Responses of subtropical deciduous and evergreen trees to varying intensities of herbivory

Xiaoyu Liu
Carri J. LeRoy
Yuan Guo
Shuwang Song
Jingfang Wu
Zhipei Wang
Fenggang Luan
Qingni Song
Xiong Fang
Qingpei Yang
Jun Liu (✉ ljaim99@163.com)
  Institute of Applied Ecology, Chinese Academy of Sciences

Research Article

Abstract

Aims

Generally, deciduous and evergreen trees coexist in subtropical forests, and both types of leaves are attacked by numerous insect herbivores. However, trees respond to and defend themselves from herbivores in different ways, and these responses may vary between evergreen and deciduous species. To understand tree responses to leaf herbivore attack under varying intensities of herbivory.

Methods

We examined both the percentage of leaf area removed by herbivores as well as the percentage of leaves attacked by herbivores to evaluate leaf herbivore damage across 15 subtropical deciduous and evergreen trees, quantified plant responses to varying intensities of herbivory and analyzed both leaf herbivore attack and leaf traits among species.

Results

We have found that there was a higher intensity of herbivory on deciduous species than evergreen species, both as percentage of leaf area removed and the percentage of leaves attacked. In addition, leaf herbivory was positively correlated with defensive traits (concentrations of tannin and lignin), and negatively correlated with nutrient content (ratios of NSC:lignin and NSC:cellulose), which may be largely attributed to long-term and frequent insect herbivore stress and potentially evolutionary arms races between plants and insects.

Conclusions

This study highlights that leaf responses to varying intensities of herbivory differed widely among subtropical plant species and there was a stronger defensive response for deciduous trees to leaf herbivore attack than that of evergreen trees. These results have implications for better understanding plant responses and defenses to varying intensities of herbivory, and reveal several underlying relationships between leaf traits and herbivory.

Highlights

1. Across 15 subtropical forest tree species, there was a higher intensity of herbivory for deciduous species than evergreen species, whether examining the percentage of leaf area removed or the percentage of herbivore-attacked leaves.
2. Leaf herbivory measurements were positively correlated with leaf level defense compound concentrations and negatively correlated with leaf level nutrient concentrations, which is consistent with long-term and frequent insect herbivory stress and suggests evolutionary arms races between trees and herbivores.

3. Leaf responses to varying intensities of herbivory resulted in wide variation among tree species in foliar traits and defenses. Overall, it appears as though leaf defensive traits of deciduous species are more sensitive to leaf herbivore attack than evergreen trees.

Introduction

Insect herbivory on trees is a common phenomenon in forests (Price 1991). Leaves are tissues with high nutrient status, are easily consumed by insects, and provide a rich resource for many consumers (Schowalter et al. 1986). At the same time, leaf herbivore damage also causes leaf area loss and tissue damage, which can negatively influence plant photosynthesis and growth (Zhang and Turner 2008, Kerchec et al. 2012, Visakorpi et al. 2020), but also affects community biodiversity (Huntly 1991, Jefferies et al. 1994). In tropical forests, it is thought that leaf herbivores can have a negative influence on interspecific competition and vegetation community structure (Hairston and Hairston 1993, Coley and Barone 1996, Kurokawa and Nakashizuka 2008). However, less is known for subtropical forests, which are composed of mixed evergreen and deciduous trees and also host numerous insect herbivores. It is well known that there are significant differences in leaf nutrients (nitrogen [N], phosphorus [P], and both structural and non-structural carbohydrates), phenology, and morphology between evergreen and deciduous trees (Coley 1988, Givnish 2002, Löiez and Piper 2022). Here, we asked, do evergreen and deciduous trees respond differently to leaf herbivores in subtropical forests?

Generally, insect herbivores prefer feeding on palatable leaves with higher nutrients and lower secondary metabolites, a pattern which is outlined in the plant vigor hypothesis (Price 1991, Cornelissen et al. 2008, Che-Castaldo et al. 2019). It is well known that evergreen and deciduous species vary greatly in leaf longevity and phenology, which contributes to different concentrations of structural or non-structural carbohydrates (Palacio et al. 2007, Michelot et al. 2012). For example, long-lived evergreen leaves invest more resources into defensive traits such as cell walls than deciduous leaves due to a potentially longer photosynthetic season (Takashima et al. 2004). In addition, the concentration of secondary metabolites (e.g, tannins) in leaves often decreases palatability and increases defensive capabilities (Harborne 1991a). The role of secondary metabolites in defense may involve herbivore deterrence, anti-consumer activity, and tissue toxicity, which can all deter further herbivore damage (Bennett and Wallsgrove 1994, Kazakou et al. 2019). Therefore, we suspected that insect herbivores would consume more palatable leaves with higher concentrations of nutrients and non-structural carbohydrates as well as lower concentrations of defensive compounds (e.g, lower concentrations of tannins and lignin). We hypothesized that deciduous tree leaves would suffer worse leaf herbivore attack with both a higher percentage of leaf area damaged and a higher percentage of leaves damaged due to their suite of leaf quality traits (Hypothesis 1).

Leaf traits determine the performance and choice of leaf herbivores to a certain extent, including nutrient status, chemical defense substances and other physical properties. Some studies indicate that the more area leaf herbivores removed can be correlated with higher concentrations of N, or lower concentrations of tannins and lignins (Coley 1988, Sagers and Coley 1995). However, we often lack a strong

understanding of the inter-relationships among leaf traits (e.g. nutrients, NSC, tannins and lignin) and their influences on herbivores and host preferences (Agrawal and Fishbein 2006). In this study, we examine both the percentage of leaf area removed by herbivores as well as the percentage of leaves attacked by herbivores to evaluate leaf herbivore damage across 15 subtropical deciduous and evergreen trees. Using two separate measures of leaf herbivory has been suggested as an approach to better demonstrate variable plant responses to herbivory (Beck and Labandeira 1998, Maldonado-López et al. 2019). Here, we hypothesized that insect herbivoress would feed preferentially on palatable leaves with higher concentrations of nutrients and NSC, and lower concentrations of defensive chemicals (tannins and lignin) (Hypothesis 2).

