New Phytologist

You are what you eat: nutrient and water relations between mistletoes and hosts

Yun-Bing Zhang[1,2], Marina Corrêa Scalon[3], Jing-Xin Liu[1], Xiao-Yang Song[1], Da Yang[1], Yong-Jiang Zhang[4], David S. Ellsworth[5] and Jiao-Lin Zhang[1,6]

[1]CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla 666303, Yunnan, China; [2]University of Chinese Academy of Sciences, No. 19(A) Yuquan Road, Shijingshan District, Beijing 100049, China; [3]Programa de Pós-graduação em Ecologia e Conservação, Universidade Federal do Paraná, Curitiba, PR 81531-990, Brazil; [4]School of Biology and Ecology, University of Maine, Orono, ME 04469, USA; [5]Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia; [6]Yuanjiang Savanna Ecosystem Research Station, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Yuanjiang, Yunnan 653300, China

Author for correspondence:
Jiao-Lin Zhang
Email: zjl@xtbg.org.cn

Received: *19 January 2022*
Accepted: *29 December 2022*

New Phytologist (2023) **238:** 567–583
doi: 10.1111/nph.18747

Key words: active uptake, carbon isotope ratio, hemiparasitic plants, mineral nutrients, nitrogen parasitism hypothesis, passive uptake.

Summary

- Mistletoes play important roles in biogeochemical cycles. Although many studies have compared nutrient concentrations between mistletoes and their hosts, no general patterns have been found and the nutrient uptake mechanisms in mistletoes have not been fully resolved.
- To address the water and nutrient relations in mistletoes compared with their hosts, we measured 11 nutrient elements, two isotope ratios and two leaf morphological traits for 11 mistletoe and 104 host species from four sites across a large environmental gradient in southwest China.
- Mistletoes had significantly higher phosphorus, potassium, and boron concentrations, nitrogen isotope ratio, and lower carbon isotope ratio ($\delta^{13}C$) indicative of lower water-use efficiency than hosts, but other elements were similar to those in hosts. Sites explained most of the variation in the multidimensional trait space. With increasing host nitrogen concentration, both mistletoe $\delta^{13}C$ and the difference between mistletoe and host $\delta^{13}C$ increased, providing evidence to support the 'nitrogen parasitism hypothesis'. Host nutrient concentrations were the best predictors for that of the mistletoe nutrient elements in most cases.
- Our results highlight the important roles of environmental conditions and host nutrient status in determining mistletoe nutrient pools, which together explain their trophic interactions with hosts in subtropical and tropical ecosystems.

Introduction

Mistletoes are obligate hemiparasitic aerial shrubs, which obtain mineral nutrients and water from their hosts via haustoria that penetrate host vascular tissues, serving as a connecting bridge between mistletoes and hosts (Kuijt, 1969; Teixeira-Costa, 2021; Teixeira-Costa & Davis, 2021). Previous studies have mainly focused on the impacts of mistletoes on their hosts and have clearly shown that mistletoes decrease host tree growth (Camarero *et al.*, 2019; Bilgili *et al.*, 2020), alter host wood and leaf anatomy (Teixeira-Costa & Ceccantini, 2015; Ozturk *et al.*, 2019), and exacerbate the water deficiency of hosts (Sangüesa-Barreda *et al.*, 2013; Tamudo *et al.*, 2021). However, mistletoes have recently been recognized as a beneficial keystone species in the ecosystem (Watson, 2009; Těšitel *et al.*, 2021), playing important roles in biogeochemical cycles (March & Watson, 2010; Ndagurwa *et al.*, 2020). Some positive influences of mistletoes include increasing the input of nutrient-rich litter due to low nutrient resorption efficiency (Scalon *et al.*, 2017), affecting the temporal–spatial litterfall patterns and nutrient dynamics due to their patchy distribution (March & Watson, 2010), hastening nutrient return by accelerating the decomposition of recalcitrant host litters (Ndagurwa *et al.*, 2020), and increasing soil nutrient concentrations beneath infected trees (Ndagurwa *et al.*, 2014; Muvengwi *et al.*, 2015). As such, mistletoes can contribute to boosting plant productivity and species composition (March & Watson, 2007; Ndagurwa *et al.*, 2016), apart from their negative effects on individual hosts as aforementioned. Therefore, a better understanding of nutritional interactions between mistletoes and hosts will provide insights into their functioning in ecosystems and mistletoe–host coevolution under global climate change scenarios (Türe *et al.*, 2010; Bell & Adams, 2011; Suriyagoda *et al.*, 2018; Fontúrbel, 2020).

Many mistletoe–host comparative studies on mineral nutrients have been conducted over the past 40 yr (Lamont, 1983; Ehleringer *et al.*, 1986; Glatzel & Geils, 2009; Scalon *et al.*, 2013; Al-Rowaily *et al.*, 2020). Theory based on such studies has posited that the higher transpiration rates of mistletoes than their hosts serve a nutrient-gathering role since mistletoes have evolved a lower energetic investment in haustoria than would be the case for free-standing plants that require structural and conducting roots (Schulze *et al.*, 1991). However, general patterns in broad-spectrum mistletoe–host mineral nutrient differences are lacking, which limits our understanding of the mechanisms underlying nutrient acquisition in mistletoes. One major reason behind this lack of knowledge lies in that few studies have included multiple nutrients (macronutrients and micronutrients) in their comparisons of mistletoes vs hosts although mistletoes would be expected to have a similar requirement for many of these nutrients as other plants. In the few such analyses, just a small number of mistletoe–host species pairs were considered (Panvini & Eickmeier, 1993; Scalon *et al.*, 2013), and hence, the robustness of these comparisons remains in question.

Regarding the mechanisms behind mistletoe–host nutrient patterns, two main competing hypotheses have been proposed involving either passive or active nutrient uptake by the mistletoe (Lamont, 1983; Panvini & Eickmeier, 1993; Scalon *et al.*, 2013). The passive nutrient uptake hypothesis suggests that nutrient acquisition is coupled with water flow into the mistletoes (see Schulze *et al.*, 1991, for example). Due to the lack of a phloem connection and no exchange of photosynthates with their hosts, nutrients are passively accumulated in mistletoes (Leonard & Hull, 1965). Many studies have found that the concentrations of a number of mineral nutrients are higher in mistletoe leaf tissues than in the hosts (Lamont, 1983; Glatzel & Geils, 2009). By contrast, the active nutrient uptake hypothesis assumes that nutrient and water uptake may not be so tightly coupled and that haustoria may play a role in selectively facilitating active nutrient acquisition and sequestration. Several case studies have indeed found that amino acids have been selectively metabolized in haustoria before their entry into the mistletoe xylem (Pate *et al.*, 1991; Escher & Rennenberg, 2006). In spite of the evidence behind passive and active nutrient uptake by mistletoes, there are still debates on mistletoe nutrient variation patterns, especially related to the uptake mechanism (Bell & Adams, 2011; Tennakoon *et al.*, 2011). More comprehensive sampling strategies, including the analyses of multiple nutrient elements and more mistletoe–host species pairs, are helpful to better understand the variation in mistletoe nutrient patterns.

Mistletoes typically have higher stomatal conductance and lower water use efficiency (WUE, as indicated by stable carbon isotope ratio: δ^{13}C) compared with their hosts (Ehleringer *et al.*, 1985; Scalon & Wright, 2015; Richards *et al.*, 2021), which could be related to their requirements to draw nutrients from their hosts (Ehleringer *et al.*, 1986; Bell & Adams, 2011) or their lower transpirational cost due to a lower investment in roots and stems compared with their hosts (Givnish, 1986; Richards *et al.*, 2021). Concerning the rate of nutrient and water uptake from hosts by mistletoes, the hypothesis that the strength of uptake by mistletoes could predict the status of mistletoe nutrients has not been rigorously examined. The uptake strength of mistletoes has been partially embodied by the difference between mistletoe and host δ^{13}C ($\delta^{13}C_{\text{M-H}}$). Only a few studies have measured both nutrient concentrations and transpiration (Glatzel, 1983; Schulze & Ehleringer, 1984), or parameters related to transpiration, such as δ^{13}C for both mistletoes and hosts (Ehleringer *et al.*, 1985; Panvini & Eickmeier, 1993; Bannister *et al.*, 2002; Tennakoon *et al.*, 2011). One hypothesis explaining the nutrient and water relations between mistletoes and their hosts is the 'N-parasitism hypothesis', which states that lower WUE and higher transpiration rate are a mechanism for mistletoes to acquire sufficient N, one of the most limiting macronutrients, from the host xylem sap (Schulze *et al.*, 1984; Schulze & Ehleringer, 1984). This hypothesis has been supported by several studies in which they found the relative WUE, as estimated by $\delta^{13}C_{\text{M-H}}$, to become smaller once mistletoes grew on nitrogen-fixing hosts or on hosts with higher N concentrations (Ehleringer *et al.*, 1985; Schulze *et al.*, 1991). By the same token, a 'P-parasitism hypothesis' has been proposed, which suggests that high transpiration by mistletoes may contribute to acquiring phosphorus from their hosts at P-limited sites (Scalon *et al.*, 2017). Also, a 'C-parasitism hypothesis' proposes that high transpiration by mistletoes is required because they can thus acquire greater amounts of heterotrophic carbon from their hosts (Marshall *et al.*, 1994; Wang *et al.*, 2008). Nevertheless, it still has not been tested whether $\delta^{13}C_{\text{M-H}}$ can predict other mistletoe element concentrations/mistletoe and host nutrient ratios.

The majority of the past δ^{13}C data on mistletoes had been sampled in semiarid (Ehleringer *et al.*, 1985; Marshall *et al.*, 1994; Wang *et al.*, 2008) or temperate areas (Panvini & Eickmeier, 1993; Bannister & Strong, 2001), with few such studies from tropical regions (Lüttge *et al.*, 1998; Tennakoon *et al.*, 2011). As a result, tropical mistletoes are still under-investigated (Těšitel *et al.*, 2021). There are large differences in water availability, seasonality, and temperature among tropical, temperate, and arid ecosystems, which may affect mistletoe physiology. Moreover, all these conditions likely affect nutrient availability to the hosts, which, in turn, will affect the vascular sap composition (Irving & Cameron, 2009). Habitat type plays a role in determining the different nutrition relationships between mistletoes and their deciduous hosts growing in wetland and semiarid habitats (Türe *et al.*, 2010), but comparative ecophysiological studies at multiple sites with different environmental conditions are relatively scant (Lüttge *et al.*, 1998; Türe *et al.*, 2010; Scalon *et al.*, 2017; Scalon & Wright, 2017; Richards *et al.*, 2021). Therefore, studies comparing nutrients and water-related parameters among different sites are needed to reveal the impacts of environmental conditions on the linkage of water and nutrient uptake between mistletoes and their hosts.

Mistletoe mineral nutrients are derived from elements dissolved in the host xylem, so the status of mistletoe nutrients should be highly related to those of the host (Glatzel & Geils, 2009). It has been assumed that the best predictor of mistletoe nutrient status is often (but not always) the host nutrient status (Glatzel & Geils, 2009). As a broad assertion for many different taxa, this mistletoe–host relation still lacks empirical evidence because many other important factors involved in mistletoe nutrient status have been neglected. For instance, legumes with high N concentrations are generally considered to be good hosts for hemiparasitic plants (i.e. *Phoradendron californiucm* Nutt.

growing on *Acacia greggii* A. Gray; Schulze & Ehleringer, 1984). Some studies have found that mistletoes have lower water flow resistance when they parasitize hosts with efficient water transport systems compared with parasitizing species with low water transport efficiencies (i.e. *Pthirusa maritima* Rizzini growing on *Conocarpus uvifera* Jacq.; Goldstein *et al.*, 1989). From this, we infer that host growth forms with different water transport efficiencies may influence the transport ability of mistletoes, thereby affecting the mistletoe leaf nutrient assimilation. Moreover, Panvini & Eickmeier (1993) have suggested that differences in climate, host resource acquisition strategy and host leaf phenology may lead to inconsistent mistletoe–host nutrient patterns. In addition, mistletoe leaf structural traits, such as specific leaf area (SLA) and leaf dry matter content (LDMC), may also influence nutrient assimilation and balance, by reducing the volume and mass of photosynthetically active, nonstorage cells, as proved in nonparasitic plants and mistletoes (Domínguez *et al.*, 2012; de la Riva *et al.*, 2018; Richards *et al.*, 2021). All these factors mentioned previously may influence mistletoe nutrient acquisition, transportation and assimilation, and ultimately mistletoe nutrient status. Until now, there is no such study integrating the host nutrient status, parameters related to transpiration (δ^{13}C or $\delta^{13}C_{M\text{-}H}$), site (a proxy for climate and soils), mistletoe leaf traits (SLA and LDMC), host growth form, leaf habit, and nitrogen-fixing type. Such a study is needed to comprehensively investigate the influences of these factors on the mistletoe nutrient concentrations and their relative importance in driving these trophic interactions. Such integrative studies can also contribute to a better understanding of the nature of nutrient interactions between mistletoes and their hosts.

