

## Greater differentiation in nitrogen sources is essential for co-occurring epiphytes under low-nitrogen canopy conditions

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### ABSTRACT

Acquiring enough nitrogen (N) to support their life processes in nutrient-poor canopies is a crucial challenge for epiphytes. When different epiphytes coexist in the same environment, they may use different strategies to acquire N. In this study, we employed stable isotope analysis to explore N acquisition and differentiation among co-occurring epiphytes in a subtropical forest in Southwest China. We found that functional group was the most important factor influencing N and  $^{15}\text{N}$  natural abundance ( $\delta^{15}\text{N}$ ) of epiphytes, and that different epiphyte groups used different N acquisition, uptake, and utilization pathways. Our analysis of the enrichment factors suggested that biological nitrogen fixation, phorophytes, canopy soil and ground soil could participate in N acquisition of epiphyte groups to varying degrees. However, epiphytes were more affected by precipitation and other canopy N sources than soil. Our structural equation models (SEMs) further showed that the potential N sources of plants and substrates affected throughfall and stemflow, thereby influenced N acquisition and preference of N forms in epiphytes. Overall, our findings indicate that there is differentiation in N sources among co-occurring epiphytes.

### 1. Introduction

Epiphytes, including lichens, bryophytes, ferns and spermatophytes, are a large and widely distributed group across many forest ecosystems. Among them, the number of vascular epiphytes can reach more than 31,000 species, accounting for about 10 % of the earth's vascular plant species (Zotz et al., 2021). The biomass of epiphytes is also substantial, and its contribution to productivity is similar to that of host plant leaves in some tropical and subtropical forests (Chen et al., 2010; Coxson and Nadkarni, 1995; Freiberg and Freiberg, 2000; Hofstede et al., 1993; Nadkarni, 1984a; b; Nakanishi et al., 2016). However, epiphytes have limited or no access to obtain water and nutrients from the soil through the root systems and are significantly limited by extreme nutrient deficiency such as N and phosphorus in nutrient-poor canopy environments (Benzing, 1990). Therefore, an interesting contradiction arises: why do the nutrient-poor canopies support such a large epiphyte community in

various forests? In other words, how do epiphytes obtain enough nutrients to fulfill their vital life processes in canopies?

N is one of the most important elements regulating the species composition and distribution of epiphytes in forest systems (Stewart et al., 1995; Wania et al., 2002; Cardelús and Mack, 2010). Epiphytes generally have lower nutrient concentrations than terrestrial plants (Hofstede et al., 1993; Wania et al., 2002; Watkins et al., 2007). For example, Hietz et al. (2022) found that the N concentration and  $\delta^{15}\text{N}$  in vascular epiphytes were commonly lower than those of terrestrial plants by collating the traits of 2882 vascular epiphytes. Epiphytes acquire N from different sources to meet their growth requirements. Canopy soil, which derived from dead organic matter (Hietz et al., 2002), is a N-rich but extremely rare resource in the canopies in many forests (Coxson and Nadkarni, 1995; Cardelús et al., 2009), while most epiphytes tend to grow on bare bark in these forests (Lesica and Antibus, 1990). Other potential N sources for epiphytes include foliar leaching of host trees

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(Hietz et al., 2002) and the decomposition of phorophyte leaves and epiphytes, as well as extra-canopy or atmospheric N obtained through wet and dry deposition (Clark et al., 1998; Liu et al., 2002). More importantly, biological nitrogen fixation is also one of the main sources of N for epiphytes (Benzing, 1990; Forman, 1975; Freiberg, 1998; Stewart et al., 1995). However, due to the high heterogeneity of the environment and the great diversity of epiphytes, the N acquisition pathways of epiphytes probably vary across different forest ecosystems (Eskov et al., 2019). Nonetheless, the N acquisition pathways and mechanisms of epiphytes remain unclear, particularly for those found in subtropical forests.

