithocarpus glaber*) with a 2-year observation. The objectives of this study were: (i) to quantify the dynamics of foliar nutrient concentrations and use; (ii) to assess drought effects on plant nutrient use and resorption efficiencies; (iii) to analyze main factors in mediating nutrient use under drought stress in this forest.

MATERIALS AND METHODS

Study site

This study was conducted in a monsoon evergreen broadleaved forest of eastern China, locating at Tiantong Forest Ecosystem Observation and Research Station ($29^{\circ}48' \text{ N}$, $121^{\circ}47' \text{ E}$, 160 m a.s.l.), Zhejiang Province, China. This region presents a typical subtropical monsoon climate. Mean annual temperature (MAT) is 16.2°C , ranging from 4.2°C in January to 28.1°C in July. Mean annual precipitation is 1374.7 mm (Song 2013). Based on a 15-year climatic record (2004–18), MAT had no significant change (Fig. 1a). However, annual and summer rainfall significantly decreased at a rate of 34.2 and 27.9 mm yr^{-1} , respectively (Fig. 1b). The stand was harvested in the 1960s and has undergone reforestation. Dominant species in the forest canopy include *L. glaber*, *S. superba* and *Castanopsis fargesii*. Total soil N, total soil P and soil pH in the top 20 cm are 0.3 mg g^{-1} , 0.2 mg g^{-1} and 4.0, respectively (Maitra *et al.* 2019).

Experimental design

A platform of throughfall exclusion experiment was conducted by a completely randomized design with three treatments (control, drought and disturbance) replicated three times in July 2013 (Supplementary Fig. S1). A total of nine 25 m \times 25 m plots were established, with at least 5 m buffer zones around each plot. To restrict horizontal water flow and lateral root growth between the transitional zones and plots, each plot was surrounded with PVC boards, which were 2 m deep in the soil. Drought treatment was carried out by fixing transparent concave polycarbonate plates on a steel frame at 1.5–3.5 m height to intercept rainfall. Each plate was 0.25 m wide and 2 m long. The spacing between plates was about 10 cm. Throughfall was consequently decreased by about 70% in the drought-treated plots every year. To estimate the effects of polycarbonate plates on surface micrometeorological conditions in

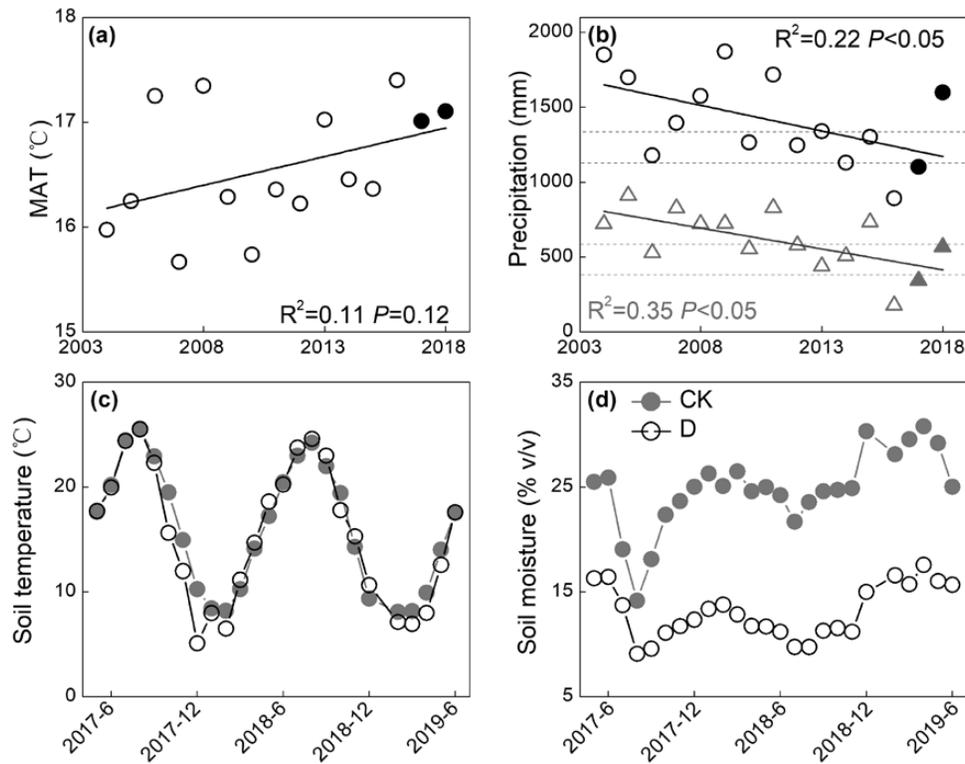


Figure 1: Changes of MAT (a, MAT, °C), annual (circle) and summer (triangle) precipitation (b, mm) from 2004 to 2018 in this study site, and monthly dynamics of soil temperature (c, °C) and moisture (d, %) under control (CK) and drought treatments (D) from May 2017 to May 2019. In panel (b), dashed lines indicate the 5th and 50th percentile (from bottom to top) of the probability distribution functions of annual (dark gray) and summer (light gray) precipitation (2004–18).