The high mistletoe diversity in southwest China provides a perfect system to investigate host and environmental determinants of mistletoe nutrient and water relations (Zhang *et al.*, 2018; Jiang & Zhang, 2021). Despite the high mistletoe diversity and endemism in China, few studies have examined the nutrient and water relations of mistletoes with few mistletoe–host species pairs included (Chen *et al.*, 2013; He *et al.*, 2021). Here, we presented the first regional data on 11 nutrient elements, two isotope ratios, and two leaf morphological traits of 121 mistletoe–host species pairs (a total of 344 individual pairs) sampled from four sites (Lijiang, Ailaoshan, Xishuangbanna, and Yuanjiang) in Yunnan Province, southwest China, in which there are distinct differences in water availability and temperature/climate. In the framework of the passive vs active nutrient uptake hypothesis, we aimed at addressing the following questions with their attendant hypotheses:

(**1**) Are mistletoes different from their hosts in terms of leaf nutrients and stable isotope ratios? Do these differences vary among sites with different environmental conditions? We hypothesized that mistletoes exhibit more nutrient accumulation and lower water use efficiency (lower δ^{13}C) compared with their hosts because mistletoes have a lower cost to acquire water and nutrients, and thus more aggressive water use (Givnish, 1986; Scalon & Wright, 2015; Richards *et al.*, 2021). We also predicted that sites may explain a high proportion of variation in nutrient and isotope comparisons because water and temperature gradients influence the capacity of hosts to acquire nutrients and the transpiration of mistletoes.

(**2**) Is the 'N-parasitism hypothesis' supported by our multiple-site data? We hypothesized that the $\delta^{13}C_{M\text{-}H}$ becomes smaller when mistletoes grow on nitrogen-fixing hosts or on hosts with higher N concentrations as previous studies demonstrated (Ehleringer *et al.*, 1985; Schulze *et al.*, 1991).

(**3**) What is the best predictor for mistletoe nutrient concentrations/mistletoe and host nutrient ratios and isotope ratios? Because mistletoes absorb mineral nutrients and water from their hosts (Kuijt, 1969; Glatzel & Geils, 2009), we hypothesized that host nutrient status and corresponding isotope ratio may be the most important predictor for mistletoe nutrient concentrations and isotope ratios, respectively. In addition, we expected that the environment (as indicated by sites in different climates) and $\delta^{13}C_{M\text{-}H}$ would play important roles in explaining mistletoe nutrient concentrations.

Materials and Methods

Sites and species

This study was conducted at four sites in Yunnan Province, southwest China: Lijiang, Ailaoshan, Xishuangbanna, and Yuanjiang (Table 1; Supporting Information Fig. S1). These four sites are characterized by distinct climates described by differences in precipitation, aridity index, and average temperature conditions. The mean annual precipitation varied more than twofold from 733 to 1778 mm across the four sites. The mean annual temperature of these sites also varied considerably, with 7.2°C, 11.3°C, 22.7°C and 24.7°C for Lijiang, Ailaoshan, Xishuangbanna, and Yuanjiang, respectively. Most of the rainfall at all these sites occurs in the rainy season (generally, May–October). More detailed site climate and soil properties are provided in Table 1.

In Lijiang, Ailaoshan and Yuanjiang, we selected mistletoe–host pairs from relatively open secondary forests, roadside and disturbed locations, in which there is high availability of mistletoe infection compared with primary forests and mistletoes were accessible. In Xishuangbanna, mistletoe–host pairs were collected from a common garden (Xishuangbanna Tropical Botanical Garden, hereafter XTBG). We chose the most common mistletoes in the four sites for this study (Table S1), including relative generalist and relative specialist mistletoes. For host species, we tried to include different growth forms (tree, shrub, and liana), different leaf habits (evergreen and deciduous), and different nitrogen-fixing types (nitrogen-fixing and non-nitrogen-fixing) to test whether this kind of host life-history classification will influence the mistletoe nutritional ecological strategies. In total, we sampled 344 mistletoe–host individual pairs (121 unique mistletoe–host species pairs), consisting of 11 mistletoe species (belonging to seven genera and two families) and 104 host species (belonging to 79 genera and 42 families). We sampled 48 mistletoe–host individual pairs (18 mistletoe–host species pairs), 74 mistletoe–host individual pairs (27 mistletoe–host species pairs), 202 mistletoe–host individual pairs (68 mistletoe–host species pairs), and 20 mistletoe–host individual pairs (eight mistletoe–host

Table 1 Basic information of the four study sites with simple indices of climate and soil characteristics.

Study site	Lijiang	Ailaoshan	Xishuangbanna Tropical Botanical Garden	Yuanjiang
Vegetation type	Subalpine cold temperate forest	Subtropical montane forest	Tropical seasonal rainforest	Valley savanna
Coordinates	27°00′N, 100°10′E	24°32′N, 101°01′E	21°41′N, 101°25′E	23°28′N, 102°10′E
Elevation (m asl)	3240	2450	580	481
Mean annual precipitation (mm)	1100	1778	1447	733
Mean annual temperature (°C)	7.9	11.3	22.7	24.7
Aridity index[1]	1.62	1.44	0.96	0.33
Soil pH	4.92	4.24	5.41	7.88
Soil organic matter (mg g^{-1})	7.2	170.4	46.91	87.73
Total soil N concentration (mg g^{-1})	0.6	5.64	3.07	3.96
Total soil P concentration (mg g^{-1})	0.1	0.97	0.69	1.3
Total soil K concentration (mg g^{-1})	486.27	9.46	10.84	12.72
Soil available N concentration (mg kg^{-1})	2.13	51.17	126.9	206.9
Soil available P concentration (mg kg^{-1})	1.1	11.10	4.22	13.28
Soil available K concentration (mg kg^{-1})	86.47	185.4	88.2	576.3

[1] Aridity index was computed according to Nastos *et al*. (2013) using the meteorological parameters at each site.

species pairs) in Lijiang, Ailaoshan, XTBG, and Yuanjiang, respectively (Table S1).

We chose the host individuals infected by mistletoes following approaches from previous studies (Scalon *et al*., 2013; Richards *et al*., 2021). Most of the sampled individuals of the host species were only parasitized by one mistletoe species, but four host species were found to be parasitized by two mistletoe species (Table S1). In most cases, we sampled three infected individuals for each mistletoe–host species pair. Due to low availability in the field, for few mistletoe–host species pairs, we found only one or two infected hosts. For each mistletoe–host species pair, we sampled mistletoe leaves from the infected branches with similar size and host leaves from uninfected branches, completely free of haustorium infection from the same host tree. All sampling was conducted in the rainy season of 2019 (from August to October).

Functional traits

We measured 15 leaf functional traits including leaf nutrient concentrations of 11 mineral elements, stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope ratios (‰), specific leaf area (SLA, $cm^2\ g^{-1}$), and leaf dry matter content (LDMC, $g\ g^{-1}$), according to the standard protocol proposed by Pérez-Harguindeguy *et al*. (2013). Two sun-exposed distal branches from the outer canopy of each individual were collected using a pole pruner. If some mistletoe-infected branches were too high to access, combined methods of climbing and a long pole pruner (> 5 m) were used.

We chose three sun-exposed, fully expanded healthy leaves in each branch to measure SLA and LDMC. For compound leaf species, all leaflets of each compound leaf were selected. The fresh leaf or leaflet area with petioles removed was determined by a flatbed scanner with 300-dpi resolution. Leaves or leaflets were then immersed in water for 12 h, and their saturated weights were weighed using a balance (AL204; Mettler Toledo, Shanghai, China). Then, leaves were oven-dried at 80°C for at least 48 h to constant mass and weighed. The scanned pictures were analyzed using the ImageJ software (National Institutes of Health, Bethesda, MD, USA). The SLA was calculated as leaf area divided by the leaf dry mass. The LDMC was determined by leaf dry mass divided by saturated weight.

Fresh leaf samples comprising > 10 leaves or > 50 leaflets for each individual were oven-dried at 80°C for at least 48 h, then finely ground, and screened through a 60-mesh sieve for further analysis. Leaf total phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), zinc (Zn), and boron (B) concentrations (mg g^{-1}) were measured using an inductively coupled plasma atomic-emission spectrometer (iCAP7400; Thermo Fisher Scientific, Bremen, Germany) after that leaf samples were digested with 8 ml HNO_3 (65–68%) + 4 ml $HClO_4$ (70–72%) and then dissolved in 4 ml HCl (3 M).

The prepared leaf powder samples screened through 100-mesh sieve were delivered to the Stable Isotope Laboratory of Shenzhen Huake Precision Testing Inc., Shenzhen, China, to analyze the total carbon (C) and nitrogen (N) concentrations (mg g^{-1}), δ^{13}C and δ^{15}N. They were measured using an elemental analyzer (Flash 2000HT; Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled with an isotope ratio mass spectrometer (Finnigan Delta V Advantage; Thermo Fisher Scientific). Stable isotope ratios were expressed in δ-unit notation, defined as follows:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}}\right) - 1\right] \times 1000‰$$

where X is ^{13}C or ^{15}N, and R_{sample} is either the sample $^{13}C/^{12}C$ ratio for carbon or $^{15}N/^{14}N$ ratio for nitrogen. The R_{standard} of the ^{13}C and ^{15}N is the Pee Dee Belemnite standard and atmospheric N_2, respectively. The analytic precision reported was ±0.1‰ for δ^{13}C and ±0.2‰ for δ^{15}N, respectively.

Statistical analysis

Natural logarithm-transformed values were used to improve the normality in all leaf functional traits, except for δ^{13}C and δ^{15}N, which were normally distributed. All the statistical analyses were based on individual data ($n = 344$), except for special cases such as for mistletoe–host species pairs where $n = 121$.

We used linear mixed-effects models (LMMs) to test whether each measured leaf trait differed between plants of different life forms (mistletoe vs host) and different sites and between species of different life forms among different sites (life forms × sites). In each model, C, N, P, K, Ca, Mg, S, Fe, Mn, Zn, B, δ^{13}C, δ^{15}N, SLA, and LDMC were dependent variables. Life forms, sites, and their interactions were fixed factors. The species pair ID (to account for the intrinsic paired data structure) and species (to account for nonindependent sampling design) were random effects in the model. If the interaction was not significant, then we refitted the model with the main effects only. The LMMs were fit using the *lmer* function of the LME4 package (Bates *et al.*, 2015). The statistical significance of fixed factors and random effects was assessed by Type III ANOVA with Satterthwaite's approximation of denominator degrees of freedom and likelihood ratio tests, respectively, in the LMERTEST package (Kuznetsova *et al.*, 2017). We used the *check_model* function in the PERFORMANCE package to check the residual normality and homoscedasticity (Lüdecke *et al.*, 2021). The results of LMMs were presented using *tab_model* function in the SJPLOT package (Lüdecke, 2021). The EMMEANS package was used to extract the predicted estimates and 95% confidence intervals to construct figures (Lenth, 2021).

We used permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) to assess whether mistletoes and hosts from different sites occupy different positions in the multidimensional trait space (number of permuted data sets = 1000) based on mistletoe–host species pair mean values ($n = 121$). The full model included life forms and sites due to their nonsignificant interactions. Analyses were conducted using the *adonis* function in the VEGAN package (Oksanen *et al.*, 2013). To summarize and visually describe the differences among traits of mistletoe–host species pairs in four study sites, a principle component analysis (PCA) was run through the *princomp* function in the STATS package.