Stable isotope analysis has been used to clarify the N sources of epiphytes (Eskov et al., 2019; Hietz et al., 2002; Houlton et al., 2007; Wania et al., 2002). In general, vascular epiphytes primarily utilize N from atmospheric N<sub>2</sub> fixation and deposition in tropical forests (Benzing, 1990; Stewart et al., 1995). In a Costa Rican cloud forest, epiphytes that root in canopy soil have high  $\delta^{15}\text{N}$  and N concentrations, while those that root on bare branches have low  $\delta^{15}\text{N}$  and may acquire N from precipitation (Hietz et al., 2002). In three tropical forests in southern Vietnam, a significant foliar  $\delta^{15}\text{N}$  correlation between epiphytes and their hosts suggests that phorophyte litter and leachates could be recognized as potential N sources for epiphytes (Eskov et al., 2019). Moreover, Wania et al. (2002) found that the  $\delta^{15}\text{N}$  of epiphytes decreases along the lower-upper trunk and from the inside-outside canopy zones in a lowland rainforest in Costa Rica, indicating that epiphytes in the upper canopy mainly obtain N from atmospheric deposition, while epiphytes in the lower canopy acquire N from litterfall, leachates and stemflow.

When considering the common co-occurrence of various epiphyte groups, including lichens, bryophytes, ferns and seed plants, and niche complementarity for N (Kahmen et al., 2006), it is reasonable to assume that these epiphytes may obtain N through different means in order to meet their individual growth needs. This may help to reduce the competition for N acquisition in the nutrient-poor canopy habitats, resulting in significant differentiation in  $\delta^{15}\text{N}$  of epiphytes within the same local habitats. The enrichment factor (or  $\Delta^{15}\text{N}$ ), an index describing  $^{15}\text{N}$  depletion of plant leaf compared to N source, is widely used to provide insight into the natural processes and dynamics of available N pools in forest ecosystems (Cheng et al., 2010; Eskov et al., 2019). However, it should be noted that  $\delta^{15}\text{N}$  of epiphytes is not only influenced by the absorption, transport and regulation of N but also by various environmental factors, such as growth height, light intensity, moisture conditions and forest type (Ah-Peng et al., 2017; Dawson et al., 2002; Gurmesa et al., 2017; Pu et al., 2021a; Wania et al., 2002). Consequently, explaining the differentiation of N sources is challenging. Further  $\delta^{15}\text{N}$  analysis of epiphytes in similar microhabitats is needed to gain insight into their N sources and N use strategies.

In subtropical montane moist evergreen broad-leaved forests in southwest China, epiphytes are abundant and often coexist in canopies and on trunks (Li et al., 2013; Pu et al., 2021b). In those forests with low background N deposition, the growth of epiphytes could continue to be N limited (Shi et al., 2017). Therefore, we conducted research in the Ailao Mountains in Yunnan Province to explore the differentiation of N sources of epiphyte groups. We collected materials from epiphytic cyanolichens, chlorolichens, bryophytes, and vascular epiphytes and potential N sources (phorophyte leaves, litterfall, canopy soil, ground soil, and precipitation) in similar microhabitats in secondary patches within primary forests and analyzed their N concentrations and  $\delta^{15}\text{N}$ . By comparing the  $\delta^{15}\text{N}$  of collected materials, we hypothesized that (1) N concentrations and  $\delta^{15}\text{N}$  of epiphytes would differ significantly among groups and between seasons; (2) the N sources of epiphyte groups would be differentiated, with variation in their N and  $\delta^{15}\text{N}$  being significantly affected by canopy-derived N.

## 2. Materials and methods

### 2.1. Study site

The study was conducted in the Xujiaba area of the Ailao Mountains National Nature Reserve (23°35'–24°44' N, 100°54'–101°30' E) in Yunnan Province, Southwest China. The reserve is at an altitude of 2400–2750 m. The annual mean air temperature is 11.3 °C, with the minimum monthly mean temperature of 5.7 °C in January and the maximum monthly mean temperature of 15.6 °C in July. The mean annual precipitation is 1841 mm, with 86 % falling in the rainy season from May to October. The mean annual relative humidity of the reserve is 85 % (Huang et al., 2019; Li et al., 2013). More than 600 epiphyte species (113 angiosperms, 117 ferns, 176 bryophytes and 217 lichens) have been recorded and identified at the species level in the Ailao Mountains. For common epiphytic species in this area, please see our previous studies (Table 1; Huang et al., 2019; Li et al., 2013). In the study plots, *Lithocarpus hancei* is the dominant canopy tree species and the understory is dominated by sparse bamboo *Sinarundinaria nitida* (Li et al., 2015).