Under herbivore attack, plants mount a defense response characterized by the accumulation of secondary metabolites and inhibitory proteins (Kerchec et al. 2012). Plants have developed herbivore-induced production of both chemical and physical defenses. Condensed tannins are a very important phenol and chemical defense substance in plants, and can deter, delay or reduce the amount of herbivore damage (Núñez-Farfán et al. 2007, Agrawal 2011, Karban 2011). However, chemical resistance is assumed to be relatively costly (Stamp 2003, Zhang et al. 2018), especially the production of high-concentrations of tannins. There are several different responses of chemical defense and nutrients to varying intensities of leaf herbivory according to the optimal defence theory (Fagerstrom et al. 1987, McCall and Fordyce 2010, Heinen et al. 2020). On the one hand, there are many examples of induced secondary plant metabolites providing effective resistance against herbivores under conditions of high levels of herbivory (Karban 2020). On the other hand, some studies found that nutrients (e.g., nitrogen and phosphorus) and non-structural carbohydrates (e.g., starch and sugars) are diverted away from the site of damage and into storage tissues, reducing nutrients and NSC in attacked plant tissues (Newingham et al. 2007, Gómez et al. 2010, Quijano-Medina et al. 2019). Therefore, we hypothesized that leaf herbivore attack would decrease the concentration of nutrients and NSC and produce more tannins for defense, particularly under heavy herbivory (Hypothesis 3a). In addition, the timing of the trade-off (chemical defence) would vary in deciduous and evergreen trees, due to different leaf traits and herbivory levels. Here, we hypothesized that chemical defense and the concentration of NSC would be more sensitive to herbivore attack in deciduous leaves compared to evergreen species (Hypothesis 3b).

To test the above three hypotheses, we analyzed herbivore damage on 15 tree species including both deciduous and evergreen trees in secondary subtropical forests. We analyzed the relationships between the percentage of leaf area damaged and percentage of damaged leaves with various leaf traits among species, and measured plant nutrient status (e.g. concentration of nutrients and nonstructural carbon) as well as defensive traits (e.g. concentration of tannins, lignin and cellulose) associated with resistance to insect herbivores. Overall, the present work builds towards a better understanding of plant responses and defenses to varying intensities of herbivory in subtropical forests.

Materials And Methods

Study site

We conducted this study in secondary forests at Guanshan National Nature Reserve in South China (28°30'~28°40'N;114°29'~114°45'E). The region has a subtropical warm and humid climate, and the mean annual air temperature is 16.2°C. The mean annual precipitation is 1,950 ~ 2,100 mm, which mainly occurs from March to August (Liu and Zhang 2017). In 2014, we established a 12 ha biodiversity monitoring plot, completed a full plant catalogue, and determined the location of all woody plants with diameter at breast height (DBH) > 1 cm. All field monitoring observations for this study were conducted in this plot. In this area, the fifteen dominant tree species were chosen based on the highest Importance Values (IV) within the plot (Table S1).

Leaf herbivory

We measured insect herbivory on fifteen dominant tree species (including seven deciduous [n = 6] and eight evergreens [n = 6]), including both young and mature leaves, in April and August 2019. We sampled trees from across the 12 ha plot, and all selected trees were sampled from similar terrain and altitude to ensure similar habitats and herbivory environments (Table S1).We picked all of the leaves off of four small branches (one facing each cardinal direction) from each sample tree, mixed all leaves together and transported them back to the laboratory for treatment. We scanned all leaves using a versatile plant image analyzer (Hangzhou WSeen Detection Technology Co., Ltd, China) and measured total leaf area as well as the area of leaf damaged by insect herbivores (wormholes, chewed edges, damaged leaf areas). In total, over 10,000 leaves from 90 trees of the 15 study species were sampled and analyzed. For each plant, percentage of leaf area removed by herbivores was calculated as: [(removed area/total area) × 100] and the percentage of leaves attacked was calculated as: [(the number of insect attacked leaves /total number of leaves) × 100].

Based on the degree of insect herbivory, we divided all sampled leaves into four categories: unattacked, mild herbivory (< 10% leaf area removed), moderate herbivory (10–50%) and heavy herbivory (> 50%). All samples were cleaned with ultra pure water and surface-sterilized at 105°C, then oven-dried at 65°C to constant weight, and ground into a fine powder using a ball mill (JX-2010, Shanghai, China) for chemical analysis. There were a total of 360 plant samples in this experiment.

Chemical analyses

The concentrations of total C and N were measured on 2 mg subsamples of leaf powder using an elemental analyzer (Thermo Fisher Scientific IR-MS) with acetanilide as external standard. All C and N measurements were run in duplicate and the average deviations of replicate analyses from the means were 1.1% for N and 0.2% for C concentrations. To extract P in samples, 150 mg of dried plant powder from each sample was digested using H_2SO_4 and H_2O_2 at 650°C with a microwave digestion system for 30 min, and then washed with deionized water and diluted up to 100 mL (final volume). Prior to analysis, all digested samples were then analyzed following the molybdenum antimony method and a molybdate–ascorbic acid procedure using ultraviolet visible (UV-Vis) spectrophotometry (880nm, UV-5100), and calibrated using a standard solution (Lu, 2000). Non-structural carbohydrates (NSC) are defined as starch and soluble sugar (glucose, fructose, and sucrose), following the enzymatic digest and UV

spectrophotometry methods, which were analysed as described in Wong (1990) and Hoch et al. (2002). The standard anthrone colorimetric method was employed to measure the contents of plant NSCs (Li et al. 2016, Dubois et al. 1956). Briefly, 100 mg ground leaf sample was placed in a 50-ml centrifuge tube and mixed with 10 ml 80% (v/v) alcohol, extracted in 90°C in hot water for 10 min three times. All the extracted solution was transferred into a 50 ml flask and we adjusted the final volume to 50 ml for the measurement of soluble sugar via the anthrone colorimetric method. The residue after three-time extraction in the centrifuge tube was dissolved using 30% (V/V) $HClO_4$ for 12 h, then extracted in 80°C hot water for 10 min. After that, the extracted residue was cooled down, filtered, and diluted to 50 ml in a flask for the determination of starch. Soluble sugars and starch contents were calculated by the dry matter of leaf (mg g − 1) respectively (Li et al. 2016, Xie et al. 2018).