To reveal factors influencing the difference between mistletoe and host δ^{13}C ($\delta^{13}C_{\text{M-H}}$), we included multiple factors in the LMMs, that is, host N, mistletoe N, sites, mistletoe SLA, mistletoe LDMC, host growth form, host leaf habit, and host nitrogen-fixing type. As statistical considerations prevented having both host δ^{13}C and mistletoe δ^{13}C simultaneously in one model for interpreting the $\delta^{13}C_{\text{M-H}}$, we also included mistletoe δ^{13}C and host δ^{13}C separately to test the influences of other factors. Thus, we performed two independent LMMs with the only difference being host δ^{13}C (model 1) or mistletoe δ^{13}C (model 2), with all other predictors kept the same. The random factors were mistletoe and host species. In both models, we used the *dredge* function of the MUMIN package (Bartoń, 2020) to select the best model. To test the 'N-parasitism hypothesis', that is, whether host N and $\delta^{13}C_{\text{M-H}}$ were positively correlated, we used the *plot_model* function of the SJPLOT package to extract the predicted results of model 1 (with host N and $\delta^{13}C_{\text{M-H}}$ having significant correlation), while controlling other factors. We finally partitioned the variance explained by each fixed variable, random effects and residuals using the *partR2* function in the PARTR2 package (Nakagawa & Schielzeth, 2013).

Mistletoe and host nutrient ratios have been used to represent whether mistletoes significantly accumulate higher nutrients than their hosts (Lamont, 1983; Panvini & Eickmeier, 1993), with the ratio > 1 indicating mistletoes accumulate more nutrients than their hosts. To evaluate which predictable variable was the best predictor for the mistletoe nutrient concentrations/mistletoe and host nutrient ratios and isotope ratios, we fitted all possible linear mixed-effects models that included combinations of eight explanatory variables. These were host nutrient concentrations (proxy for each corresponding nutrient of mistletoe, i.e. for mistletoe C, the corresponding host nutrient concentration was host C), $\delta^{13}C_{\text{M-H}}$, sites, mistletoe LDMC and SLA, host growth form, leaf habit, and nitrogen-fixing type. These eight variables were fixed factors; host species and mistletoe species were random factors; each mistletoe nutrient concentration/mistletoe and host nutrient ratio or isotope ratio was the response variable. The automated model selection procedures were performed using the *dredge* function of the MUMIN package. We determined the random effects using restricted maximum likelihood (REML) together with the full fixed effects model. Subsequently, fixed effects were selected using maximum likelihood with the chosen random effects. Coefficients for parameters of the final models were estimated using REML (Baayen *et al.*, 2008). We also checked multicollinearity among predicted variables with variance inflation factor (VIF) values > 3 (Zuur *et al.*, 2010) using the *vif* function in the CAR package. Because there were no variables with VIF > 3 in this study, none of the variables were excluded. We selected the best-fitting model with the lowest Akaike information criterion (AICc) value. We only reported the most parsimonious model instead of model averaging for those with several models with delta AICc < 2. After model selection, we refitted the final model with the chosen variables using REML and then partitioned the variance explained by each fixed variable, random effects and residuals using the *partR2* function in the PARTR2 package.

The LMMs were fitted to test whether regression relationships between mistletoe and host nutrient and isotope ratios differed among four sites. Host nutrient concentrations, sites, and their interactions were fixed effects; mistletoe and host species were random effects; each mistletoe nutrient concentration was the dependent variable. The predicted results of LMMs were presented using the *ggpredict* function of the GGEFFECTS package (Lüdecke, 2018). All analyses were performed in R v.4.0.2 (R Core Team, 2020).

Results

Differences in nutrients, stable isotope ratios, and leaf morphological traits between mistletoes and hosts

Leaf C, K, Ca, Mn, and SLA showed significant interactions between life forms (mistletoe vs host) and four sites (Fig. 1; Tables 2, S2). There were significant differences in P, K, B, δ^{13}C,

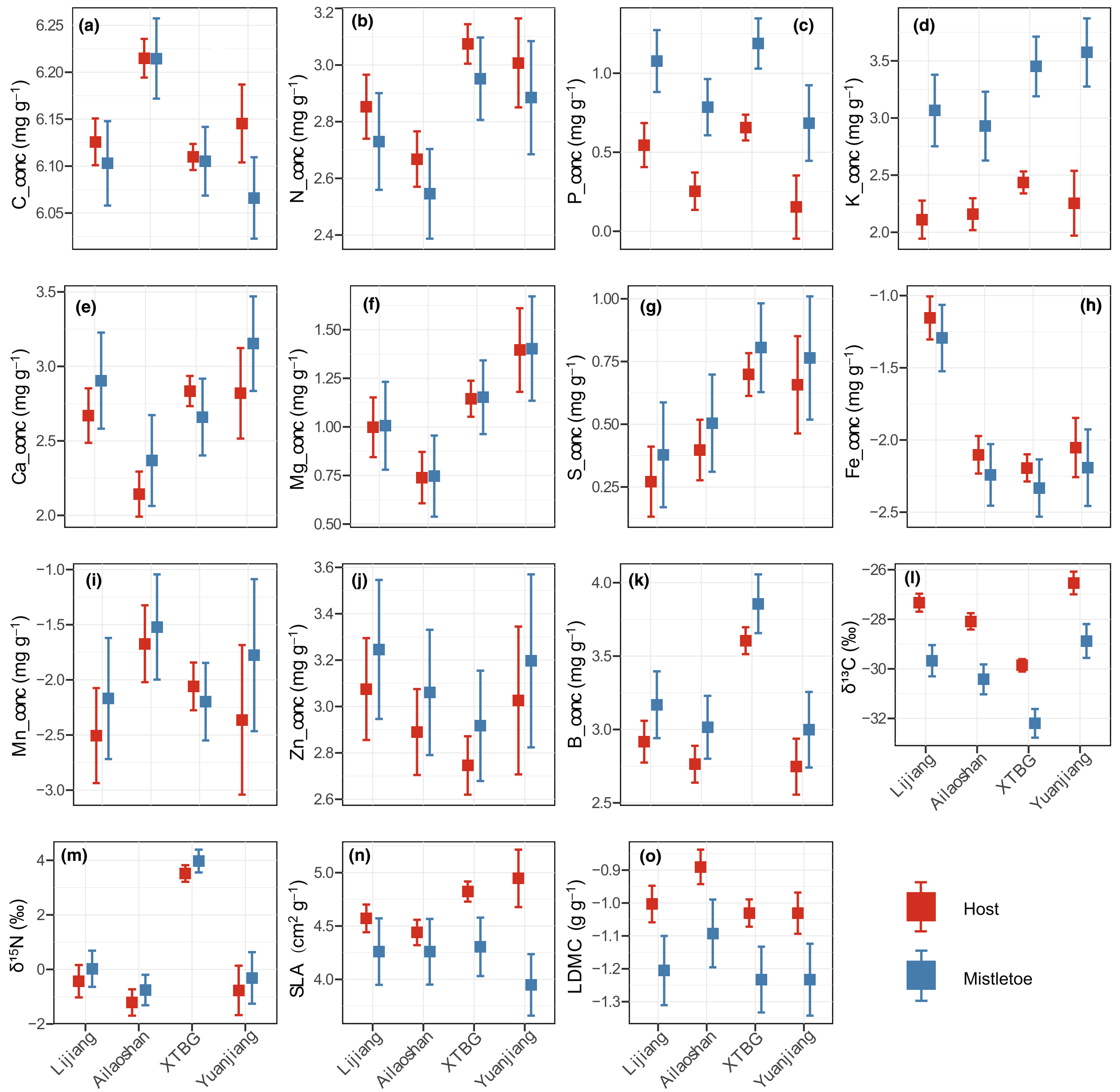

Fig. 1 Comparison of leaf chemical and functional traits among mistletoes and hosts from four sites, with 'conc' indicating concentration. (a) carbon (C), (b) nitrogen (N), (c) phosphorus (P), (d) potassium (K), (e) calcium (Ca), (f) magnesium (Mg), (g) sulfur (S), (h) iron (Fe), (i) manganese (Mn), (j) zinc (Zn), (k) boron (B), (l) carbon isotope ratios (δ^{13}C), (m) nitrogen isotope ratios (δ^{15}N), (n) specific leaf area (SLA), and (o) leaf dry matter content (LDMC). Means and 95% confidence intervals (as shown by horizontal bars) are shown using the prediction results based on linear mixed-effects models (Supporting Information Table S2). Red and blue colors indicate hosts and mistletoes, respectively.

δ^{15}N, SLA, and LDMC between mistletoes and hosts (all $P < 0.05$), but not in the other nutrients. Specifically, mistletoes had significantly higher P, K, B, and δ^{15}N, but lower δ^{13}C, SLA, and LDMC than their hosts. By contrast, all traits exhibited significant differences among sites, except for SLA with a marginally significant difference ($P = 0.091$). The influence of sites on SLA could not be excluded because of the significant interactions between life forms and sites in SLA ($P < 0.001$). In addition, random effects, namely species pair ID and species, showed significant influences on the comparison of nutrients, stable isotope ratios, and leaf morphological traits between mistletoes and hosts (both $P < 0.05$; Tables 2, S2). Moreover, models with the random components had better fits (with the lowest AIC values) than models without them in all cases (Table S3).

Table 2 ANOVA results of linear mixed-effects model for each trait based on individual data.

	Fixed effects						Random effects			
	Life forms		Sites		Life forms × Sites		Species pair ID		Species	
Trait	*F*	*P*	*F*	*P*	*F*	*P*	LRT	Pr (> χ^2)	LRT	Pr (> χ^2)
C	3.07	0.084	34.49	< **0.001**	4.49	**0.005**	45.95	< **0.001**	142.46	< **0.001**
N	2.95	0.090	15.36	< **0.001**	ns		109.19	< **0.001**	140.25	< **0.001**
P	48.50	< **0.001**	17.06	< **0.001**	ns		181.59	< **0.001**	91.21	< **0.001**
K	88.57	< **0.001**	4.24	**0.006**	2.99	**0.033**	92.01	< **0.001**	195.19	< **0.001**
Ca	2.10	0.151	16.21	< **0.001**	4.94	**0.003**	120.72	< **0.001**	120.02	< **0.001**
Mg	0.01	0.934	11.55	< **0.001**	ns		224.97	< **0.001**	187.02	< **0.001**
S	1.53	0.220	9.85	< **0.001**	ns		187.46	< **0.001**	245.12	< **0.001**
Fe	1.99	0.164	60.71	< **0.001**	ns		32.14	< **0.001**	77.21	< **0.001**
Mn	3.25	0.076.	3.11	**0.026**	6.27	**0.001**	527.69	< **0.001**	74.19	< **0.001**
Zn	2.28	0.135	2.86	**0.037**	ns		190.42	< **0.001**	99.87	< **0.001**
B	6.43	**0.013**	60.74	< **0.001**	ns		125.23	< **0.001**	195.95	< **0.001**
$\delta^{13}C$	64.11	< **0.001**	110.94	< **0.001**	ns		12.60	< **0.001**	147.85	< **0.001**
$\delta^{15}N$	7.83	**0.010**	118.73	< **0.001**	ns		411.27	< **0.001**	15.02	< **0.001**
SLA	20.21	< **0.001**	2.18	0.091.	7.46	< **0.001**	208.00	< **0.001**	612.08	< **0.001**
LDMC	15.85	< **0.001**	13.35	< **0.001**	ns		5.33	**0.021**	326.71	< **0.001**

Life forms (mistletoe vs host), sites (Lijiang, Ailaoshan, Xishuangbanna, and Yuanjiang) and their interactions were fixed factors, and their values were *F*- and *P*-values. Species pair ID (an intrinsic paired attribute) and species (nonindependent sampling design) were random factors, and their values were likelihood ratio and Pr (> χ^2)-values. Degrees of freedom for fixed effects were estimated using Satterthwaite estimations. 'ns' indicates nonsignificant interaction term, which was dropped off during model selection. Bold indicates significant term.

Life forms and sites were separated in the multidimensional functional space we sampled (both $P < 0.05$; Table 3). The proportions of the variation explained by sites and life forms were 41.0% and 10.5%, respectively. Results of PCA based on 15 functional traits of 121 mistletoe–host species pairs from four sites (Fig. 2) supported the results of LMMs based on individual data (Table 2) and of PERMANOVA based on mistletoe–host pair mean data (Table 3). The first and second principal components accounted for 27.0% and 16.2% of the total variation, respectively (Fig. 2). Species distribution along the first axis was positively associated with C and LDMC and negatively correlated with P, K, and B (Fig. 2; Table S4). Along the second axis, there was a separation between mistletoes and hosts, with mistletoe species associated with higher K, P, and lower $\delta^{13}C$, N, and SLA, while hosts were on the opposite side.