the drought, the steel frame with convex polycarbonate plates that are the same size and spacing as the concave plates was settled as the disturbance. Three automatic meteorological data collectors (CR1000, Campbell Scientific Inc., USA) were used to measure and record soil temperature (ST) and moisture at 5 cm depth every 30 s. Five 1 m × 1 m litterfall traps were established 1 m off the ground or on the PVC plates in each plot, and litterfall was monthly collected.

Leaf sampling and chemical analysis

By the preliminary species investigation, *S. superba* and *L. glaber* are dominant species and have important contributions to the carbon stock of the subtropical forest in East Asia. Both of them not only show great abundances in this study (Supplementary Fig. S2), but also widely distribute in the subtropical forest ecosystem (Song 2013). Besides, they have high growth rates (Chi et al. 2017) and greatly contribute to carbon stocks of subtropical forests, especially *S. superba* (52.84%, Ali et al. 2014). Although the contribution of *L. glaber* to carbon stocks is relatively low (4.3%), it ranks in the top five of typical subtropical species in this region (Ali et al. 2014). Thus, *S. superba* and *L. glaber* were chosen and leaf samples were collected in 2016 (October and November), 2017 (May, August and November), 2018 (April, July and October) and 2019 (January and May).

For previous analysis showed no significant difference in soil conditions between control and disturbance treatments (Bu et al. 2018), leaves were sampled from control and drought plots in this study. For each species, three normal individuals of diameter at breast height approaching the averages of all plots (19.9 cm for *S. superba* and 14.9 cm for *L. glaber*) were randomly selected and labeled for repetitive samplings to minimize sampling variations in each plot. Fully expanded leaves were sampled using a pole with a sickle head, which resulted in

10–20 leaves per tree. Senesced leaves were collected from the ground near the labeled tree, with identifications that the base of leaf petioles was new and fresh, or leaf color was red or yellow. It is impossible to collect a standard number of leaves from each plot, as the amount of senesced leaves varies among species and months. Therefore, 10–45 senesced leaves per plot were obtained for each species.

The leaf samples were taken to the laboratory, desiccated at 105°C for 30 min, and dried at 75°C for more than 48 h to a constant weight in a drying oven. Dried samples were then weighted and ground to fine powder to pass through a 100-µm mesh sieve. After that, subsamples were digested in $H_2SO_4-H_2O_2$. Total N concentration was determined using the Kjeldahl method with an automatic Kjeldahl analyzer (K9840 Kjeldahl Distillation Unit; Hanon Instruments, Jinan, China). Total P concentration was measured colorimetrically by the molybdenum blue method with a spectrophotometer (745 Spectrophotometer; Sunny Hengping Instrument, Shanghai, China). Meanwhile, leaf litters of *S. superba* and *L. glaber* were collected from litterfall traps and weighted.

Calculation

Leaf nutrient resorption efficiency (NuRE) refers to the percentage differences of nutrient concentration between green and senesced leaves (Aerts 1996). For example, N resorption efficiency (NRE) is calculated as:

$$NRE = \frac{N_g - N_s}{N_g} \times 100\% \quad (1)$$

where N_g and N_s represent N concentration in green and senesced leaves, respectively.

Leaf nutrient use efficiency (NuUE) means the efficiency of biomass produced per unit nitrogen and can be defined as the inverse

of nutrient concentration in senesced leaves (Vitousek 1982; Yuan and Li 2007). For example, N use efficiency (NUE) is estimated as:

$$NUE = \frac{1}{N_s} \times 1000 \quad (2)$$

Drought effect is the relative change in drought-treated plots compared with control plots as follows:

$$\text{Drought effect} = \frac{N_D - N_{CK}}{N_{CK}} \quad (3)$$

where N_D and N_{CK} represent leaf nutrients in the drought and control, respectively.