### 2.2. Sample collection

Based on the life forms and functional traits (Li et al., 2015; Huang et al., 2019), common epiphytes were classified into four groups, including vascular plants (ferns and seed plants), bryophytes, chlorolichens (lichens with green algae), cyanolichens (lichens with cyanobacteria) in this area. Cyanolichens were further subdivided into two groups according to the ecophysiological and morphological traits (Li et al., 2013, 2015; Liu et al., 2018): dominant *Lobaria retigera* (have large thallus loosely adnate on bark) and other non-dominant cyanolichen species (often have small thallus closely adnate on bark). Moreover, all cyanolichens and some bryophyte species can function as the primary participants in forest ecosystem N fixation (Fan, 2022; Liu et al., 2018; Song et al., 2016). All epiphyte samples were collected had to meet the criteria as described below:

- (I) In order to minimize the effects associated with habitat heterogeneity, targeted epiphytes were planning to collect from similar forest patches. Since almost all macrolichens grow in the canopies in primary forests (Li et al., 2013, 2015), five small secondary patches with similar conditions (patch size 0.4–0.9 ha, canopy openness 20–30 %, and detailed information are described in Li et al. (2013, 2015) were selected, in which five epiphyte groups can be found on lower tree trunks. The plots were established within the primary forests around the Ailao Mountains Station for Subtropical Forest Ecosystem Studies (2450 m altitude, 24°32' N, 101°01' E), and were 200–500 m away from each other (Fig. S8).
- (II) In order to minimize the effects of individual characteristics of the hosts and grow position of epiphytes (Wania et al., 2002), healthy-looking and mature leaves of vascular epiphytes (10–20 g dry weight), bryophyte shoots (10–20 g), chlorolichens thalli (10–20 g), *L. retigera* thalli (3–5 g) and other cyanolichens thalli (2–3 g) were collected from 30 to 50 epiphyte-colonized trunks at a height between 1 and 3 m above ground in each plot (Table 1). The number of selected trunks varied according to how many materials could be collected within the plot, most of which were *L. hancei*.
- (III) To evaluate the effect of season, epiphytes were sampled twice and collection times were April–May (dry season) and July (wet season) of 2020. However, leaves of deciduous vascular epiphytes were sampled only during the growing (wet) season.

Meanwhile, samples of potential N sources were collected from every plot. On the forest floor, litterfall was collected from five 20 cm × 20 cm subplots with a distance of > 1 m from the nearest trunk, and topsoil

**Table 1**

Investigated epiphytes and potential N sources and their  $\delta^{15}\text{N}$  and N% (mean  $\pm$  standard error, n = 10, different lowercase letters represent significant differences,  $p < 0.05$ ) in selected plots in the Ailao Mountains National Nature Reserve.