Parameters related to the 'N-parasitism hypothesis'

We attempted to integrate multiple factors to interpret the difference between mistletoe and host $\delta^{13}C$ ($\delta^{13}C_{M\text{-}H}$). We did find a positive correlation between host N and $\delta^{13}C_{M\text{-}H}$ ($P < 0.001$, Fig. 3; Table 4, model 1). However, host growth form, nitrogen-fixing type, and leaf habit did not influence the $\delta^{13}C_{M\text{-}H}$ (each showed $P > 0.05$; Table 4, model 1; Fig. S2). In model 2, host leaf habit had a significant influence on $\delta^{13}C_{M\text{-}H}$, despite little variance explained ($r^2 = 0.0138$). The $\delta^{13}C_{M\text{-}H}$ declined with host $\delta^{13}C$ (model 1), but increased with mistletoe $\delta^{13}C$ (model 2; Table 4), with the variance explained by host $\delta^{13}C$ and mistletoe $\delta^{13}C$ being 50.07% and 34.01%, respectively. Although sites had a significant influence in both models, the variance explained can be ignored ($r^2 < 0.0001$). In addition, we found that host N was positively correlated with mistletoe $\delta^{13}C$ ($P < 0.001$), while mistletoe N was not correlated with mistletoe $\delta^{13}C$ ($P > 0.05$, Table 5).

Table 3 PERMANOVA on the Euclidean distance of 15 functional traits for 121 mistletoe–host species pair mean values.

Predictors	Degree of freedom	Sum of squares	Mean squares	*F*	r^2	*P*
Life forms	1	444.9	444.95	51.551	0.105	**0.001**
Sites	3	1729.3	576.43	66.785	0.410	**0.001**
Residual	237	2045.6	8.63		0.485	
Total	241	4219.8			1	

Life forms and sites were predictable variables. The response variables were 15 traits. The interaction term was not significant. Bold indicates significant term.

Predictors for the mistletoe nutrient status and isotope ratios

Host $\delta^{13}C$, host N, mistletoe SLA, and sites significantly influenced mistletoe $\delta^{13}C$, while sites explained the highest proportion of the variation ($P < 0.05$, Table 5). Although host nitrogen-fixing type and sites influenced mistletoe $\delta^{15}N$, host $\delta^{15}N$ explained most of the variation, with mistletoe $\delta^{15}N$ increasing with host $\delta^{15}N$ ($P < 0.05$, Table 5). In most cases, we found that host nutrient concentrations played the most important role in explaining mistletoe nutrient concentrations (all $P < 0.001$, Fig. 4; Tables 6, S5, S6). However, host nutrients alone did not explain the pattern of mistletoe nutrients, and other variables also explained parts of the variation. The variation explained by sites was larger than the host nutrient status for mistletoe C, K, and Fe concentrations. Mistletoe C and K concentrations decreased with $\delta^{13}C_{M\text{-}H}$, but mistletoe

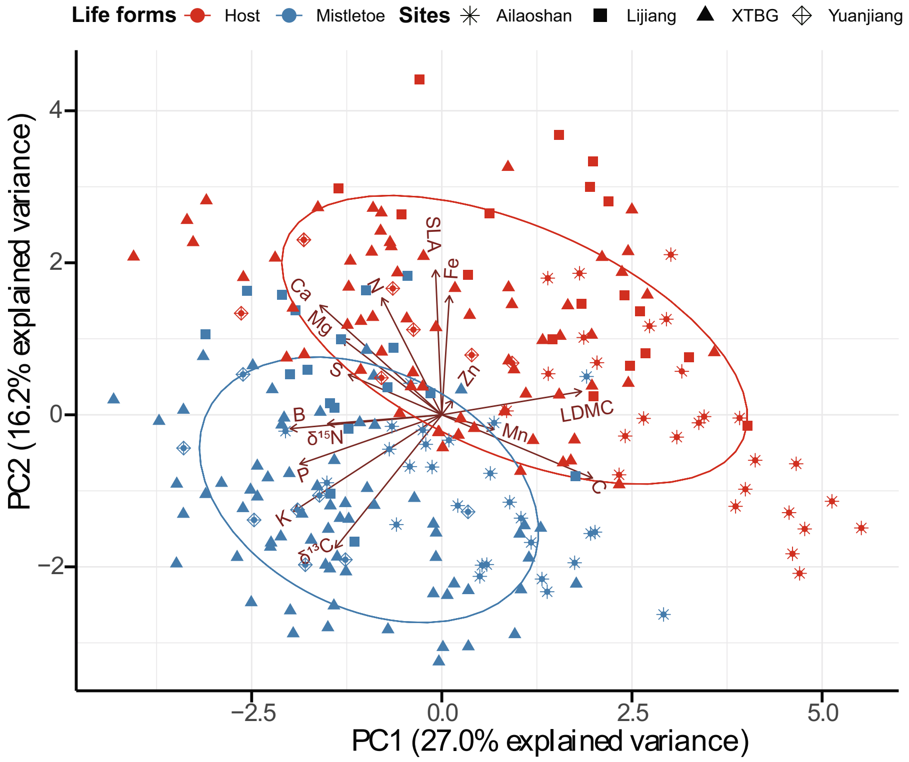

Fig. 2 Biplot of the first two axes of the principal component (PC) analysis for the 15 leaf functional traits and the loadings of the 121 mistletoe–host species pairs from Lijiang (square, $n = 18$), Ailaoshan (star, $n = 27$), Xishuangbanna Tropical Botanical Garden (triangle, $n = 68$), and Yuanjiang (diamond, $n = 8$). Red and blue colors indicate hosts and mistletoes, respectively. All variables were log-transformed before analysis, except for δ^{13}C and δ^{15}N.

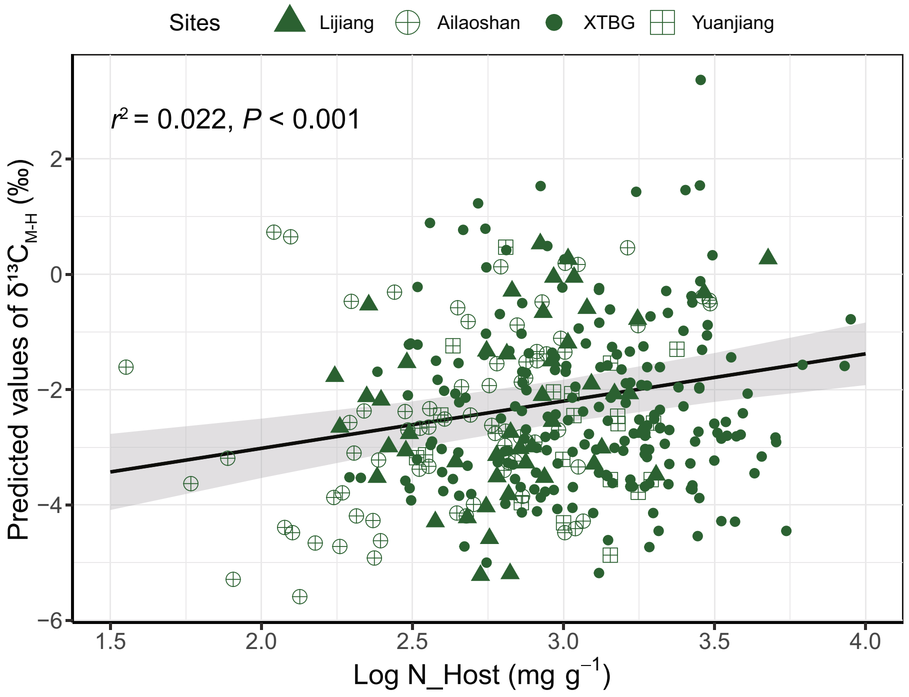

Fig. 3 Regression relationship between the difference between mistletoe and host δ^{13}C ($\delta^{13}C_{M\text{-}H}$) and host N, based on the predicted results of the best model of linear mixed-effects models (see model 1 in Table 4). The black line represents model-fitted slope, gray shading denotes 95% confidence intervals, and each point indicates an individual value for 344 mistletoe–host individual pairs (Lijiang: triangle, $n = 48$; Ailaoshan: open circle, $n = 74$; Xishuangbanna Tropical Botanical Garden: solid circle, $n = 202$; Yuanjiang: square, $n = 20$). r^2 is the variation explained by host N for the best model. P-value is the result of LMM in model 1 in Table 4.

N concentration increased with $\delta^{13}C_{M\text{-}H}$. However, only a small part of the variation (< 2%) in mistletoe nutrient concentrations was explained by $\delta^{13}C_{M\text{-}H}$ (Fig. 4; Table S5). Despite the low variation explained, mistletoe LDMC influenced nine out of 11 mistletoe nutrient concentrations, while mistletoe SLA only influenced two mistletoe nutrients. Host growth form, leaf habit, and nitrogen-fixing type also explained the variation in some of the mistletoe nutrient concentrations. The random effects (host species and mistletoe species) explained a great proportion of the variation (Fig. 4; Table S5). The prediction of eight factors on mistletoe and host nutrient ratios was similar to that on mistletoe nutrient concentrations, despite different proportions of variation explained (Fig. S3; Table S7).

Table 4 Results of linear mixed-effects model predicting the effects of the fixed variables for the difference between mistletoe and host δ^{13}C ($\delta^{13}C_{\text{M-H}}$) based on possible subset combinations of all the fixed variables using *dredge* function of the MuMIn package.

	Model 1: $\delta^{13}C_{\text{M-H}}$					Model 2: $\delta^{13}C_{\text{M-H}}$			
Predictors	Estimates	CI	*P*	r^2	Predictors	Estimates	CI	*P*	r^2
(Intercept)	−25.32	−28.70 to −21.94	< **0.001**		(Intercept)	22.86	19.09 to 26.63	< **0.001**	
Host δ^{13}C	−0.9	−0.98 to −0.82	< **0.001**	0.5007	Mistletoe δ^{13}C	0.85	0.73 to 0.98	< **0.001**	0.3401
Site (Ailaoshan)	−0.61	−1.07 to −0.16	**0.009**	0.0000	Site (Ailaoshan)	0.29	−0.29 to 0.87	0.32	0.0000
Site (XTBG)	−2.06	−2.92 to −1.20	< **0.001**		Site (XTBG)	1.89	1.26 to 2.52	< **0.001**	
Site (Yuanjiang)	0.74	−0.29 to 1.77	0.161		Site (Yuanjiang)	−0.53	−1.43 to 0.38	0.256	
Host N	0.82	0.44 to 1.20	< **0.001**	0.0217	Host N			–	
Mistletoe SLA	−0.97	−1.54 to −0.40	**0.001**	0.0272	Mistletoe SLA			–	
Host leaf habit (Evergreen)			–		Host leaf habit (Evergreen)	0.58	0.17 to 0.99	**0.006**	0.0138
Mistletoe N			–		Mistletoe N			–	
Mistletoe LDMC			–		Mistletoe LDMC			–	
Host growth form			–		Host growth form			–	
Host nitrogen-fixing			–		Host nitrogen-fixing			–	
Random effects					Random effects				
σ^2	0.36				σ^2	0.67			
τ_{00} host species	0.50				τ_{00} host species	0.82			
τ_{00} mistletoe species	0.24				τ_{00} mistletoe species				
ICC	0.67				ICC	0.55			
N mistletoe species	11				N mistletoe species				
N host species	104				N host species	104			
Observations	344				Observations	344			
Marginal r^2/ Conditional r^2	0.550/0.853				Marginal r^2/ Conditional r^2	0.354/0.709			

The only difference between model 1 and model 2 was host δ^{13}C and mistletoe δ^{13}C, respectively; other predictors were all the same. Degrees of freedom for fixed effects were estimated using Satterthwaite estimations and their values were *P*-values. '–' indicates the variable was not selected. r^2 was the variance explained by each selected fixed variable. Marginal r^2 and conditional r^2 were the variation explained by fixed effects and the variation explained both by fixed and random effects, respectively. Bold indicates significant term.

Table 5 ANOVA results of linear mixed-effects models predicting the effects of the fixed variables for mistletoe isotope ratios (δ^{13}C and δ^{15}N) based on all possible subset combinations of all the fixed variables using *dredge* function of the MuMIn package.