The concentration of lignin, cellulose and hemicellulose were assayed using HPLC (Sluiter et al. 2005, Sluiter et al. 2008). Briefly, 200 mg subsamples were extracted with ferrous ammonium sulfate hexahydrate ($H_8FeN_2O_8S_2 \cdot 6H2O$) and CuO in tube, then analyzed on a HPLC (Brand, Location) using an 10 μL. The concentrations of condensed tannins were determined by using the Folin Ciocalteu assay. Samples (200 mg) of leaf powder were extracted in water bath heating with 80°C, then a 100 μL aliquot of extract was added to 750 μL of distilled water, 500 μL Folin-Ciocateu reagent and 1000 μL of 35% sodium carbonate (Na_2CO_3) were added. The mixture was shaken vigorously after diluting to 10 mL of distilled water. The mixture was incubated for 30 min at room temperature and read at 725 nm using a UV-Vis spectrophotometer. Gallic acid (GA) standard curve was first prepared from 0−200 mg/L and tannin content was expressed in mg gallic acid equivalent / g dry matter. The total tannin content was expressed as gallic acid equivalents (GAE)/ g dry matter, as calculated from the prepared standard curve with 0−100 mg/ GA (Tamilselvi et al. 2012). All chemical analyses were run in duplicate. Additionally, we calculated the ratios of C:N, NSC:lignin, and NSC:cellulose as indicators of leaf quality.

Leaf Response to Herbivory

We calculated leaf response to herbivory (LRH) by comparing the concentrations of nutrients and defense compounds in damaged and undamaged leaves. We measured this response to determine if a tree species increased (positive values) or decreased (negative values) production of a compound under varying levels of herbivory. We calculated the LRH for each compound, N, P, NSC, tannins, lignin, and cellulose, using the equation below:

LRH = 1- (content in herbivore-attacked leaves / content in undamaged leaves) × 100%

where content = concentrations of N, P, NSC, tannins, lignin, or cellulose.

Data analysis

We determined the concentrations of nutrients (N, P and NSC) and foliar defensive compounds (tannins, lignin and cellulose), then compared these traits between deciduous and evergreen trees, among tree species, and across levels of herbivory (low, medium, high). Differences in foliar chemical traits between deciduous and evergreen species were detected using Student's t-test. Analyses of variance (ANOVAs)

were used to detect significant differences in the concentrations of C, N, P, non-structural carbohydrates, lignin, cellulose, hemicellulose and tannins among species and herbivore damage levels. We used correlation analysis and Pearson's r to determine linear relationships between percentage of leaf area removed, percentage of leaves attacked, and all possible leaf traits across all species. Alpha = 0.05 was the criterion for significant differences for all tests. Statistical analyses were performed using SPSS 24.0 for Windows.

Results

Levels of herbivory differed between deciduous and evergreen subtropical tree species as a whole, and among species within each plant type. Mean percentage of leaf area removed by herbivores ranged from 4–19% for deciduous trees, and from < 1–12% for evergreen trees (Fig. 1a). Overall, mean percentage of leaf area removed for deciduous species (10.0%) was significantly higher than for evergreen species (7.9%) (t = 2.069, P = 0.042) (Fig. 1a'). In addition, the percentage of leaves attacked by herbivores ranged from 40% to > 90% for deciduous species and from 2–90% for evergreen species (Fig. 1b), with a higher mean percentage of deciduous leaves damaged (73.5%) compared to evergreen leaves (54.3%, Fig. 1b', t = 3.41, P = 0.001).

Percentage of leaf area removed varied among species, with highest damage levels (18%) for *C. axillaris* and lowest levels (0.5%) for bamboo (*P. pubescens*, Fig. 1a). Percentage of leaves attacked also varied widely among species, with herbivore damage frequencies as high as 90%, for some deciduous species: *C. tibetana*, *C. axillaris*, *L. formosana*, and the lowest values again for bamboo (Fig. 1b). These results indicated that leaf herbivore damage for bamboo was much lower than other subtropical trees, whether the percentage of leaf area removed or the percentage of leaves attacked was measured.

There was a significant positive correlation between the percentage of leaf area damaged and the concentration of both tannins (Fig. 2a, R^2 = 0.4892, P = 0.012) and lignin (Fig. 2b, R^2 = 0.3066, P = 0.062) across all subtropical species. In contrast, there was a significant negative correlation between percentage of leaf area damaged and the NSC:lignin ratio (Fig. 2i, R^2 = 0.3023, P = 0.007), but no other measures of leaf quality showed linear relationships with % leaf area removed (Fig. 2). Moreover, we found that the percentage of leaves damaged by herbivores was positively correlated with the concentrations of both tannins (Fig. 2a', R^2 = 0.3842, P = 0.032) and lignin in leaves (Fig. 2b', R^2 = 0.3674, P = 0.037), but uncorrelated with any measure of nutrient status or quality (Fig. 2').