Its standard deviation (SD) was calculated as:

$$SD = \text{drought effect} \times \sqrt{\left(\frac{\sqrt{S_D^2 + S_{CK}^2}}{N_D - N_{CK}}\right)^2 + \left(\frac{S_{CK}}{N_{CK}}\right)^2} \quad (4)$$

in which S_D and S_{CK} are the standard deviation of the leaf nutrients in the drought and control, respectively.

Statistical analysis

Based on the meteorological data from 2004 to 2018, we calculated the probability distribution functions of annual and summer rainfall. To assess the dynamics of nutrient concentrations and use in whole years, we analyzed a 2-year data from May 2017 to May 2019. Here, we defined 2017 and 2018 as a period of May 2017–May 2018 and May 2018–May 2019, respectively. Multiple comparisons tested differences of leaf nutrients among months in a year. Repeated-measure analyses of variance (RMANOVAs) were used to test the main and interactive effects of drought and sampling months on leaf nutrients with the 2-year data. *T*-tests examined drought effects in each sampling month. Linear regressions examined the relationship between leaf nutrients and soil microclimate. Analyses were conducted with the statistical software SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Besides, correlations between drought effects and soil microclimate were analyzed with the data from October 2016 to May 2019 in software Origin 8.5 (OriginLab Corporation, Northampton, USA).

RESULTS

Environmental factors

During the 2 years, MAT was 17.0°C in 2017 and 17.1°C in 2018 (Fig. 1a). Compared with the long-term average (1377.4 mm), precipitation in 2017 was low (1102.3 mm) especially in summer (near the 5th percentile of the probability distribution functions of summer rainfall from 2004 to 2018), but it was high in 2018 (1598.0 mm)

(Fig. 1b). ST showed a one-peak pattern, which was high in summer and low in winter (Fig. 1c). Soil moisture (SM) was low in summer, which may be attributed to the concurrent high air temperature and low natural precipitation (Fig. 1d).

Besides, drought treatment did not change ST but significantly reduced SM by 47.7% (Fig. 1c and d). Due to the deficiency of natural precipitation in the summer of 2017, SM in both control (14.1 v/v%) and drought (9.1 v/v%) plots was the lowest in August during the 2-year observation. Hence, drought-treated plots experienced a severe natural drought at the same time.

Temporal variability in leaf nutrient concentrations and use

During the 2 years, nutrient concentrations and use varied with species and sampling months (Table 1). In the control, Ng and Pg were 17.14 and 0.65 mg g⁻¹ in *L. glaber*, greater than 15.05 and 0.53 mg g⁻¹ in *S. superba* (Fig. 2a and c). Similarly, Ns and Ps were higher in *L. glaber* (13.86 and 0.39 mg g⁻¹) than *S. superba* (7.22 and 0.17 mg g⁻¹). In contrast to leaf nutrient concentrations, higher NRE and phosphorus resorption efficiency (PRE) were observed in *S. superba* (51.22%, 68.02%) than in *L. glaber* (18.93%, 41.07%; Fig. 3a). Besides, NUE and phosphorus use efficiency (PUE) were 144.26 and 6789.19 g g⁻¹ in *S. superba*, about 2-fold higher than 73.57 and 3005.40 g g⁻¹ in *L. glaber* (Fig. 3c). Though nutrient use and concentrations were temporally dynamic, no consistent dynamics was observed in these 2 years (Table 2).

Drought effects on nutrient concentrations and use

The RMANOVAs showed that species responded differently to drought in nutrient concentrations and use during the 2 years (Table 1). For *L. glaber*, NUE, NRE and PRE were significantly reduced by 3.4%, 20.2% and 7.1%, respectively, due to the significantly increased Ns (+3.4%), Pg (+14.1%) and Ps (+23.5%). However, no response was observed in the nutrient use and concentrations of *S. superba* under drought treatment.

Furthermore, *T*-tests showed that Ns and Ps of *L. glaber* were significantly increased from 11.01 and 0.20 mg g⁻¹ in the control to 13.30 and 0.46 mg g⁻¹ in the drought, respectively, in August 2017 when natural drought occurred (Fig. 2b and d). As a result, NUE and PUE were depressed by 17.2% and 58.1%, while NRE and PRE were reduced by 56.5% and 53.8%, respectively (Fig. 3b and d).

Variation of drought effects with soil microclimate

Regression analysis showed that ST and SM had neither effect on nutrient concentrations in green and senesced leaves, nor on nutrient use and resorption efficiencies (Supplementary Table S2). Analyzing the relationship between SM and the drought effects on leaf nutrient concentrations and use, no significant correlation was observed for *S. superba*. However, for *L. glaber*, a negative correlation was observed in Ns, and positive correlations were observed in NRE, PRE and NUE (Fig. 4).