	Mistletoe δ^{13}C				Mistletoe δ^{15}N		
Predicted variables	*F*	*P*	r^2	Predicted variables	*F*	*P*	r^2
Host δ^{13}C	5.89	**0.016**	0.0114	Host δ^{15}N	690.1	< **0.001**	0.7766
Sites	23.81	< **0.001**	0.4604	Sites	7.65	< **0.001**	0.0916
Host N	18.17	< **0.001**	0.0311	Host N			–
Mistletoe SLA	11.16	**0.001**	0.0143	Mistletoe SLA			–
Host Nitrogen-fixing			–	Host Nitrogen-fixing	4.86	**0.03**	0.0115
Mistletoe N			–	Mistletoe N			–
Mistletoe LDMC			–	Mistletoe LDMC			–
Host leaf habit			–	Host leaf habit			–
Host growth form			–	Host growth form			–
Random effects	LRT	*P*	0.3256	Random effects	LRT	*P*	0.0409
Mistletoe species	44.77	< **0.001**		Mistletoe species	–	–	
Host species	99.35	< **0.001**		Host species	36.45	< **0.001**	
Residuals			0.1571	Residuals			0.0794

Degrees of freedom for fixed effects were estimated using Satterthwaite estimations and their values were *F*- and *P*-values. For random factors, likelihood ratio and Pr (> χ^2)-values were provided. '–' indicates the variable was not selected. The r^2 was variation explained by each variable selected, random effects and residuals. Bold indicates significant term.

We presented the predicted regression relationships between mistletoe and host nutrients and isotope ratios among four sites and the results of LMMs for a better understanding of their shifts in nutrient acquisition strategy (Fig. 5; Table S8). The pairwise nutrition relationships between mistletoes and hosts in Fe, Zn, and δ^{15}N exhibited different slopes among four sites.

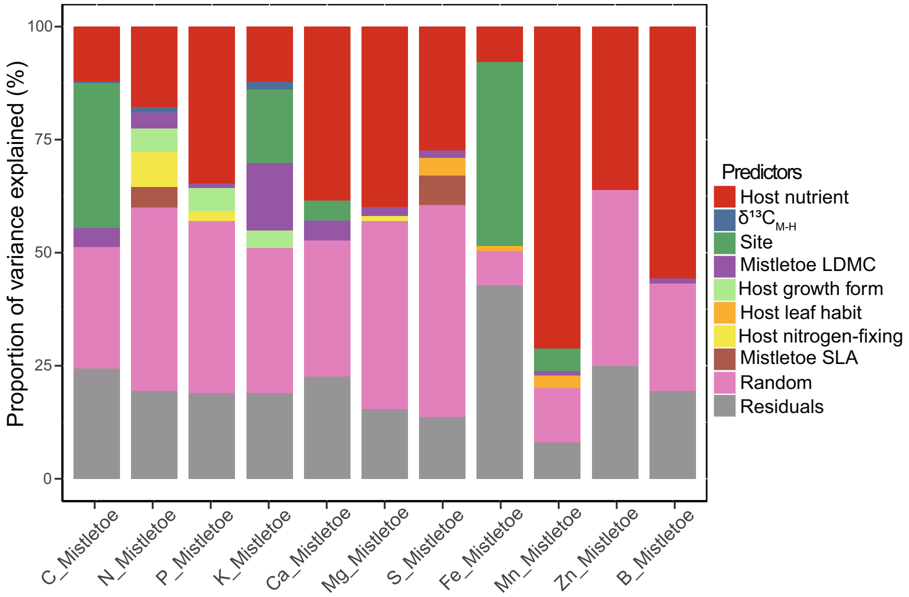

Fig. 4 Variance partitioning results of linear mixed-effects models for 11 mistletoe nutrient concentrations. LDMC, leaf dry matter content; SLA, specific leaf area.

The nutritional relationships of K, Ca, Mn, and $\delta^{13}C$ showed different intercepts, but the nutritional relationships of N, P, Mg, S, and B did not vary across sites.

Discussion

Differences in nutrients and stable isotope ratios between mistletoes and hosts

In support of our hypothesis that the differences between mistletoes and hosts shifted among sites, leaf C, K, Ca, Mn, and SLA showed significant interactions between life forms (mistletoe vs host) and sites. Moreover, sites explained much larger variation than that of life forms in the multidimensional trait space, indicating that sites play an important role in ecophysiological and morphological comparison between mistletoes and hosts. Our results using a large number of mistletoe–host pairs along large environmental gradients extend the findings from a previous study (Türe *et al.*, 2010). Previous mistletoe and host nutrient comparative studies were limited to only a few mistletoe species in a specific location with few mistletoe–host pairs, which may lead to contradictory results among different studies (Panvini & Eickmeier, 1993; Tennakoon *et al.*, 2011; Scalon *et al.*, 2013). By taking the effect of sites into account, our study highlights the importance of sites (a proxy for climate) for nutrient and/or physiological comparisons between mistletoes and hosts.

Meanwhile, we found that two random factors, mistletoe–host pair ID and species, had significant effects on mistletoe nutrient status, indicating that the uniqueness of each mistletoe–host relationship matters a lot in studying the physiology, ecology, and anatomy of mistletoes. Previous studies also found similar results (Teixeira-Costa & Ceccantini, 2016; Teixeira-Costa *et al.*, 2017). For example, *Phoradendron perrottetii* Nutt. and *P. bathyoryctum* Eichler growing on different host species show distinct anatomical features in the haustorium interface (Teixeira-Costa & Ceccantini, 2016), suggesting that particular characteristics may be associated with the unique interaction between mistletoes and hosts. Moreover, mistletoe *Struthanthus martianus* Dettke & Waechter infecting a deciduous host shows significantly higher leaf flush than that infecting an evergreen host, indicating that mistletoe phenology is influenced by host phenology (Teixeira-Costa *et al.*, 2017). Additionally, it has been well demonstrated that the impact of mistletoe infection may differ among host species, even among individuals within the same species (Cuevas-Reyes *et al.*, 2011). Therefore, the uniqueness of each mistletoe and host interaction should be included in future studies.

Mistletoes had significantly higher P and K concentrations than hosts in our study, which agrees with most previous comparative studies between mistletoes and hosts (Glatzel & Geils, 2009; Scalon *et al.*, 2013; Kubov *et al.*, 2020). This could be because mistletoes usually have a higher stomatal conductance and transpiration rate, facilitating nutrient uptake (Zweifel *et al.*, 2012; Yang *et al.*, 2017), and there is a lack of nutrient relocation from mistletoes back to the hosts (Leonard & Hull, 1965). Higher P in the form of acid-soluble P compounds may play a key role in mistletoe respiration (Kumar, 1975), probably explaining higher dark respiration rates in mistletoes than in their hosts (Scalon & Wright, 2017). It has been well documented that K plays a pivotal role in maintaining osmotic potentials (Benlloch-González *et al.*, 2008). Apart from passive nutrient accumulation (Lamont, 1983; Glatzel & Geils, 2009), higher P and K in mistletoes may also be associated with selectively active uptake of mineral nutrients through the haustoria (Lamont, 1983).

Mistletoes had lower N concentrations than hosts (a marginal significance of $P = 0.090$) in our study, which agrees with previous studies showing that mistletoes have similar (Bannister *et al.*, 2002; Scalon *et al.*, 2013; Scalon & Wright, 2015) or lower N than their hosts (Kubov *et al.*, 2020; Richards *et al.*, 2021). Also, we found that there were no differences in leaf Ca, Mg, and S concentrations between mistletoes and hosts, which is in line

Table 6 ANOVA results of linear mixed-effects model predicting the effects of the eight fixed variables on 11 mistletoe nutrient concentrations based on all possible subset combinations of all eight fixed variables using *dredge* function of the MuMIn package.

	Macronutrients														Micronutrients							
Variables	Mistletoe C		Mistletoe N		Mistletoe P		Mistletoe K		Mistletoe Ca		Mistletoe Mg		Mistletoe S		Mistletoe Fe		Mistletoe Mn		Mistletoe Zn		Mistletoe B	
Fixed effects	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Host nutrient	45.25	**< 0.001**	53.62	**< 0.001**	180.83	**< 0.001**	65.36	**< 0.001**	128.69	**< 0.001**	208.55	**< 0.001**	125.28	**< 0.001**	31.52	**< 0.001**	851.17	**< 0.001**	133.41	**< 0.001**	225.44	**< 0.001**
Mistletoe LDMC	24.28	**< 0.001**	24.94	**< 0.001**	21.36	**< 0.001**	99.09	**< 0.001**	11.84	**0.001**	9.52	**0.002**	12.41	**< 0.001**	–		5.38	**0.021**	–		20.31	**0.011**
Sites	16.79	**< 0.001**	–		–		3.97	**0.015**	7.55	**< 0.001**	–		4.06	**0.016**	27.43	**< 0.001**	6.54	**0.001**	–		–	
$\delta^{13}C_{M\text{-}H}$	10.56	**0.001**	4.72	**0.030**	–		4.14	**0.043**	–		–		–		–		–		–		–	
Host growth form	–		7.72	**0.001**	6.55	**0.002**	6.90	**0.002**	–		–		–		–		–		–		–	
Host nitrogen-fixing	–		12.12	**0.001**	5.42	**0.022**	–		–		4.80	**0.031**	–		–		–		–		–	
Mistletoe SLA	–		6.40	**0.012**	–		–		–		–		16.18	**< 0.001**	–		–		–		–	
Host leaf habit	–		–		–		–		–		–		12.08	**0.001**	4.24	**0.043**	11.16	**0.001**	–		–	
Random effects	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*
Mistletoe species	20.35	**< 0.001**	38.83	**< 0.001**	51.37	**< 0.001**	39.66	**< 0.001**	13.45	**< 0.001**	44.82	**< 0.001**	61.74	**< 0.001**	–		28.93	**< 0.001**	24.90	**< 0.001**	–	
Host species	44.81	**< 0.001**	86.23	**< 0.001**	84.40	**< 0.001**	62.59	**< 0.001**	69.21	**< 0.001**	127.66	**< 0.001**	109.31	**< 0.001**	5.46	**0.019**	70.26	**< 0.001**	93.55	**< 0.001**	97.87	**< 0.001**

The fixed effects were host nutrient, $\delta^{13}C_{M\text{-}H}$, mistletoe leaf dry matter content (LDMC), mistletoe specific leaf area (SLA), sites, host growth form, host leaf habit, and host nitrogen-fixing. The host nutrient was a proxy for each corresponding mistletoe nutrient, for example, for mistletoe C, the corresponding host nutrient was host C. $\delta^{13}C_{M\text{-}H}$ was the difference between mistletoe and host $\delta^{13}C$. '–' indicated the variable was not selected. Degrees of freedom for fixed effects were estimated using Satterthwaite estimations and their values were *F*- and *P*-values. For random factors, likelihood ratio and Pr (> χ^2)-values were provided. Bold indicates significant term.