Material type	No. of samples (dry/wet season)	Common species	Sample source	$\delta^{15}\text{N}$ (‰)	N (%)
Large cyanolichens	5/5	<i>Lobaria retigera</i>	<i>Lobaria retigera</i>	-2.44 $\pm$ 0.10 <sup>c</sup>	3.87 $\pm$ 0.05 <sup>b</sup>
Other cyanolichens	5/5	<i>Coccocarpia erythroxyli</i> , <i>Leptogium menziesii</i> , <i>Sticta duplolibata</i> , <i>Sticta gracilis</i>	Mixed	-2.78 $\pm$ 0.11 <sup>c</sup>	4.31 $\pm$ 0.07 <sup>a</sup>
Chlorolichens	5/5	<i>Anzia hypomelaena</i> , <i>Cetrelia braunsiana</i> , <i>Cetrelia olivetorum</i> , <i>Everniastrum nepalense</i> , <i>Parmotrema reticulatum</i>	Mixed	-8.78 $\pm$ 0.41 <sup>f</sup>	1.13 $\pm$ 0.05 <sup>g</sup>
Bryophytes	5/5	<i>Bazzania praerupta</i> , <i>Bazzania ovistipula</i> , <i>Homaliodendron flabellatum</i> , <i>Plagiochila assamica</i>	Mixed	-5.94 $\pm$ 0.19 <sup>e</sup>	1.72 $\pm$ 0.07 <sup>e</sup>
Leaves of vascular epiphytes	5/5	<i>Lepisorus loriformis</i> , <i>Lepisorus bicolor</i> , <i>Lepisorus scolopendrium</i> , <i>Davallia perdurans</i> , <i>Briggsia longifolia</i> , <i>Aeschynanthus buxifolius</i> , <i>Begonia yui</i>	Mixed	-4.32 $\pm$ 0.19 <sup>d</sup>	2.06 $\pm$ 0.18 <sup>d</sup>
Phorophyte leaves	5/5	<i>Lithocarpus hancei</i>	<i>Lithocarpus hancei</i>	-2.66 $\pm$ 0.40 <sup>c</sup>	2.03 $\pm$ 0.06 <sup>d</sup>
Litterfall	5/5		Mixed	-2.63 $\pm$ 0.18 <sup>c</sup>	1.38 $\pm$ 0.06 <sup>f</sup>
Canopy soil	5/5		Mixed	-1.01 $\pm$ 0.24 <sup>b</sup>	2.51 $\pm$ 0.06 <sup>c</sup>
Ground soil	5/5		Mixed	3.69 $\pm$ 0.26 <sup>a</sup>	0.73 $\pm$ 0.05 <sup>h</sup>

cores (5 cm diameter, 0–10 cm) were collected underneath the litterfall. Phorophyte leaves and canopy soil were collected from the crowns of canopy tree species accessed by free climbing. For practical and safety reasons, three large individuals of *L. hancei* were selected, 30 healthy and well-grown mature leaves (a total of 90 leaves) were picked from different canopy zones of each tree individual using a pole pruner. Canopy soil (about 10 g dry weight) was collected with a fine brush on the surface of different large branches and under moss layers within the interior crown. Rainwater including bulk precipitation, throughfall and stemflow was collected twice a month using polythene containers during the growing seasons (from May to August). Bulk precipitation was collected at five open sites (spaced 100–200 m) near the ecological station. In each plot, stemflow was collected from with five *L. hancei* trunks and diverted to a polyethylene bag at a height of 2 m, and throughfall was collected under the canopies (1 m above the ground). The sampling containers must be carefully cleaned and washed with deionized water and were set before the rain event, and rain samples were collected within two hours after each rain event.

All samples were transferred to the station laboratory after collection. Litterfall was cleaned to remove soil, small rocks, roots and other contaminants, and fresh plant materials were washed with deionized water, dried at 65 °C for 48 h, and then were ground with a grinder and sift through 100 meshes. Canopy and ground soils were naturally air-dried and sifted through a 2-mm sieve. Rainwater was filtered through a 0.45  $\mu\text{m}$  membrane and stored at  $-20$  °C before chemical analysis.

For each type of plant and soil samples, materials collected from the same plot were combined to form one composite sample during each sampling season. In order to capture the monthly variability of rainfall in the study area, rainwater collected from the same month was mixed to form a composite sample per plot/site, with those in May were categorized as samples of the dry season and those from June to August as samples of the wet season (Fig. S4). A total of 150 samples, including 50 epiphyte (five duplicates  $\times$  five groups  $\times$  two seasons), 40 plant and soil N (five duplicates  $\times$  four types  $\times$  two seasons) and 60 rainwater (60 ml/sample, five duplicates  $\times$  three types  $\times$  four months) materials, were subjected to subsequent analysis.

### 2.3. Chemical analysis

For plant and soil samples, C and N concentrations and isotopic composition of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) were determined with an elemental analyzer (Finnigan DELTA V Advantage, Isotope Ratio Mass Spectrometer, Thermo Fisher Scientific Inc., USA) connected to a continuous flow isotope ratio mass spectrometer (Flash IRMS, Elemental

Analyzer, Thermo Fisher Scientific Inc., USA). All  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values are expressed in delta notation (‰) relative to the internationally accepted standards for C (PeeDee Belemnite, PDB) and N (Atmosphere, Atm).