Table 1: Results of RMANOVAs for the main effects of drought (D) and sampling months (Month), and their interactions on nutrient use and concentrations of *L. glaber* and *S. superba* from May 2017 to May 2019

	NRE	PRE	NUE	PUE	Ng	Pg	Ns	Ps
<i>Lithocarpus glaber</i>								
D	<0.05	<0.05	<0.01	0.106	0.275	<0.05	<0.01	<0.05
Month	<0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.01
D × Month	0.428	0.085	0.118	<0.01	0.177	0.553	0.173	<0.05
<i>Schima superba</i>								
D	0.818	0.360	0.305	0.853	0.189	0.682	0.370	0.567
Month	<0.01	<0.01	<0.001	<0.001	<0.05	<0.001	<0.001	<0.001
D × Month	0.477	0.771	0.677	0.906	0.274	0.510	0.530	0.795

P value is shown as numbers. Bold texts indicate significance at *P* < 0.05.

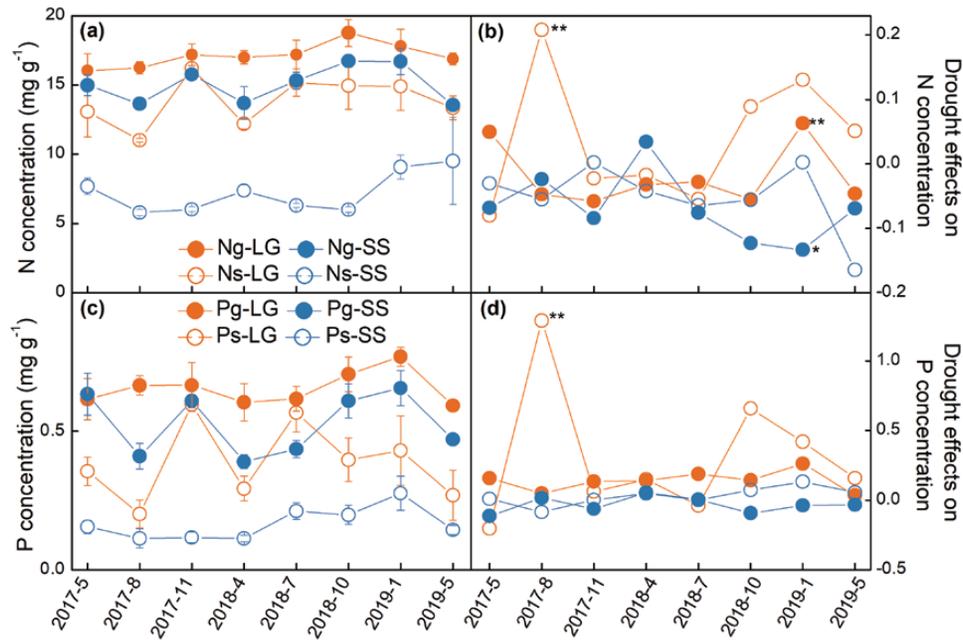


Figure 2: Dynamics of N and P concentrations in the control treatment (a, c) and drought effects on the N and P concentrations (b, d) of *L. glaber* (LG) and *S. superba* (SS). Values in panels (a) and (c) represent the mean \pm SD ($n = 3$). The marks next to the dots indicate its significance (* $P < 0.05$; ** $P < 0.01$).