When comparing nutrient concentrations in attacked vs. unattacked leaves from the same species using calculation of leaf response to herbivores (LRH), we found some interesting variation both among species, and between deciduous and evergreen species. The difference between the nutrient content in herbivore-attacked leaves compared to undamaged leaves tended to be higher (more negative) as herbivore intensity increased, but more strongly for deciduous species (Fig. 3). For example, significantly negative response to herbivory for N were found in leaves undergoing either medium or high intensity herbivory, for four of seven deciduous species, and 3 of eight evergreen species (Fig. 3a). Similarly, for

three of seven deciduous species, significantly lower concentrations of phosphorus were found for herbivore-attacked leaves (mainly medium and high intensity herbivory, but even for low herbivory intensity on *Q. acutissima* leaves), and for two of eight evergreen species (even at low levels of herbivory for *R. latoucheae* leaves, Fig. 3b). Interestingly, one species of evergreen, *D. oldhamii*, showed a significant increase in P concentrations under high intensity herbivory (Fig. 3b). Patterns are more mixed when examining differences in NSC across intensities of herbivory. In this case, low or medium levels of herbivory resulted in significantly lower concentrations of NSC for two of seven deciduous species (*P. buergeriana* and *A. fortunei*, Fig. 3c) and high levels of herbivory caused no significant effects. Low levels of herbivory actually increased NSC for one deciduous species (*A. wilsonii*, Fig. 3c). For evergreen species, low levels of herbivory were related to significantly lower concentrations of NSC for *D. oldhami*, *S. superba*, *C. tibetana*, and *C. carlesii*, Fig. 3c), and high levels of herbivory resulted in lower concentrations of NSC for *D. oldhami* and *M. thunbergii*, Fig. 3c). In contrast, only *P. pubescens* showed significantly higher levels of NSC, and only under low levels of herbivory (Fig. 3c). These results suggest that there is a stronger response for N to insect herbivores in deciduous species, but a stronger response for NSC to insect herbivores in evergreen leaves.

For defensive traits of tree leaves (i.e., the concentration of tannin, lignin and cellulose), we found that medium or high intensity herbivory (> 10%) increased foliar tannins for three of seven decidous species (*L. formosana*, *A. fortnuei*, *S. discolor*) and four of eight evergreen species (*E. japonicus*, *D. oldhamii*, *R. latoucheae*, *M. thunbergii*, Fig. 4a). For all other species, there were indications that insect herbivory did not significantly stimulate the production of foliar tannin (Fig. 3a). The concentration of lignin was stimulated at high levels of herbivory for almost all deciduous and evergreen species. Five of seven deciduous species and five of seven evergreen species showed significantly higher concentrations of lignin under mainly high, but ocassionally low or medium intensity herbivory (Fig. 4b). Only the deciduous species *S. discolor* and *C. axillaris*, and the evergreen species *E. japonicus* and *S. superba* did not show an induction of lignin for high intensity herbivory (Fig. 4b). Interestingly, the patterns for cellulose are opposite, with most species showing lower concentrations of cellulose, especially under low or medium levels of herbivory intensity (Fig. 4c). Only *P. pubescans* showed a significant increase in cellulose under high intensity herbivory (Fig. 4c).

Discussion

In our study, we found that the mean overall percentage of leaf area removed was only 7%-10% in these subtropical tree species, which is lower than that seen in tropical forests (11%-15%; Coley and Barone 1996). We suspect that leaf herbivory varies between subtropical and tropical tree species due to differences in plants traits (phenology, morphology, nutrient status) and insect communities between forest types. However, we found that the mean percentage of leaves attacked was high (73% and 53% in deciduous and evergreen trees respecitively), especially for some subtropical trees such as *C. tibetana*, *C. axillaris*, *L. formosana*, which exceeded 90%. These results indicate that the percentage of leaf area removed does not fully account for the influence of herbivores at this site, and we should equally emphasize the importance of leaf-herbivore damage as an important factor, even when the area removed

by herbivores is low. Moreover, we found that the percentage of leaf area removed for deciduous species (10%) was higher than evergreen species (7%), which supports Hypothesis 1, and is consistent with other studies in tropical forests that evergreen species tend to exhibit lower intensity of leaf herbivory than deciduous species (Pringle et al. 2011, Silva et al. 2015), due to the higher toughness, lower water content, and higher C:N ratios in evergreen leaves (Schuldt et al. 2010). Interestingly, we also found that leaf herbivore damage for bamboo was much lower than other subtropical trees (using either measure of herbivory), which may be attributed to the silicon defense mechanisms of bamboo (Emamverdian et al. 2020). Studies suggest that high Si deposition in some plant tissues not only decreases palatability, but also enhances strength and rigidity, improving plant defenses against both biotic and abiotic stressors (Ma and Yamaji 2006, Mandlik et al. 2020).

In general, foliar secondary metabolites determine both leaf palatability and defensive capabilities (Kessler and Baldwin 2002, Kazakou et al. 2019), and contribute to decreased leaf herbivore attack (White and Whitham 2000). However, we found that the percentage of leaf area damaged was positively correlated with the concentrations of both tannins and lignin in leaves, and negatively correlated with the ratio of NSC:lignin, which was inconsistent with Hypothesis 2. We suspect that this pattern was mostly attributed to either the induction of defense compounds in attacked leaves or to long-term and frequent insect herbivore stress in several species, potentially demonstrating evidence of evolutionary arms races between trees and herbivores (Mello and Silva-Filho 2002, Becklin 2008, Anderson et al. 2010). Long-term co-evolutionary adaptations between phytochemical defenses and herbivory attack have been reported elsewhere (Arora 2012, Wöll et al. 2013, Gripenberg et al. 2010, Forister et al. 2012). In addition, our results suggest higher costs of construction and maintenance due to evolutionary adaptations, such as higher concentrations of tannins and lignin which agrees with other studies (Mithöfer and Boland 2012, Brandenburger et al. 2020). For example, Silva et al. (2015) found that the percentage of leaf area removed on each plant was positively related to the concentrations of both phenolic compounds and nitrogen.

In our study, we found that leaf herbivore attack decreased the concentrations of nutrients and NSC, and increased the concentrations of tannin and lignin for defense in some species, particularly under high herbivore intensities, which supports Hypothesis 3a. Moreover, the leaf defensive response of some deciduous species was stronger than most evergreen trees, particularly the increased concentrations of lignin, which supports Hypothesis 3b. Generally, plants develop chemical defenses through the production of secondary metabolic compounds to resist insect herbivory (Mitchell et al. 2016, Vidal and Murphy 2018). The concentration of defense compounds within the plant may well be a crucial factor in determining whether a leaf is eaten or not (Harborne 1991). Here, the degree of herbivory and the effectiveness of defenses varies widely among plant species due to variation in defense mechanisms. We found that the concentration of tannins increased greatly in response to higher levels of herbivory (> 50%), especially for species such as *L. formosana*, *S. discolor*, *E. japonicus*, *D. oldhamii*, which supports Hypothesis 3a. It is well known that chemical defense is relatively costly (Stamp 2003), and plants will make trade-offs between growth and chemical defense. Induced secondary plant metabolites may provide effective resistance against herbivores mostly when herbivory levels are high, which increases

defense and is in line with the optimal defence theory (Coley et al. 1985, Chapman et al. 2006, Ballaré and Austin 2019).