The calculation equation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  is as follows:

$$\delta (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the isotope ratios ( $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ ) in the sample and standard substance, respectively, and the analytical errors are less than 0.1‰ for  $^{13}\text{C}$  and 0.2‰ for  $^{15}\text{N}$ .

For precipitation and soil samples, isotopic composition of ammonium and nitrate was determined with an elemental analyzer (MAT 253, Isotope Ratio Mass Spectrometer, Thermo Fisher Scientific Inc., USA), using azide reduction methods (hypobromite oxidation for  $\text{NH}_4^+$  preprocessing; cadmium power reduction for  $\text{NO}_3^-$  preprocessing; Zhang et al., 2007; Tu et al., 2016).  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N of samples were chemically converted to  $\text{N}_2\text{O}$ . By detecting  $^{15}\text{N}/^{14}\text{N}$  of  $\text{N}_2\text{O}$  and comparing it with international standard material (atmospheric  $\text{N}_2$ ), the  $\delta^{15}\text{N}$  values of inorganic N were calculated and analyzed, and the analytical error is 0.2‰. However, inorganic N and its  $\delta^{15}\text{N}$  could not be detected in canopy soil due to very high percentage of organic N. N concentrations of ammonium and nitrate in precipitation and soil extract samples were determined by a continuous flow analytical system (AutoAnalyzer 3, Germany).

### 2.4. Statistical analysis

All data analysis was processed by R 4.2.0 (R Core Team, 2022).

#### 2.4.1. Differences in elemental content and isotopic composition of epiphytes and potential N sources between groups and seasons

To test elemental content and isotopic composition of epiphytes and potential N sources among types, one-way ANOVA, and Tukey's HSD test for multiple pairwise comparisons were used. All data were checked for normal distribution using Shapiro-Wilk test and homogeneity of variance using Bartlett's test. When the assumptions could not be satisfied after transformation, comparisons of non-normal data were made using the non-parametric Kruskal-Wallis test and Wilcoxon rank sum test. For each sample type, paired t-test was performed to compare the elemental content and isotopic composition between seasons.

As the results showed that the  $\delta^{15}\text{N}$  of litterfall and phorophytes was too similar (Table 1), we thus chose phorophyte leaves as a proxy of N supply from phorophytes and excluded litterfall from the relevant subsequent models. *L. retigera* and other cyanolichens were combined into

cyanolichens, given their comparable habitats and  $\delta^{15}\text{N}$ . The values of bulk precipitation indicated that background N input via precipitation in this area and were not included in the subsequent models.

#### 2.4.2. The effects of potential N sources on N and $\delta^{15}\text{N}$ of epiphytes

First, we used a simple linear regression model (LRM) to analyze the relationship between  $\delta^{15}\text{N}$  and N in epiphyte groups. Then we calculated the modified enrichment factor (modified  $\delta^{15}\text{N}_{\text{epiphyte}} - \delta^{15}\text{N}_{\text{N source}}$ ) and performed the potential correlations between this factor and  $\delta^{15}\text{N}_{\text{epiphyte}}$  and  $\text{N}_{\text{epiphyte}}$ . The modified enrichment factor potentially represents the ability of epiphytes to acquire N from potential sources (Cheng et al., 2010; Eskov et al., 2019).