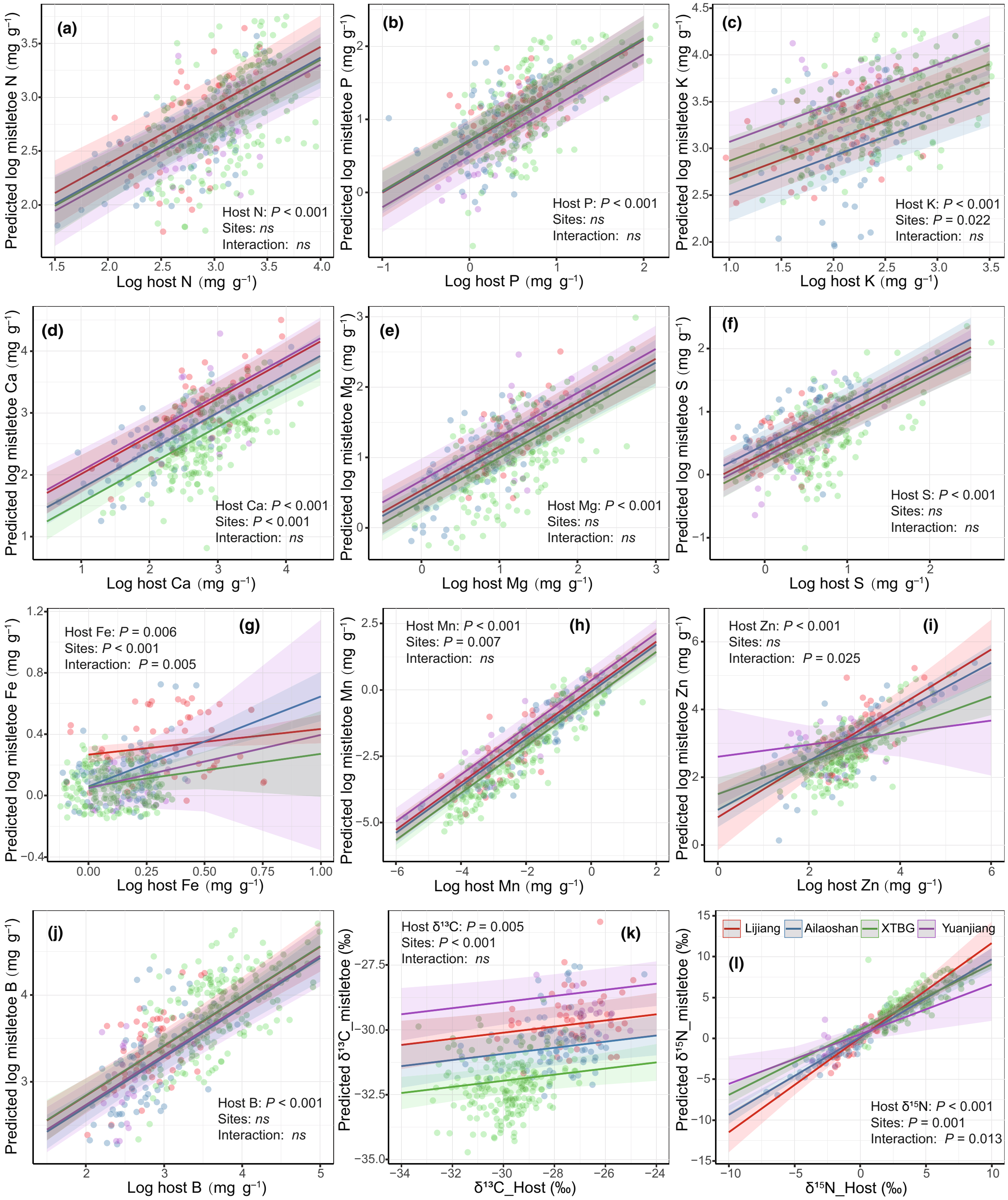

Fig. 5 Model predicted regressions for (a) nitrogen (N), (b) phosphorus (P), (c) potassium (K), (d) calcium (Ca), (e) magnesium (Mg), (f) sulfur (S), (g) iron (Fe), (h) manganese (Mn), (i) zinc (Zn), (j) boron (B), (k) carbon isotope ratios ($\delta^{13}C$), and (l) nitrogen isotope ratios ($\delta^{15}N$) between mistletoes and hosts among four sites. The lines with different colors represent model-fitted slopes, shading areas denote 95% confidence intervals, and each point is an individual value for 344 mistletoe–host individual pair (Lijiang, red circle and regression line, $n = 48$; Ailaoshan, blue circle and regression line, $n = 74$; Xishuangbanna Tropical Botanical Garden: green circle and regression line, $n = 202$; Yuanjiang: purple circle and regression line, $n = 20$). ns indicates nonsignificant term. See Supporting Information Table S8 for the statistical results for host nutrient concentrations, sites, and their interactions.

with some previous findings (Goldstein *et al.*, 1989; Bannister *et al.*, 2002; Scalon *et al.*, 2013; but see Glatzel, 1983; Lamont, 1983; Gebauer *et al.*, 2018). The reasons for the contradicting results may lie in previous analyses relying on few mistletoe species, narrow host species ranges, or a single site included in those past studies (Panvini & Eickmeier, 1993; Türe *et al.*, 2010). Micronutrients showed little variation between mistletoes and hosts, except for B, which was higher in mistletoes than in hosts. B is important for membrane integrity and turgor pressure (Hänsch & Mendel, 2009; Lambers & Oliveira, 2019), but the accumulation of B in mistletoes is still unclear. Higher K may be coupled with higher B demands, and this may be one of the reasons why there is higher B in mistletoes than in hosts.

Based on our regional data, mistletoes had more negative $\delta^{13}C$ than corresponding hosts across all sites, suggesting that they maintain a higher stomatal conductance than their hosts, which is in line with previous local, regional, and global studies (Ehleringer *et al.*, 1985; Givnish, 1986; Scalon & Wright, 2015; Mostaghimi *et al.*, 2021; Richards *et al.*, 2021). These results from the present study, temperate Australia, and a global study suggest a remarkable convergence in different studies in terms of lower $\delta^{13}C$ and water use efficiency of mistletoes, supporting the 'transpirational cost hypothesis' across diverse sites and taxa (Givnish, 1986; Scalon & Wright, 2015; Richards *et al.*, 2021). However, several studies found contrasting results that similar $\delta^{13}C$ values were found between mistletoes and hosts in temperate environments (Panvini & Eickmeier, 1993; Bannister & Strong, 2001; Bannister *et al.*, 2002). Bannister & Strong (2001) contended that high water availability might result in similar $\delta^{13}C$ between mistletoes and hosts. Yet, our results did not support their argument. For instance, mistletoes in Lijiang and Ailaoshan with high water availability exhibited much more negative $\delta^{13}C$ than their hosts.

It seems that both mistletoes and hosts respond to environmental gradients in the same way. For instance, mistletoes and hosts in Yuanjiang with the lowest precipitation exhibited less negative $\delta^{13}C$ than that of other sites, supporting the findings that $\delta^{13}C$ increases with decreasing precipitation (Prentice *et al.*, 2011). Our results, therefore, supported previous findings that mistletoe and host exhibit similar responses to the environment (Scalon & Wright, 2015, 2017; Richards *et al.*, 2021). Moreover, sites rather than host $\delta^{13}C$ explained the largest variation in mistletoe $\delta^{13}C$, suggesting the sensitivity of mistletoe's water use efficiency to the environment. Furthermore, there were no differences in $\delta^{13}C_{M\text{-}H}$ among four sites (Fig. S2), in contrast to the postulation that $\delta^{13}C_{M\text{-}H}$ should be larger in arid sites (Bannister & Strong, 2001). We found that mistletoe $\delta^{15}N$ was higher than that of hosts, in contrast with other studies in which mistletoe and host $\delta^{15}N$ are similar (Bannister & Strong, 2001; Richards *et al.*, 2021). In Xishuangbanna, mistletoe and host $\delta^{15}N$ were larger than that of other sites. This may be because tropical mistletoes prefer nitrate reductase activity due to high leaching of organic N and ammonium ions (NH_4^+), whereby NO_3^- rather than organic N or NH_4^+ is used during amino acid metabolism, but temperate mistletoes may prefer organic N or NH_4^+ (Tennakoon *et al.*, 2011).

Is the 'N-parasitism hypothesis' supported?

The 'N-parasitism hypothesis' was proposed to interpret the higher transpiration and lower water use efficiency in mistletoes (Schulze *et al.*, 1984; Schulze & Ehleringer, 1984). According to this hypothesis, the $\delta^{13}C_{M\text{-}H}$ should become smaller when the mistletoe parasitizes nitrogen-fixing hosts or hosts with higher N concentrations (Ehleringer *et al.*, 1985; Schulze *et al.*, 1991; Bannister & Strong, 2001). Indeed, a significantly positive relationship between host N and $\delta^{13}C_{M\text{-}H}$ was found in our multiple linear mixed-effects model selection, indicating that higher host N drives smaller $\delta^{13}C_{M\text{-}H}$ (more similar $\delta^{13}C$). However, other studies found no correlation between host N and $\delta^{13}C_{M\text{-}H}$ (Scalon & Wright, 2015; Richards *et al.*, 2021). Scalon & Wright (2015) found that $\delta^{13}C_{M\text{-}H}$ was not significantly different between nitrogen-fixing and non-nitrogen-fixing hosts, and host N was marginally related to $\delta^{13}C_{M\text{-}H}$, concluding lack of support for the 'N-parasitism hypothesis' in a global analysis. In our model selection, host growth form, nitrogen-fixing, and leaf habit exhibited nonsignificant effects on the $\delta^{13}C_{M\text{-}H}$ (Table 4, model 1). Taken together, we provided evidence to support the 'N-parasitism hypothesis' using a more robust regression framework to exclude the confounding effects, after considering the exact host N concentrations and controlling for host life-history classification and host- and mistletoe-species-specific influences.

Differences in $\delta^{13}C_{M\text{-}H}$ may be more related to the host gas exchange characteristics rather than that of mistletoes (Givnish, 1986). The $\delta^{13}C$ has been shown to increase with the increasing N values in nonparasitic plants (Sparks & Ehleringer, 1997), and here, we found that $\delta^{13}C$ was not significantly correlated with N concentration across all species, with nonsignificant correlations between $\delta^{13}C$ and N in either hosts or mistletoes (Fig. S4). Yet, we found that host N rather than mistletoe N was positively correlated with mistletoe $\delta^{13}C$ values, which was also found by Schulze *et al.* (1991). This might suggest that mistletoes regulate their water use efficiency according to host N supply. Moreover, host $\delta^{13}C$ was negatively correlated with $\delta^{13}C_{M\text{-}H}$, but mistletoe $\delta^{13}C$ was positively linked with $\delta^{13}C_{M\text{-}H}$. It means that smaller $\delta^{13}C_{M\text{-}H}$ depends on mistletoe $\delta^{13}C$ rather than host $\delta^{13}C$. Together with the positive relationship between host N and mistletoe $\delta^{13}C$, we concluded that host N does drive smaller $\delta^{13}C_{M\text{-}H}$. Thus, our study comprising 11 mistletoe species growing on 104 host species from four sites, 344 mistletoe and host pairs in total, provided evidence for 'N-parasitism hypothesis' (Schulze *et al.*, 1984; Schulze & Ehleringer, 1984). Our results did not support the 'P-parasitism hypothesis' and 'C-parasitism hypothesis' because $\delta^{13}C_{M\text{-}H}$ was not related to mistletoe P, but negatively related to mistletoe C, implying that mistletoes did not regulate their water use efficiency according to P or C demands. Besides, the method for calculating mistletoe heterotrophy is still on debate (Bannister & Strong, 2001; Tennakoon *et al.*, 2011). More work is needed to falsify the 'P-parasitism hypothesis' and 'C-parasitism hypothesis'.

Determinants of the mistletoe nutrient status and isotope ratios

The host nutrient concentration (a proxy for each corresponding mistletoe nutrient concentration) was the best predictor of mistletoe nutrient status in most cases. Given that we integrated multiple factors and random effects in one model and conducted robust model selection, we empirically tested previous assumptions that the best predictor of mistletoe nutrient status is often (but not always) the host nutrient status (Panvini & Eickmeier, 1993; Glatzel & Geils, 2009; Gebauer *et al.*, 2012). Mistletoes absorb all their essential mineral nutrients and water from their hosts via haustoria connected to their hosts (Kuijt, 1969; Glatzel & Geils, 2009). Therefore, element concentrations in mistletoes might be expected to highly correlate with those in the hosts, especially when the nutrient uptake is mainly passive (Glatzel & Geils, 2009).

Apart from host nutrient concentration, other variables also explained parts of the variation in mistletoe nutrient concentration. For instance, sites explained much more variation than host nutrient concentration in mistletoe C, K, and Fe. Indeed, hosts may acquire nutrients more easily from the soil in an environment with higher water availability. Similarly, other environmental factors, such as soil type, mean annual precipitation and temperature, and vegetation type are all likely to affect the nutrients available to the hosts, which will, in turn, affect the vascular sap composition (Irving & Cameron, 2009). Therefore, considering site effects may contribute to our understanding of nutritional interactions between mistletoes and hosts, which is in line with a previous study (Türe *et al.*, 2010).

We found that the $\delta^{13}C_{M\text{-}H}$ only had a significant influence on mistletoe C, N, and K concentrations and their corresponding mistletoe and host nutrient ratios. Panvini & Eickmeier (1993) investigated one mistletoe species growing on seven deciduous host species (in total 46 mistletoe–host pairs) and found that the $\delta^{13}C_{M\text{-}H}$ was only correlated with mistletoe Ca concentration and mistletoe and host P ratio. They suggested that water and nutrient uptake of mistletoes were not tightly coupled. Previous studies also found that mistletoe and host nutrient ratios for Ca, Mg, and P were associated with host transpiration or host $\delta^{13}C$ rather than mistletoe transpiration or $\delta^{13}C$ (Bannister *et al.*, 2002). Yet, we found that both mistletoe and host water use efficiencies ($\delta^{13}C$) only significantly affected the status of Ca, Fe, and B of mistletoes (Figs S5, S6). Accordingly, we concluded that mistletoe water and nutrient acquisition may not be tightly coupled because nutrient acquisition may only be partly passive through the apoplast pathway. There are abundant parenchyma cells in the haustorium interface (Cocoletzi *et al.*, 2016; Lambers & Oliveira, 2019), with interesting variation among different haustorium morphotypes (Teixeira-Costa *et al.*, 2020), suggesting potential existence of active and selective uptake. Overall, we found little evidence of transpiration-controlled nutrient acquisition in mistletoes except for N, as proposed by other studies that high transpiration in mistletoes seems more likely to reflect other physiological processes (Richards *et al.*, 2021).