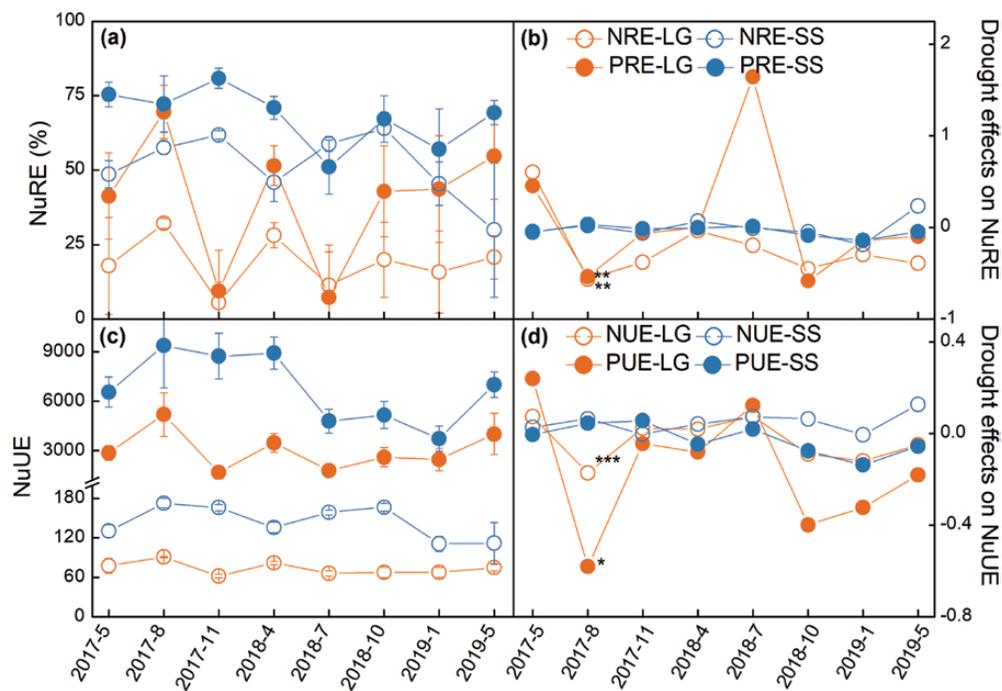


Figure 3: Dynamics of nutrient resorption and use efficiencies in the control treatment (a, c) and drought effects on the nutrient resorption and use efficiencies (b, d) of *L. glaber* (LG) and *S. superba* (SS). Values in panels (a) and (c) represent the mean \pm SD ($n = 3$). The marks next to the dots indicate its significance (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

DISCUSSION

Temporal and species variability in leaf nutrient use

It has been suggested that the environment and leaf growth stage are important for the dynamics of nutrient concentrations and uses (Fife *et al.* 2008; Yuan and Chen 2009). However, no consistent dynamics was observed in this study during the 2 years. On the one hand, no

correlation between leaf nutrient use and ST or SM suggests that soil microclimate may not account for the temporal variability of nutrient use in this forest. On the other hand, the samplings with low frequency and in different months between 2017 and 2018 may contribute to the absence of consistent dynamics (Fife *et al.* 2008; Xue and Shao 2002). Therefore, more frequent samplings are recommended in studying the nutrient dynamics of subtropical forests.

Table 2: Results of multiple comparisons for nutrient concentrations and use of *L. glaber* and *S. superba* in 2017 (May 2017–May 2018) and 2018 (May 2018–May 2019)