To test the effects of potential N sources on N and  $\delta^{15}\text{N}$  of epiphytes, a set of linear mixed models (LMMs) were chosen. First, LMMs were implemented using the *lmer* function in the R package *lme4* (Douglas et al., 2015) to evaluate the performance of random effects. The random effect variance of epiphyte group, season, and plot ID approached zero or was very small, so they were excluded in most cases. LMMs with 'epiphyte group' included as the random effect were only adopted to evaluate the potential effects of N sources on all non- $\text{N}_2$ -fixing epiphytes, and  $R_m^2$  (Marginal  $R^2$ , represents the proportion of all fixed effects that explain the response variables in total) and  $R_c^2$  (Conditional  $R^2$ , represents the proportion of all fixed effects and random effects that explain the response variables in total) were calculated (Nakagawa and Schielzeth, 2013). In terms of each group of epiphytes, multivariate LMMs were used to discern the effects of potential N sources (mentioned as full models). To identify the best predictors of N and  $\delta^{15}\text{N}$  of epiphytes, model selections were further conducted based on the corrected Akaike's information criterion (AICc;  $\Delta\text{AICc} < 2$ ) using the function *dredge* in the R package *MuMIn* (Bartoń, 2022) (mentioned as optimal models). Model averaging was performed based on AICc weights when multivariate LMMs were selected. All predictors were standardized before analyses using the Z-score to interpret parameter estimates on a comparable scale and were checked for collinearity and the variance inflation factor (VIF) (Fig. S1). When a pair had high Pearson correlation coefficient ( $|r| \geq 0.7$ ), one of the two variables was first removed, the remaining variable with the greatest VIF was then removed and the model was refitted. This process was repeated until all variables had a VIF  $< 10$ . Model residuals were checked for normality.

Finally, to estimate the individual fixed effects of predictors, *glmm.hp* package was performed for variance partitioning analysis of both full and optimal LMMs and LRMs (Lai et al., 2022).

#### 2.4.3. Importance assessment of N source characteristics and experimental design on $\delta^{15}\text{N}$ and N of epiphytes

To evaluate the importance of biological characteristics (N,  $^{15}\text{N}$ , C,  $^{13}\text{C}$ ) of potential N sources and experimental design (epiphyte group, season, and plot ID) on N and  $\delta^{15}\text{N}$  of epiphytes, we performed the random forest regression using the function *randomForest* in the R package *randomForest* (Liaw and Wiener, 2002). The random forest algorithm is highly flexible because it can capture nonlinear relationships between response and explanatory variables as well as hierarchical interactions between explanatory variables (Henderson et al., 2014). This method is also suitable for small samples and performs well in such scenarios (Rodríguez-Galiano et al., 2012). In cases where the explanatory power of a particular factor was found to be negative, the factor was eliminated, and the equation was recalculated accordingly.

#### 2.4.4. Direct and indirect influencing paths of potential N sources on N and $\delta^{15}\text{N}$ of epiphytes

To further evaluate the effects of potential N sources on N and  $\delta^{15}\text{N}$  of epiphytes and the complex causality between N sources, we generated SEMs for each group of epiphytes using the *lavaan* package (Rosseeel, 2012). In the models, we assuming that stemflow and throughfall influence the N and  $\delta^{15}\text{N}$  of epiphytes directly, whereas the other N sources could influence those of epiphytes both directly and indirectly

via their effects on stemflow and throughfall (Fig. S2). We used  $\chi^2$  test ( $P > 0.05$ ), goodness of fit (GIF) index ( $> 0.9$ ), and approximate root mean square error (RMSEA) index ( $< 0.08$ ) to judge the model fit (Spitale et al., 2009).

### 3. Results

#### 3.1. N concentration and $\delta^{15}\text{N}$ values in plants and substrates

The N and  $\delta^{15}\text{N}$  varied significantly among epiphyte groups (Table 1,  $\chi^2 = 78.369$ ,  $p < 0.001$ ). *Lobaria retigera* (N  $3.87 \pm 0.1\%$ ;  $\delta^{15}\text{N} -2.44 \pm 0.31\%$ ) and other cyanolichens (N  $4.31 \pm 0.25\%$ ;  $\delta^{15}\text{N} -2.78 \pm 0.35\%$ ) had higher N and  $\delta^{15}\text{N}$  than others, while chlorolichens had the lowest values (N  $1.13 \pm 0.14\%$ ;  $\delta^{15}\text{N} -8.78 \pm 1.29\%$ ). Based on these results, chlorolichens, bryophytes and vascular epiphytes could be classified as non- $\text{N}_2$ -fixing epiphytes and *L. retigera* and other cyanolichens could be classified as a  $\text{N}_2$ -fixing N source. However, the differences in N and  $\delta^{15}\text{N}$  of epiphytes were small between seasons within each group (Fig. 1). Only the N of *L. retigera* and vascular epiphytes differed greatly between the dry and wet seasons.