Here, we also found that there were no significant responses of foliar tannin production to leaf herbivory for some evergreen species, such as *P. pubescens*, *S. superba*, and *C. tibetana*, possibly due to reliance on different defensive mechanisms, such as silicon defense (Ma and Yamaji 2006, Mandlik et al. 2020) in bamboo or constitutive physical defense in some *Castanopsis* species (Peeters 2002, Onoda et al. 2011), which was not predicted by Hypothesis 3a. Indeed, leaves with high concentrations of Si, high toughness, many trichomes, or waxy surfaces, might negatively affect herbivore attack by decreasing palatability and digestibility (Yamawo et al. 2012).

In addition, we also found that high levels of herbivory (> 50% leaf area removed) greatly increased the concentrations of foliar lignin in most species, which was in line with Hypothesis 3a. Other studies have also found increased lignin produced by leaves following insect herbivory, contributing to increased leaf toughness, lower palatability, and lower digestibility (Marler and Dongol 2016). It is also possible that lignin values were higher because the more palatable tissues of the leaf were largely consumed by leaf herbivores, and higher lignin-containing veins remain, resulting in increased concentrations of lignin post-herbivory (Beck and Labandeira 1998). Overall, the leaf defensive response to herbivore attack for deciduous species was stronger than evergreen trees, particularly the concentration of lignin, which supports Hypothesis 3b. Some studies found that long-lived evergreen leaves have higher costs of construction and maintenance than leaves of deciduous species (Chaturvedi et al. 2011; Eamus 1999; Sobrado 1991), which may contribute to lower responses of defensive traits to herbivore attack in evergreen species.

Interestingly, we found that leaf herbivory decreased the concentration of foliar NSC in most species (Najar et al. 2014, Piper and Fajardo 2014), which supports Hypothesis 3a. Previous studies have shown that NSC was reduced in herbivore-attacked plant tissues, because resources are diverted away from the site of damage and into storage tissues (Newingham et al. 2007, Gómez et al. 2010, Quijano-Medina et al. 2019). On the other hand, herbivore attack can greatly weaken plant photosynthesis due to leaf area loss and tissue damage (Coley 1988, Zhang and Turner 2008, Bilgin et al. 2010, Visakorpi et al. 2020). It is also possible that plants will invest more in defense under leaf herbivore attack, instead of growth (Herms and Mattson 1992). In our study, we found that insect herbivory reduced foliar cellulose for most species, which was in agreement with other studies (Onoda et al. 2011). Cellulose, is a major component of plant cell walls, and can influence plant growth and stability (Taylor 2008). It has been reported that the enzymes secreted by insect herbivores can destroy the physical tissues of plant leaves, especially the plant cell wall, which is dominated by cellulose (Schowalter et al. 1986, Onoda et al. 2011), making the leaves more susceptible to pathogen infection due to weaker physical defenses.

Conclusion

Our study has revealed a higher intensity of herbivory for deciduous species than evergreen species in subtropical forests, whether examining the percentage of leaf area removed or percentage of leaves attacked. Moreover, leaf herbivore damage was positively correlated with the concentrations of tannins and lignin in leaves, and negatively correlated with the ratio of NSC:lignin, which confirms long-term and frequent insect herbivory stress and potentially evidence for evolutionary arms races between plant species and herbivores. Here, the degree of herbivory and the effectiveness of defenses varied widely among subtropical tree species due to variation in defensive mechanisms. Leaf defensive traits of deciduous species were more sensitive to leaf herbivore attack than evergreen trees. Collectively, the present work builds toward a better understanding of plant responses and defenses to varying intensities of herbivory, and explores underlying relationships between leaf traits and herbivore attack in subtropical forests.

Declarations

Acknowledgments

Conflict of interest

The authors declare they have no conflict of interest.

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Tables

Table 1. The comparison of foliar chemical traits between deciduous and evergreen subtropical tree species. Abbreviations: carbon (C), nitrogen (N), phosphorus (P), Non-structural carbohydrates (NSC). Significantly higher values are shown in bold.

| Chemical traits	Mean		t	Sig. (2-tailed)
	deciduous	evergreen		
C (%)	55.28	56.45	-1.357	0.179
N (%)	**2.22**	1.51	5.876	<0.001***
P (g/kg)	**1.40**	1.06	4.441	<0.001***
NSC (%)	**11.78**	9.30	3.718	<0.001***
Tannin (%)	1.38	1.34	0.224	0.823
Lignin (%)	23.67	23.84	-0.202	0.841
Cellulose (%)	21.99	21.84	0.166	0.869
Hemicellulose (%)	18.64	**19.72**	-2.691	0.009**
C/N	26.53	**40.91**	-5.686	<0.001***
N/Lignin	**0.10**	0.06	4.974	<0.001***
NSC/Lignin	**0.51**	0.39	3.96	<0.001***
NSC/Cellulose	**0.55**	0.43	3.785	<0.001***

*P<0.05, **P<0.01, ***P<0.001.