		NRE	PRE	NUE	PUE	Ng	Pg	Ns (%)	Ps (%)
<i>Lithocarpus glaber</i>	2017	17.9 ± 16.3 ^{ab}	41.3 ± 14.5 ^b	77.6 ± 11.4 ^b	2860.5 ± 447.9 ^{bc}	16.0 ± 1.2 ^a	0.6 ± 0.1 ^a	13.1 ± 1.8 ^b	0.4 ± 0.1 ^b
		32.2 ± 1.6 ^a	69.5 ± 9.0 ^a	90.8 ± 1.0 ^a	5194.8 ± 1331.5 ^a	16.2 ± 0.4 ^a	0.7 ± 0.0 ^a	11.0 ± 0.1 ^c	0.2 ± 0.1 ^c
		5.5 ± 6.9 ^b	9.4 ± 13.7 ^c	61.8 ± 2.5 ^c	1679.0 ± 72.8 ^c	17.2 ± 0.8 ^a	0.7 ± 0.1 ^a	16.2 ± 0.7 ^a	0.6 ± 0.0 ^a
		28.2 ± 4.2 ^a	51.5 ± 6.6 ^{ab}	82 ± 2.5 ^{ab}	3475.1 ± 578.1 ^b	17.0 ± 0.5 ^a	0.6 ± 0.1 ^a	12.2 ± 0.4 ^{bc}	0.3 ± 0.0 ^b
	2018	11.3 ± 11.3 ^a	15.5 ± 17.5 ^b	66.1 ± 4.2 ^a	1782.3 ± 234.0 ^b	17.2 ± 1.0 ^{ab}	0.6 ± 0.0 ^b	15.2 ± 1.0 ^a	0.6 ± 0.1 ^a
		19.9 ± 12.7 ^a	42.9 ± 15.2 ^a	67.5 ± 8.2 ^a	2596.2 ± 581.8 ^{ab}	18.8 ± 1.0 ^a	0.7 ± 0.1 ^a	14.9 ± 1.7 ^a	0.4 ± 0.1 ^{ab}
		15.8 ± 13.8 ^a	43.6 ± 17.9 ^a	67.7 ± 7.7 ^a	2456.2 ± 695.9 ^b	17.8 ± 1.2 ^{ab}	0.8 ± 0.0 ^a	14.9 ± 1.7 ^a	0.4 ± 0.1 ^{ab}
		20.7 ± 7.3 ^a	54.7 ± 14.5 ^a	75.1 ± 4.7 ^a	3999.1 ± 1259.5 ^a	16.9 ± 0.4 ^b	0.6 ± 0.0 ^b	13.4 ± 0.9 ^a	0.3 ± 0.1 ^b
		48.7 ± 4.6 ^b	75.4 ± 4.2 ^a	130.7 ± 9.6 ^b	6556.6 ± 929.6 ^a	15.0 ± 0.7 ^a	0.6 ± 0.1 ^a	7.7 ± 0.6 ^a	0.2 ± 0.0 ^a
		57.5 ± 2.2 ^a	72.2 ± 9.5 ^a	172.9 ± 7.1 ^a	9388.4 ± 2575 ^a	13.6 ± 0.3 ^b	0.4 ± 0.0 ^b	5.8 ± 0.2 ^b	0.1 ± 0.0 ^a
<i>Schima superba</i>	2017	61.8 ± 1.4 ^a	80.9 ± 3.4 ^a	166.1 ± 4.9 ^a	8748.2 ± 1393.3 ^a	15.8 ± 0.1 ^a	0.6 ± 0.0 ^a	6.0 ± 0.2 ^b	0.1 ± 0.0 ^a
		45.9 ± 6.5 ^b	71.0 ± 3.8 ^a	136.1 ± 8.4 ^b	8927.6 ± 985.5 ^a	13.7 ± 1.2 ^b	0.4 ± 0.0 ^b	7.4 ± 0.4 ^a	0.1 ± 0.0 ^a
		58.8 ± 2.5 ^a	51.1 ± 9.0 ^b	158.9 ± 3.7 ^a	4795.6 ± 724.6 ^{bc}	15.3 ± 0.6 ^b	0.4 ± 0.0 ^b	6.3 ± 0.1 ^b	0.2 ± 0.0 ^{ab}
		64.1 ± 2.2 ^a	67.2 ± 7.8 ^{ab}	166.6 ± 5.8 ^a	5158.5 ± 821.7 ^b	16.7 ± 0.5 ^a	0.6 ± 0.1 ^a	6.0 ± 0.2 ^b	0.2 ± 0.0 ^b
	2018	45.4 ± 7.3 ^{ab}	57.0 ± 13.5 ^{ab}	110.9 ± 11.3 ^b	3732.6 ± 765.3 ^c	16.7 ± 0.9 ^a	0.7 ± 0.1 ^a	9.1 ± 0.9 ^{ab}	0.3 ± 0.1 ^a
		29.9 ± 22.5 ^b	69.3 ± 4.1 ^a	112 ± 31.1 ^b	7006.0 ± 774.2 ^a	13.6 ± 0.4 ^c	0.5 ± 0.0 ^b	9.5 ± 3.1 ^a	0.1 ± 0.0 ^b

Values indicate the mean ± SD. Different letters indicate the differences among sampling months for each year.