Mistletoe nutrient status could also be influenced by mistletoe leaf structural traits like LDMC and SLA. Mistletoe LDMC influenced nine out of 11 mistletoe nutrient concentrations, while mistletoe SLA influenced only two. Mistletoes had much lower SLA and LDMC than their hosts in this study, suggesting a succulent leaf structure, which is in consistent with other studies (Scalon & Wright, 2017; Mostaghimi *et al.*, 2021; Richards *et al.*, 2021). We postulate that succulent leaves and higher water content might be a strategy involved in balancing nutrient concentrations in mistletoes. Besides, host growth form, nitrogen-fixing, and leaf habit also influenced mistletoe nutrient status, as well as mistletoe and host nutrient ratios. Yet, the variation explained by these variables was relatively small compared with host nutrient concentrations, site-specific or species-specific (mistletoe- and host-specific) sources of variation. This suggests that quantifying site-specific or species-specific uncertainty in nutrient variation will be the way forward.

Mistletoe and host $\delta^{13}C$ were positively correlated, which is in accordance with previous studies (Bannister & Strong, 2001; Scalon & Wright, 2015), but in contrast with studies in Australia (Ziegler, 1995), Brazil (Lüttge *et al.*, 1998) and Borneo (Tennakoon *et al.*, 2011). Tennakoon *et al.* (2011) have postulated that the absence of correlation may result from a small sample size or may be derived from the differences between species and sites or the limited range of hosts (Bannister & Strong, 2001). In our study, host $\delta^{13}C$, host N, sites, and mistletoe SLA showed significant influences on mistletoe $\delta^{13}C$, with sites explaining the largest variation, followed by random effects (mistletoe and host species). We, therefore, supported this previous postulation using robust regression models and large data sets. Mistletoe $\delta^{15}N$ showed a significant correlation with host $\delta^{15}N$, which explained most of the variation, in support of the results of numerous studies in which mistletoe N is entirely derived from their hosts (Bannister & Strong, 2001; Tennakoon *et al.*, 2011; Mostaghimi *et al.*, 2021).

Conclusions

We found that mistletoe nutrient concentrations mainly depend on host nutrients, but other factors, such as environmental conditions, host life-history classification, and mistletoe morphological traits, also play important roles for some nutrients. Moreover, our results supported the 'N-parasitism hypothesis' with a robust regression model framework across 11 mistletoe species and 104 different host species. Mistletoes had significantly higher P, K, and B than their hosts, which may be interpreted by active and selective uptake, in addition to passive uptake driven by higher transpiration than hosts. Moreover, transpiration-related parameters (either mistletoe/host $\delta^{13}C$ or $\delta^{13}C_{M\text{-}H}$) explained little variation in mistletoe nutrient concentrations, implying that water and nutrient uptake may not be tightly coupled in mistletoes. These results suggest that selective uptake of nutrient elements might exist in the haustoria of mistletoes. Additionally, mistletoe and host species explained a large proportion of the variation in mistletoe nutrient elements, stable isotope ratios, and leaf morphological traits. Overall, our findings shed light on the importance of combining sites, species, transpiration-related parameters, host life-history traits in understanding the water and nutrient relations of mistletoes and nutritional interactions between mistletoes and hosts.

Acknowledgements

We thank Wen-Hua Li, Shi-Hua Qi, Xiao-Long Bai and Qi-Cai Zhu for field assistance and lab experiments and Yun-Hong Tan and Ren-Bin Zhu for species identification. The Institutional Center for Shared Technologies and Facilities of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, analyzed the foliar nutrient concentrations. The Lijiang Forest Ecosystem Research Station, the Xishuangbanna Station for Tropical Rainforest Ecosystem Studies, the Ailaoshan Station for Subtropical Forest Ecosystem Studies, and the Yuanjiang Savanna Ecosystem Research Station provided the climate and soil data. We are grateful to the Center for Gardening and Horticulture, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences for collection permit. We thank the reviewers for their constructive comments, which significantly improved this manuscript. This work was supported by the National Natural Science Foundation of China (32171507, 31870385 and 31901285) and the CAS 'Light of West China' program to J-LZ.

Competing interests

None declared.

Author contributions

Y-BZ and J-LZ conceived and designed the experiment; Y-BZ and J-LZ collected the data; Y-BZ, J-XL, X-YS, DY and J-LZ analyzed the data; and Y-BZ, MCS, DSE, Y-JZ and J-LZ led the writing. All authors contributed critically to the drafts and gave final approval for publication.

ORCID

Marina Corrêa Scalon https://orcid.org/0000-0003-2069-8226
David S. Ellsworth https://orcid.org/0000-0002-9699-2272
Jing-Xin Liu https://orcid.org/0000-0003-4127-7252
Xiao-Yang Song https://orcid.org/0000-0001-9529-1418
Da Yang https://orcid.org/0000-0001-7306-3289
Jiao-Lin Zhang https://orcid.org/0000-0003-3693-7965
Yong-Jiang Zhang https://orcid.org/0000-0001-5637-3015
Yun-Bing Zhang https://orcid.org/0000-0002-2739-4324

Data availability

The data that support the results of this study are available from the corresponding author upon reasonable request.

References

Al-Rowaily SL, Al-Nomari GSS, Assaeed AM, Facelli JM, Dar BM, El-Bana MI, Abd-ElGawad AM. 2020. Infection by *Plicosepalus curviflorus* mistletoe affects the nutritional elements of *Acacia* species and soil nutrient recycling in an arid rangeland. *Plant Ecology* **221**: 1017–1028.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**: 32–46.

Baayen RH, Davidson DJ, Bates DM. 2008. Mixed-effects modeling with crossed random effects for subjects and items. *Journal of Memory and Language* **59**: 390–412.

Bannister P, Strong GL. 2001. Carbon and nitrogen isotope ratios, nitrogen content and heterotrophy in New Zealand mistletoes. *Oecologia* **126**: 10–20.

Bannister P, Strong GL, Andrew I. 2002. Differential accumulation of nutrient elements in some New Zealand mistletoes and their hosts. *Functional Plant Biology* **29**: 1309–1318.

Bartoń K. 2020. *MuMIn: multi-model inference.* [WWW document] URL https://CRAN.R-project.org/package=MuMIn [accessed 1 September 2022].

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using LME4. *Journal of Statistical Software* **67**: 1–48.

Bell TL, Adams MA. 2011. Attack on all fronts: functional relationships between aerial and root parasitic plants and their woody hosts and consequences for ecosystems. *Tree Physiology* **31**: 3–15.

Benlloch-González M, Arquero O, Fournier JM, Barranco D, Benlloch M. 2008. K^+ starvation inhibits water-stress-induced stomatal closure. *Journal of Plant Physiology* **165**: 623–630.

Bilgili E, Coskuner KA, Baysal I, Ozturk M, Usta Y, Eroglu M, Norton D. 2020. The distribution of pine mistletoe (*Viscum album* ssp. *austriacum*) in Scots pine (*Pinus sylvestris*) forests: from stand to tree level. *Scandinavian Journal of Forest Research* **35**: 20–28.

Camarero JJ, González de Andrés E, Sangüesa-Barreda G, Rita A, Colangelo M. 2019. Long- and short-term impacts of a defoliating moth plus mistletoe on tree growth, wood anatomy and water-use efficiency. *Dendrochronologia* **56**: 125598.

Chen LZ, Huang L, Li XF, You SY, Yang SC, Zhang YH, Wang WQ. 2013. Water and nutrient relationships between a mistletoe and its mangrove host under saline conditions. *Functional Plant Biology* **40**: 475–483.

Cocoletzi E, Angeles G, Ceccantini G, Patron A, Francisco OJ. 2016. Bidirectional anatomical effects in a mistletoe-host relationship: *Psittacanthus schiedeanus* mistletoe and its hosts *Liquidambar styraciflua* and *Quercus germana*. *American Journal of Botany* **103**: 986–997.

Cuevas-Reyes P, Fernandes GW, González-Rodríguez A, Pimenta M. 2011. Effects of generalist and specialist parasitic plants (Loranthaceae) on the fluctuating asymmetry patterns of ruprestrian host plants. *Basic and Applied Ecology* **12**: 449–455.

Domínguez MT, Aponte C, Pérez-Ramos IM, García LV, Villar R, Marañón T. 2012. Relationships between leaf morphological traits, nutrient concentrations and isotopic signatures for Mediterranean woody plant species and communities. *Plant and Soil* **357**: 407–424.

Ehleringer JR, Cook CS, Tieszen LL. 1986. Comparative water use and nitrogen relationships in a mistletoe and its host. *Oecologia* **68**: 279–284.

Ehleringer JR, Schulze ED, Ziegler H, Lange OL, Farquhar GD, Cowar IR. 1985. Xylem-tapping mistletoes – water or nutrient parasites. *Science* **227**: 1479–1481.

Escher P, Rennenberg H. 2006. Influx of double labelled glutamine into mistletoes (*Viscum album*) from the xylem sap of its host (*Abies alba*). *Plant Physiology and Biochemistry* **44**: 880–884.

Fontúrbel FE. 2020. Mistletoes in a changing world: a premonition of a non-analog future? *Botany* **98**: 479–488.

Gebauer R, Volarik D, Urban J. 2012. *Quercus pubescens* and its hemiparasite *Loranthus europaeus*: nutrient dynamics of leaves and twigs. *Acta Physiologiae Plantarum* **34**: 1801–1809.

Gebauer R, Volarik D, Urban J. 2018. Seasonal variations of sulphur, phosphorus and magnesium in the leaves and current-year twigs of hemiparasitic mistletoe *Loranthus europaeus* Jacq. and its host *Quercus pubescens* Willd. *Journal of Forest Science* **64**: 66–73.

Givnish TJ. 1986. Optimal stomatal conductance, allocation of energy between leaves and roots, and the marginal cost of transpiration. In: Givnish TJ, ed. *On the economy of plant form and function*. Cambridge, UK: Cambridge University Press, 171–213.

Glatzel G. 1983. Mineral nutrition and water relations of hemiparasitic mistletoes: a question of partitioning. Experiments with *Loranthus europaeus* on *Quercus petraea* and *Quercus robur*. *Oecologia* **56**: 193–201.

Glatzel G, Geils BW. 2009. Mistletoe ecophysiology: host-parasite interactions. *Botany* **87**: 10–15.

Goldstein G, Rada F, Sternberg L, Burguera JL, Burguera M, Orozco A, Montilla M, Zabala O, Azocar A, Canales MJ *et al.* 1989. Gas-exchange and water-balance of a mistletoe species and its mangrove hosts. *Oecologia* **78**: 176–183.

Hänsch R, Mendel RR. 2009. Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Current Opinion in Plant Biology* **12**: 259–266.

He XF, Wang SW, Körner C, Yang Y. 2021. Water and nutrient relations of mistletoes at the drought limit of their hosting evergreen oaks in the semiarid upper Yangtze region, SW China. *Trees* **35**: 387–394.

Irving LJ, Cameron DD. 2009. You are what you eat: interactions between root parasitic plants and their hosts. *Advances in Botanical Research* **50**: 87–138.

Jiang RY, Zhang GF. 2021. Distribution patterns and influencing factors of different parasitic angiosperm types in China. *Global Ecology and Conservation* **27**: e01533.

Kubov M, Fleischer P, Rozkošný J, Kurjak D, Konôpková A, Galko J, Húdoková H, Lalík M, Rell S, Pittner J *et al.* 2020. Drought or severe drought? hemiparasitic yellow mistletoe (*Loranthus europaeus*) amplifies drought stress in sessile oak trees (*Quercus petraea*) by altering water status and physiological responses. *Water* **12**: 2985.

Kuijt J. 1969. *The biology of parasitic flowering plants.* Berkeley, CA, USA: University of California Press.

Kumar NC. 1975. Eco-physiological studies on *Dendrophthoe falcata* infection. *Bulletin of the Botanical Society of Bengal* **29**: 33–38.

Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software* **82**: 1–26.

Lambers H, Oliveira RS. 2019. Mineral nutrition. In: Lambers H, Oliveira RS, eds. *Plant physiological ecology.* Cham, Switzerland: Springer Nature Switzerland AG, 301–384.

Lamont B. 1983. Mineral nutrition of mistletoes. In: Calder DM, Bernhardt P, eds. *The biology of mistletoes.* New York, NY, USA: Academic Press, 185–204.