Figures
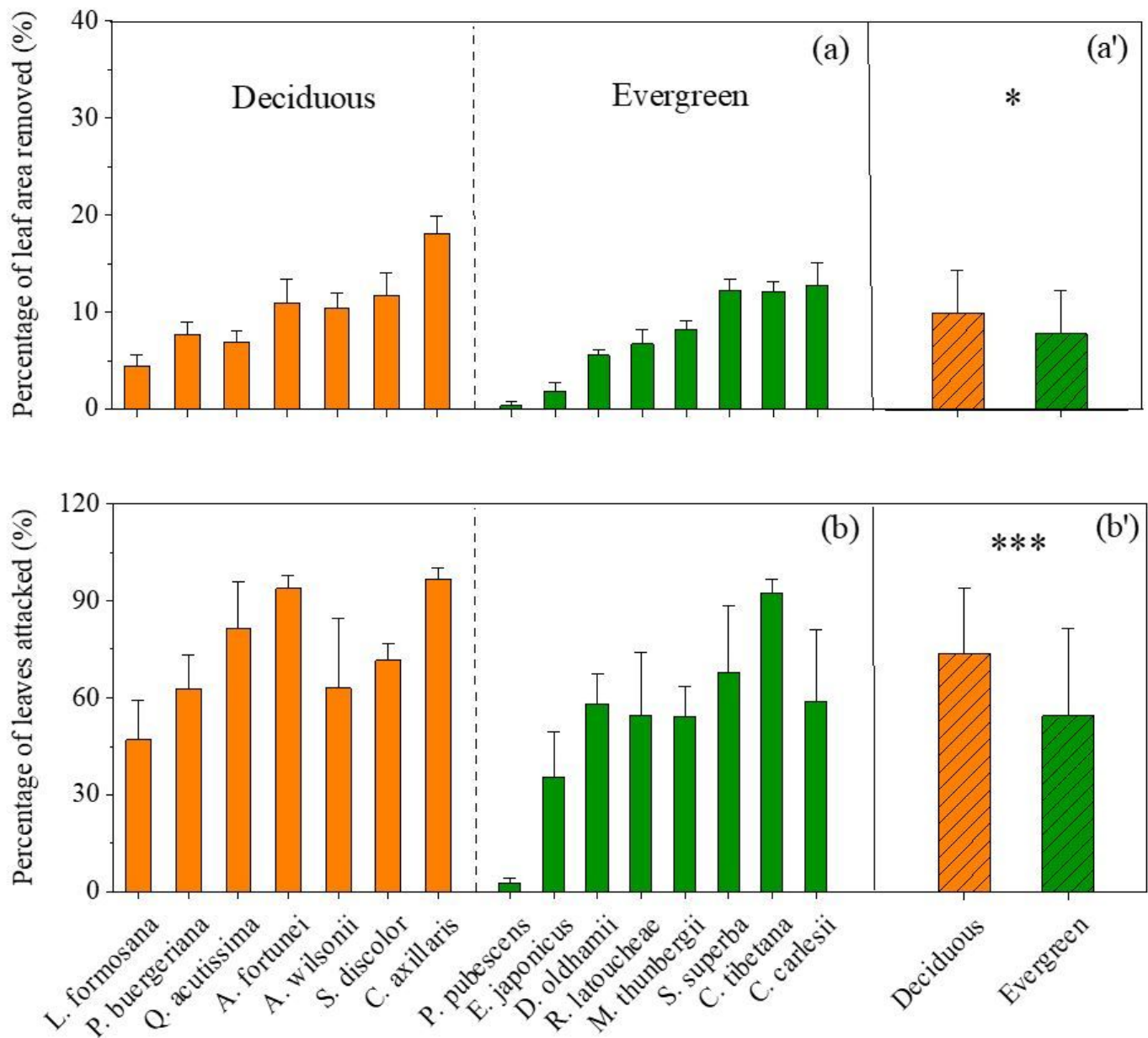
Figure 1

Mean (± 1 SE) percentage of leaf area removed (a, a') and the percentage of leaves attacked (b, b') in the Guanshan National Nature Reserve for leaves of 15 study species in 2020. Both deciduous (orange, n = 42) and evergreen (green, n = 48) species were included: *Liquidambar formosana*, *Padus buergeriana*, *Quercus acutissima*, *Alniphyllum fortunei*, *Acer wilsonii*, *Sapium discolor*, *Choerospondias axillaris*, and *Phyllostachys pubescens*, *Elaeocarpus japonicus*, *Daphniphyllum oldhamii*, *Rhododendron latoucheae*, *Machilus thunbergii*, *Schima superba*, *Castanopsis tibetana*, *Castanopsis carlesii*. Significant differences are denoted with *P<0.05, **P<0.01, ***P < 0.001.
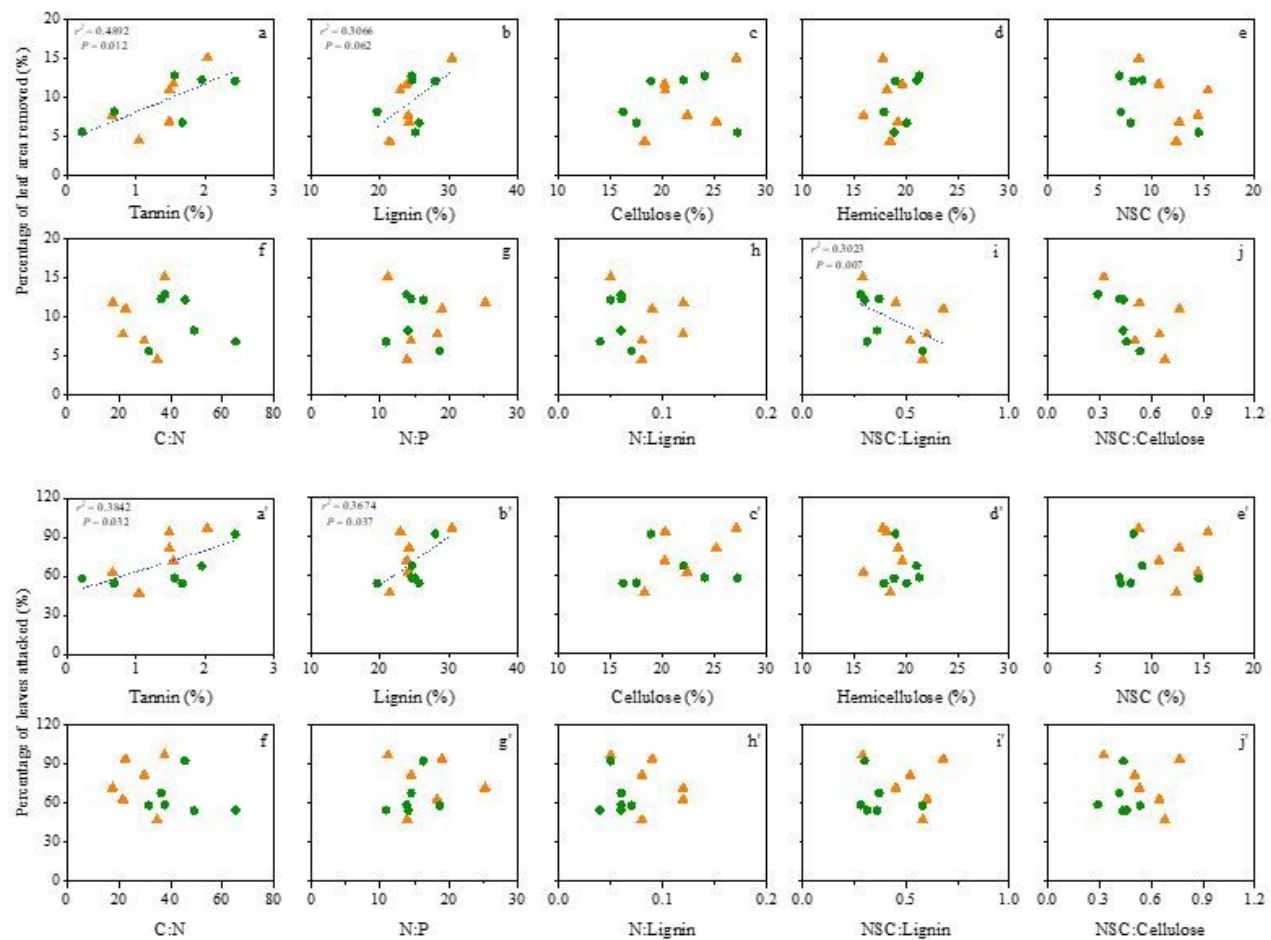
Figure 2 Linear relationships (Pearson's *r* and r^2) between percentage of leaf area removed (a-j), and the percentage of leaves attacked (a'-j') with various leaf traits for both deciduous (▲) and evergreen (●) subtropical species. Abbreviations: carbon (C), nitrogen (N), phosphorus (P), Non-structural carbohydrates (NSC). The data represent mean values per tree species (n = 6), and correlation lines are shown for significant relationships.