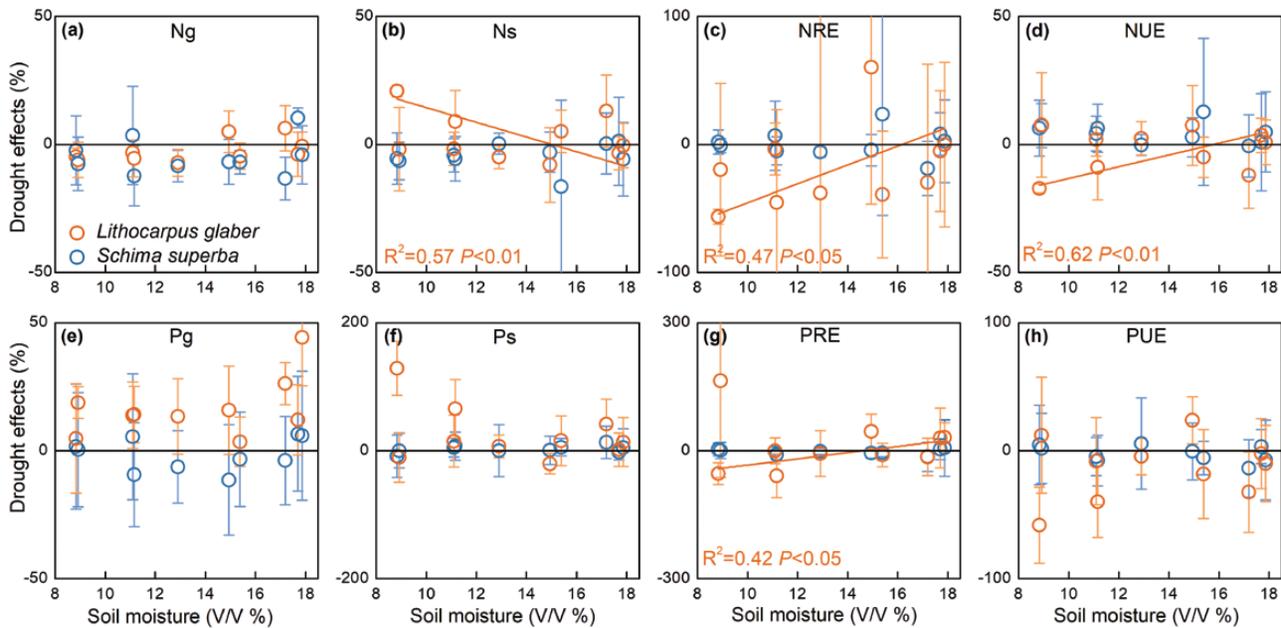


Figure 4: Correlations between SM and drought effects on nutrient concentrations and use of *S. superba* and *L. glaber* from October 2016 to May 2019. Values represent the mean ± SD ($n = 3$).

Though belonging to the same plant functional type, *S. superba* and *L. glaber* take diverse nutrient use strategies. Lower N and P concentrations in green leaves of *S. superba* indicate its weaker nutrient demands than *L. glaber*. According to nutrient concentrations in senesced leaves, Killingbeck (1996) identified resorption potential of evergreen plants and suggested N and P concentrations below 7 and 0.4 mg g⁻¹ as ‘complete resorption’, and above 10 and 0.5 mg g⁻¹ as ‘incomplete resorption’, respectively. Hence, *S. superba* has a stronger potential for conserving nutrients than *L. glaber* (Fig. 2). Meanwhile, greater nutrient use and resorption efficiencies of *S. superba* (Fig. 3) imply higher carbon gain per unit nutrient and stronger internal nutrient cycles. Therefore, *S. superba* presents a superior nutrient use strategy than *L. glaber*.

Interspecific variability in responses of nutrient use to drought treatment

Nutrient use and resorption efficiencies, as important traits of nutrient conservation in plants, are complex in response to drought. Numerous studies have reported that plants will improve nutrient use and resorption efficiencies under drought (Rentería and Jaramillo 2011; Suseela et al. 2015). Controversial results have also been observed, with negative (Minoletti and Boerner 1994; Pugnaire and Chapin 1992) and neutral responses (Sanz-Pérez et al. 2009; Yuan and Li 2007). Besides, drought effects on nutrient use can differentiate with species (Khasanova et al. 2013; Marchin et al. 2010). In this study, drought decreased nutrient use and resorption efficiencies of *L. glaber* by increasing nutrient losses, but had no effect on *S. superba*, owing

to the unchanged plant nutrient uptake and loss during the 2 years (Figs 2 and 3).

It has been suggested that drought can affect nutrient resorption efficiencies through altering nutrient concentrations of green leaves (Khasanova *et al.* 2013; Luo *et al.* 2018). As we know, nutrient acquisitions in leaves are largely supplied through root uptake (Cleveland *et al.* 2013), which mainly depends on the availability of soil nutrients and the ability of root uptake. Although soil nutrient availability was limited by drought at this site (Bu *et al.* 2018), N and P concentrations of green leaves were unaffected or even increased in our study. This suggests that the ability of root uptake may play a key role under drought. Unfortunately, in this experiment, we had no relevant data to test this hypothesis. However, recent studies have confirmed that *S. superba* can improve the ability of root uptake under drought, via increasing specific surface area (Liao 2017) or adjusting root osmotic pressure through accumulating proline in roots (Kuang *et al.* 2017). Besides, arbuscular mycorrhizal symbiosis may also make a contribution (Maitra *et al.* 2019). Hence, *S. superba* and *L. glaber* have the potential to hold nutrient concentrations in green leaves under long-term drought by improving the ability of root uptake. On the other hand, changing nutrient concentrations in senesced leaves can also alter drought effects on nutrient resorption efficiencies. The increased N (3.4%) and P concentrations (23.5%) in senesced leaves of *L. glaber* indicate that drought reduced nutrient resorption of *L. glaber* by impairing the capacity of phloem loading and transferring nutrients from senesced leaves to plants (Pugnaire and Chapin 1992). Our results suggest species-specific nutrient use under long-term drought.