Lenth RV. 2021. *emmeans: estimated marginal means, aka least-squares means. R package version 1.5.5-1.* [WWW document] URL https://CRAN.R-project.org/package=emmeans [accessed 27 October 2022].

Leonard OA, Hull RJ. 1965. Translocation relationships in and between mistletoes and their hosts. *Hilgardia* **37**: 115–153.

Lüdecke D. 2018. ggeffects: tidy data frames of marginal effects from regression models. *Journal of Open Source Software* **3**: 772.

Lüdecke D. 2021. *sjPlot: data visualization for statistics in social science.* R package v.2.8.10. [WWW document] URL https://CRAN.R-project.org/package=sjPlot [accessed 19 November 2022].

Lüdecke D, Ben-Shachar MS, Patil I, Waggoner P, Makowski D. 2021. performance: an R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software* **6**: 3139.

Lüttge U, Haridasan M, Fernandes GW, de Mattos EA, Trimborn P, Franco AC, Caldas LS, Ziegler H. 1998. Photosynthesis of mistletoes in relation to their hosts at various sites in tropical Brazil. *Trees* **12**: 167–174.

March WA, Watson DM. 2007. Parasites boost productivity: effects of mistletoe on litterfall dynamics in a temperate Australian forest. *Oecologia* **154**: 339–347.

March WA, Watson DM. 2010. The contribution of mistletoes to nutrient returns: evidence for a critical role in nutrient cycling. *Austral Ecology* **35**: 713–721.

Marshall JD, Ehleringer JR, Schulze ED, Farquhar G. 1994. Carbon-isotope composition, gas-exchange and heterotrophy in Australian mistletoes. *Functional Ecology* **8**: 237–241.

Mostaghimi F, Seyedi N, Banj Shafiei A, Correia O. 2021. How do leaf carbon and nitrogen contents of oak hosts affect the heterotrophic level of *Loranthus europaeus*? Insights from stable isotope ecophysiology assays. *Ecological Indicators* **125**: 107583.

Muvengwi J, Ndagurwa HGT, Nyenda T. 2015. Enhanced soil nutrient concentrations beneath-canopy of savanna trees infected by mistletoes in a southern African savanna. *Journal of Arid Environments* **116**: 25–28.

Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution* **4**: 133–142.

Nastos PT, Politi N, Kapsomenakis J. 2013. Spatial and temporal variability of the Aridity Index in Greece. *Atmospheric Research* **119**: 140–152.

Ndagurwa HGT, Dube JS, Mlambo D. 2014. The influence of mistletoes on nutrient cycling in a semi-arid savanna, southwest Zimbabwe. *Plant Ecology* **215**: 15–26.

Ndagurwa HGT, Maponga TS, Muvengwi J. 2020. Mistletoe litter accelerates the decomposition of recalcitrant host litter in a semi-arid savanna, south-west Zimbabwe. *Austral Ecology* **45**: 1080–1092.

Ndagurwa HGT, Ndarevani P, Muvengwi J, Maponga TS. 2016. Mistletoes via input of nutrient-rich litter increases nutrient supply and enhance plant species composition and growth in a semi-arid savanna, southwest Zimbabwe. *Plant Ecology* **217**: 1095–1104.

Oksanen J, Blanchet FG, Kindt R, Legendre P, O'Hara RB, Simpson GL, Solymos P, Stevens H, Wagner H. 2013. *vegan: community ecology package.* [WWW document] URL https://cran.r-project.org/web/packages/vegan/index.html [accessed 11 October 2022].

Ozturk M, Coskuner KA, Usta Y, Serdar B, Bilgili E. 2019. The effect of mistletoe (*Viscum album*) on branch wood and needle anatomy of Scots pine (*Punts sylvestris*). *IAWA Journal* **40**: 352–365.

Panvini AD, Eickmeier WG. 1993. Nutrient and water relations of the mistletoe *Phoradendron leucarpum* (Viscaceae): how tightly are they integrated? *American Journal of Botany* **80**: 872–878.

Pate JS, True KC, Rasins E. 1991. Xylem transport and storage of amino-acids by SW Australian mistletoes and their hosts. *Journal of Experimental Botany* **42**: 441–451.

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE *et al.* 2013. New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* **61**: 167–234.

Prentice IC, Meng T, Wang H, Harrison SP, Ni J, Wang G. 2011. Evidence of a universal scaling relationship for leaf CO_2 drawdown along an aridity gradient. *New Phytologist* **190**: 169–180.

R Core Team. 2020. *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. [WWW document] URL https://www.R-project.org [accessed 22 June 2020].

Richards JH, Henn JJ, Sorenson QM, Adams MA, Smith DD, McCulloh KA, Givnish TJ. 2021. Mistletoes and their eucalypt hosts differ in the response of leaf functional traits to climatic moisture supply. *Oecologia* **195**: 759–771.

de la Riva EG, Villar R, Pérez-Ramos IM, Quero JL, Matías L, Poorter L, Marañón T. 2018. Relationships between leaf mass per area and nutrient concentrations in 98 Mediterranean woody species are determined by phylogeny, habitat and leaf habit. *Trees* **32**: 497–510.

Sangüesa-Barreda G, Linares JC, Camarero JJ. 2013. Drought and mistletoe reduce growth and water-use efficiency of Scots pine. *Forest Ecology and Management* **296**: 64–73.

Scalon MC, Haridasan M, Franco AC. 2013. A comparative study of aluminium and nutrient concentrations in mistletoes on aluminium-accumulating and non-accumulating hosts. *Plant Biology* **15**: 851–857.

Scalon MC, Wright IJ. 2015. A global analysis of water and nitrogen relationships between mistletoes and their hosts: broad-scale tests of old and enduring hypotheses. *Functional Ecology* **29**: 1114–1124.

Scalon MC, Wright IJ. 2017. Leaf trait adaptations of xylem-tapping mistletoes and their hosts in sites of contrasting aridity. *Plant and Soil* **415**: 117–130.

Scalon MC, Wright IJ, Franco AC. 2017. To recycle or steal? Nutrient resorption in Australian and Brazilian mistletoes from three low-phosphorus sites. *Oikos* **126**: 32–39.

Schulze ED, Ehleringer JR. 1984. The effect of nitrogen supply on growth and water-use efficiency of xylem-tapping mistletoes. *Planta* **162**: 268–275.

Schulze ED, Lange OL, Ziegler H, Gebauer G. 1991. Carbon and nitrogen isotope ratios of mistletoes growing on nitrogen and non-nitrogen fixing hosts and on CAM plants in the Namib desert confirm partial heterotrophy. *Oecologia* **88**: 457–462.

Schulze ED, Turner NC, Glatzel G. 1984. Carbon, water and nutrient relations of 2 mistletoes and their hosts – a hypothesis. *Plant, Cell & Environment* **7**: 293–299.

Sparks JP, Ehleringer JR. 1997. Leaf carbon isotope discrimination and nitrogen content for riparian trees along elevational transects. *Oecologia* **109**: 362–367.

Suriyagoda LDB, Rajapaksha R, Pushpakumara G, Lambers H. 2018. Nutrient resorption from senescing leaves of epiphytes, hemiparasites and their hosts in tropical forests of Sri Lanka. *Journal of Plant Ecology* **11**: 815–826.

Tamudo E, Camarero JJ, Sangüesa-Barreda G, Anadón JD. 2021. Dwarf mistletoe and drought contribute to growth decline, dieback and mortality of Junipers. *Forests* **12**: 1199.

Teixeira-Costa L. 2021. A living bridge between two enemies: haustorium structure and evolution across parasitic flowering plants. *Brazilian Journal of Botany* **44**: 165–178.

Teixeira-Costa L, Ceccantini G. 2015. Embolism increase and anatomical modifications caused by a parasitic plant: *Phoradendron crassifolium* (Santalaceae) on *Tapirira guianensis* (Anacardiaceae). *IAWA Journal* **36**: 138–151.

Teixeira-Costa L, Ceccantini GCT. 2016. Aligning microtomography analysis with traditional anatomy for a 3D understanding of the host-parasite interface – *Phoradendron* spp. case study. *Frontiers in Plant Science* **7**: 1340.

Teixeira-Costa L, Coelho FM, Ceccantini GCT. 2017. Comparative phenology of mistletoes shows effect of different host species and temporal niche partitioning. *Botany* **95**: 271–282.

Teixeira-Costa L, Davis CC. 2021. Life history, diversity, and distribution in parasitic flowering plants. *Plant Physiology* **187**: 32–51.

Teixeira-Costa L, Ocampo G, Ceccantini G. 2020. Morphogenesis and evolution of mistletoes' haustoria. In: Demarco D, ed. *Plant ontogeny*. New York: Nova Science, 107–157.

Tennakoon KU, Chak WH, Bolin JF. 2011. Nutritional and isotopic relationships of selected Bornean tropical mistletoe-host associations in Brunei Darussalam. *Functional Plant Biology* **38**: 505–513.

Těšitel J, Li AR, Knotková K, McLellan R, Bandaranayake PCG, Watson DM. 2021. The bright side of parasitic plants: what are they good for? *Plant Physiology* **185**: 1309–1324.

Türe C, Böcük H, Aşan Z. 2010. Nutritional relationships between hemiparasitic mistletoe and some of its deciduous hosts in different habitats. *Biologia* **65**: 859–867.

Wang L, Kgope B, D'Odorico P, Macko SA. 2008. Carbon and nitrogen parasitism by a xylem-tapping mistletoe (*Tapinanthus oleifolius*) along the Kalahari Transect: a stable isotope study. *African Journal of Ecology* **46**: 540–546.

Watson DM. 2009. Parasitic plants as facilitators: more Dryad than Dracula? *Journal of Ecology* **97**: 1151–1159.

Yang D, Goldstein G, Wang M, Zhang WW, Wang AY, Liu YY, Hao GY. 2017. Microenvironment in the canopy rivals the host tree water status in controlling sap flow of a mistletoe species. *Tree Physiology* **37**: 501–510.

Zhang GF, Li Q, Sun SC. 2018. Diversity and distribution of parasitic angiosperms in China. *Ecology and Evolution* **8**: 4378–4386.

Ziegler H. 1995. Deuterium content in organic material of hosts and their parasites. In: Schulze ED, Caldwell MM, eds. *Ecophysiology of photosynthesis*. Berlin, Germany: Springer, 393–408.

Zuur AF, Ieno EN, Elphick CS. 2010. A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution* **1**: 3–14.

Zweifel R, Bangerter S, Rigling A, Sterck FJ. 2012. Pine and mistletoes: how to live with a leak in the water flow and storage system? *Journal of Experimental Botany* **63**: 2565–2578.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Locations of the four study sites.

Fig. S2 Difference between mistletoe and host $\delta^{13}C$ ($\delta^{13}C_{M\text{-}H}$) among different host leaf habits, nitrogen-fixing types, growth forms, and sites.

Fig. S3 Variance partitioning results of linear mixed-effects model for 11 nutrient ratios between mistletoes and hosts.

Fig. S4 Pearson's correlation between $\delta^{13}C$ and N based on mistletoe and host species pair mean data.

Fig. S5 Relationships between mistletoe $\delta^{13}C$ and 11 mistletoe nutrient concentrations among four sites.

Fig. S6 Relationships between host $\delta^{13}C$ and 11 mistletoe nutrient concentrations among four sites.

Table S1 Mistletoe–host (M-H) species pairs, haustorium types, host leaf habits and numbers of M-H individual pairs used for measurements of leaf nutrients, stable isotope ratios, and morphology.

Table S2 Results of linear mixed-effects model with both species pair ID and species as random effects for 15 leaf functional traits of all the species as response variables.

Table S3 Results of simple linear model, linear mixed-effects models with species pair ID as random effects, and linear mixed-effects model with both species pair ID and species as random effects for 15 leaf functional traits of all the species as response variables.

Table S4 Loadings of 15 functional traits on the first four principal components for 121 mistletoe–host species pairs among four sites.

Table S5 Model with the lowest AIC from model selection based on linear mixed-effects models, showing the effects of the eight fixed variables for 11 mistletoe nutrient concentrations and two isotope ratios.

Table S6 Variance partitioning results for each mistletoe nutrient concentration using PARTR2 package.

Table S7 Variance partitioning results for each mistletoe and host nutrient ratio using PARTR2 package.

Table S8 Results of linear mixed-effects model with both mistletoe and host species as random effect for leaf nutrient elements and stable isotope ratios as response variables.