Figure 2

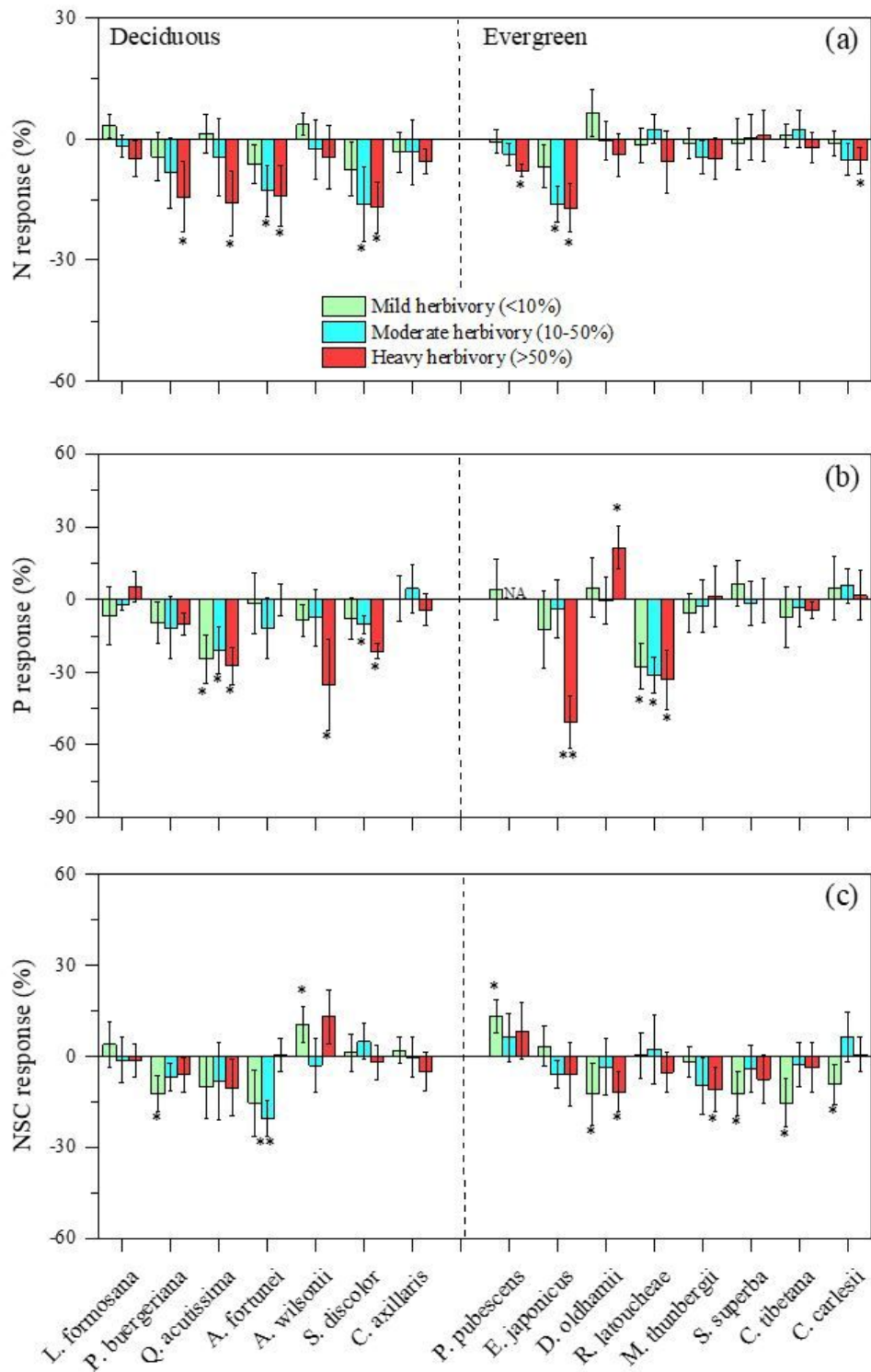See image above for figure legend.