Nutrient loss caused by drought was not only estimated by the quality but also by the quantity of litter. An enhancement in senesced leaf N concentration relates to a fast litter N mineralization (Deng *et al.* 2018). Besides, Macinnis-Ng and Schwendenmann (2015) found that a 72% increase in litterfall quantity stimulated by drought contributed to more than 50% increase in nitrogen cycling in a Zealand forest. In this study, the patterns and amounts of leaf litter for *S. superba* and *L. glaber* were unaffected by drought (Supplementary Fig. S3). Hence, the increased nutrient concentrations in senesced leaves of *L. glaber* suggest an accelerated feedback in the responses of nutrient cycling to drought.

Responses of nutrient use varied with months and species

Drought effects on nutrient use and resorption efficiencies of *L. glaber* varied with the conditions of SM of different months. In this study, when natural precipitation was deficient and lead to the lowest SM (near 9 v/v%) in August 2017, the drought effects on nutrient use and resorption efficiencies of *L. glaber* switched from unresponsive to negative (Figs 3 and 4). It indicates the importance of natural water conditions on the drought effects and the existence of SM threshold which determines the direction of drought effects on tree leaf nutrient use. Similar variability of nutrient-use responses over time was observed in water addition experiments. For example, moderate and high water supply increased the NRE of *Lucerne* in branching and squaring growth stages, respectively, but weakened it in flowering stages (Lu *et al.* 2019). Hence, responses of nutrient use to water availability depend on time. Since precipitation changes are flexible in magnitude and direction, experiments within a wide range of precipitation amounts can help us better understand complex ecosystem processes (De Boeck *et al.* 2020). However, precipitation manipulative experiments (increased or reduced precipitation) were less conducted in wet zones. Given the spatial and temporal variability of wet zones, more studies considering multilevel precipitation over multisites can contribute to a full picture of impacts of changes in water availability on nutrient use in wet zones.

Plants exposed to the summer severe drought will also experience concomitant high temperature. The combination of drought and

heat stress will induce an interactive effect on nutrient use, which is different from i.e. elicited by either factor alone (Suseela *et al.* 2015). While we did not prove this possible mechanism behind the contrasting responses, if it is true, the future climate scenarios in this region, decreased precipitation with a high temperature in summer, will limit plant nutrient use and accelerate the feedbacks between biogeochemical cycles and climate changes. Furthermore, the characteristics of plants can mediate drought impacts on plant nutrient use. Schwinning *et al.* (2005) have suggested that deep-root species can absorb deep soil water and maintain plant growth in extreme drought, whereas shallow-root species may die or dormant. Compared with *L. glaber*, *S. superba* with deeper roots is more accessible to water and nutrients in the deep soil to alleviate their limitations on plants (Supplementary Table S1). Besides, *S. superba* shows a superior nutrient use strategy than *L. glaber*. As a result, *S. superba* is less sensitive than *L. glaber* in the responses of nutrient use to drought stress. Therefore, forest dominated with sensitive species, e.g. *L. glaber* in this region, would be more vulnerable in the stability and productivity under future climate scenarios.

CONCLUSIONS

To the best of our knowledge, this study provides the first experimental evidence on the temporal dynamics of drought effects on tree nutrient use and resorption efficiencies in subtropical forests. Interestingly, the nutrient use and resorption efficiencies varied between species and months. *Schima superba* was more resistant than *L. glaber* in maintaining foliar nutrient concentrations and nutrient use under drought. Besides, significant negative drought effects on the nutrient use of *L. glaber* were found when natural drought lead to the lowest SM. Our findings demonstrate the species-specific threshold of drought-induced reductions in leaf nutrient use and resorption efficiencies in this forest. Besides, drought effects on nutrient use depend on natural precipitation conditions. This study recommends more research efforts on detecting the responses of different tree species to precipitation changes in different months, to provide more comprehensive perspectives for the importance of water–nutrient interactions in managing the subtropical forests.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Figure S1: Design of the throughfall exclusion experiment (a), and three treatments as control (b; 7,8,9), disturbance (c; 3,5,6) and drought (d; 1,2,4).

Figure S2: The composition and abundance of species in the control (CK) and drought (D) treatments.

Figure S3: Dynamics of the litterfall amount for *L. glaber* (a) and *S. superba* (b) in the litter traps from May 2017 to May 2019 under control (blue) and drought (orange) treatments.

Table S1: Comparisons of living conditions and species characteristics between *Schima superba* and *Lithocarpus glaber*.

Table S2: Results of relationships between leaf nutrient variables and soil temperature (ST) and soil moisture (SM), respectively.

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