Figure 3

Variation in leaf responses to herbivores presented as increased concentrations (positive values) or decreased concentration (negative values) of leaf: a) nitrogen (N), b) phosphorus (P), and c) Non-structural carbohydrates (NSC) for 15 subtropical tree species under varying intensities of herbivory (mean ± SE, n=6. Herbivory intensities were classified as low (<10%), medium (10-50%) and high (>50%). Both deciduous and evergreen species were included: *Liquidambar formosana*, *Padus buergeriana*,

Quercus acutissima, Alniphyllum fortunei, Acer wilsonii, Sapium discolor, Choerospondias axillaris, and *Phyllostachys pubescens, Elaeocarpus japonicus, Daphniphyllum oldhamii, Rhododendron latoucheae, Machilus thunbergii, Schima superba, Castanopsis tibetana, Castanopsis carlesii*. Significant deviations from zero are denoted as *P<0.05, **P<0.01. NA, not available
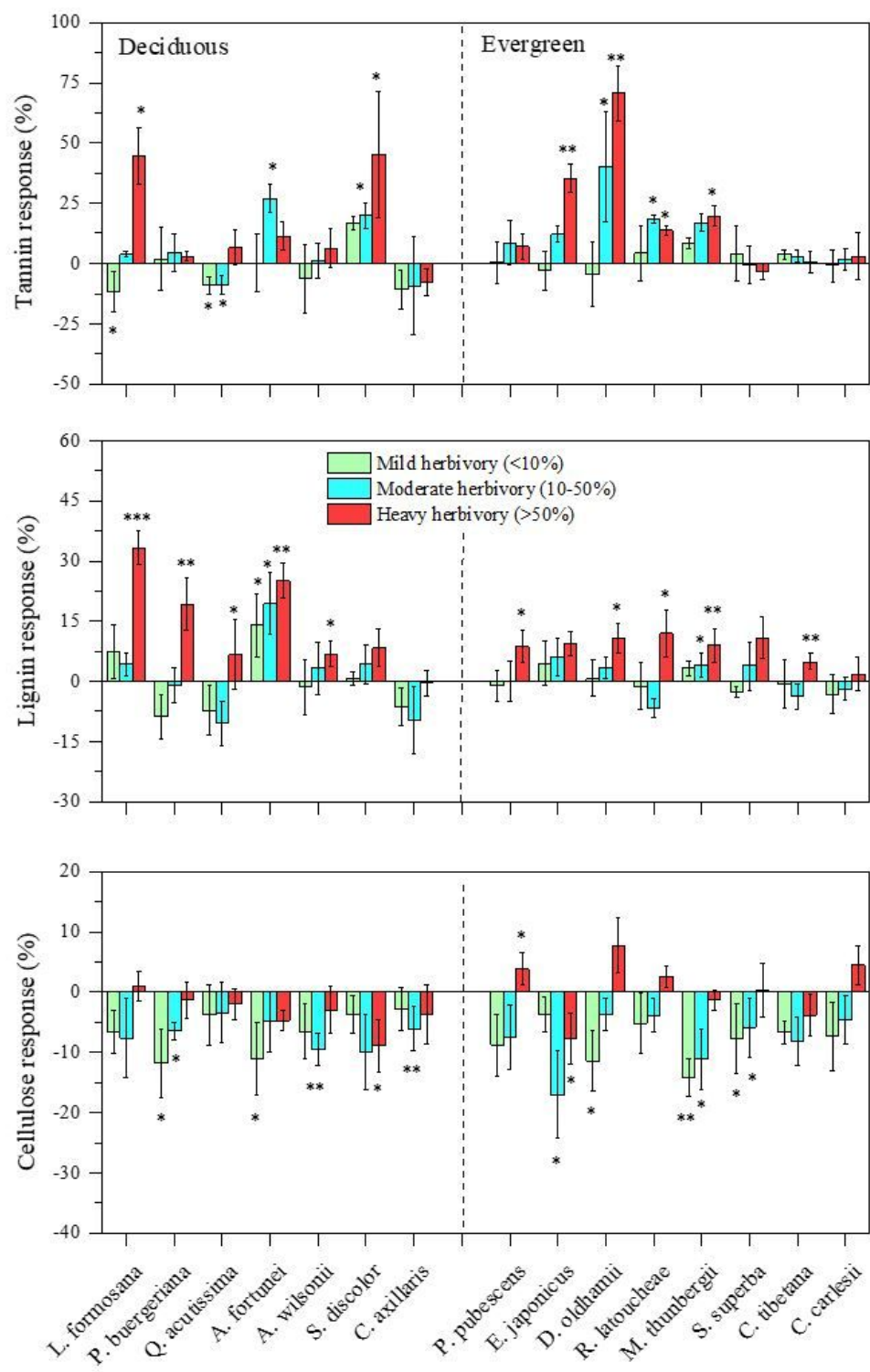

Figure 4

Variation in leaf response to herbivores presented as increased concentrations (positive values) or decreased concentrations (negative values) of leaf: a) tannins, b) lignin, and c) cellulose for 15 subtropical tree species under varying intensities of herbivory (mean ± SE, n=6. Herbivory intensities were classified as low (<10%), medium (10-50%) and high (>50%). Both deciduous and evergreen species were included: *Liquidambar formosana*, *Padus buergeriana*, *Quercus acutissima*, *Alniphyllum fortunei*, *Acer wilsonii*, *Sapium discolor*, *Choerospondias axillaris*, and *Phyllostachys pubescens*, *Elaeocarpus japonicus*, *Daphniphyllum oldhamii*, *Rhododendron latoucheae*, *Machilus thunbergii*, *Schima superba*, *Castanopsis tibetana*, *Castanopsis carlesii*. Significant deviations from zero are denoted as *P<0.05, **P<0.01.

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