For substrates (Table 1), the  $\delta^{15}\text{N}$  of canopy soil ( $-1.01 \pm 0.77\%$ ) was close to that of atmospheric N (0‰), but  $\delta^{15}\text{N}$  of ground soil ( $3.69 \pm 0.76\%$ ) was above 0‰. In addition, the N of canopy soil ( $2.51 \pm 0.18\%$ ) was significantly higher than that of ground soil ( $0.73 \pm 0.17\%$ ). The  $\delta^{15}\text{N}$  of litterfall ( $-2.63 \pm 0.59\%$ ) and phorophytes ( $-2.66 \pm 1.19\%$ ) were close, but the N of phorophyte leaves was significantly higher than that of litterfall ( $1.38 \pm 0.14\%$ ). For rainwater, the  $\delta^{15}\text{N}_{\text{NH}_4^+}$  and  $\delta^{15}\text{N}_{\text{NO}_3^-}$  of throughfall and stemflow varied greatly among months (Fig. S4).

#### 3.2. Relationships between N and $\delta^{15}\text{N}$ of epiphytes and potential N sources

The  $\delta^{15}\text{N}$  value was strongly and positively associated with N across epiphyte groups (Fig. 2). There was a clear trend from epiphytic group with low N and depleted  $^{15}\text{N}$  to those with high N and enriched  $^{15}\text{N}$ . Moreover, the cyanolichens formed a cluster, while most points of chlorolichens and vascular epiphytes fell outside the confidence interval of the regression.

Our results showed that N and  $\delta^{15}\text{N}$  of epiphytes were positively correlated with enrichment factors (Fig. 3), indicating that phorophyte leaves, canopy soil, ground soil and biological nitrogen fixation were probably involved in N acquisition of epiphytes to various degrees. The regression coefficient between epiphytes and enrichment factor was the highest between  $\delta^{15}\text{N}_{\text{Non-N}_2\text{-fixing epiphytes}}$  and  $\delta^{15}\text{N}_{\text{Cyanolichens}}$  ( $R_{\text{adj.}}^2 = 0.993$ ), followed by that  $\delta^{15}\text{N}_{\text{Epiphytes}}$  and  $\delta^{15}\text{N}_{\text{Canopy soil}}$  ( $R_{\text{adj.}}^2 = 0.915$ ), and between  $\delta^{15}\text{N}_{\text{Epiphytes}}$  and  $\delta^{15}\text{N}_{\text{Ground soil}}$  ( $R_{\text{adj.}}^2 = 0.900$ ), and the lowest between  $\delta^{15}\text{N}_{\text{Epiphytes}}$  and  $\delta^{15}\text{N}_{\text{Phorophyte leaves}}$  ( $R_{\text{adj.}}^2 = 0.792$ ). Significant and lower coefficients ( $R_{\text{adj.}}^2 = 0.446-0.900$ ) were observed between N of epiphytes and enrichment factors. However, all regression relationships between N and  $\delta^{15}\text{N}$  of epiphytes and  $\delta^{15}\text{N-NH}_4^+$  and  $\delta^{15}\text{N-NO}_3^-$  of precipitation and ground soil were not significant (Fig. S5-7).

The variations in  $\delta^{15}\text{N}$  and N in non- $\text{N}_2$ -fixing of epiphytes were explained to a small extent by fixed effects ( $R_m^2$ ) only, but to a large extent by both fixed and random effects ( $R_c^2$ ) (Table 2). These results were consistent with those obtained from Fig. 1, suggesting that N acquisition pathways differed significantly among epiphyte groups. For each epiphyte group,  $\delta^{15}\text{N}_{\text{Chlorolichens}}$  were mainly affected by  $\delta^{15}\text{N-NO}_3^-$  of stemflow and throughfall (53.42 % proportion of  $R_{\text{adj.}}^2$ ), canopy soil (20.68 %) and phorophyte (20.11 %).  $\delta^{15}\text{N}_{\text{Bryophytes}}$  was mainly influenced by  $\delta^{15}\text{N}_{\text{Cyanolichens}}$  (51.77 %) and  $\delta^{15}\text{N}_{\text{Ground soil}}$  (26.67 %).  $\delta^{15}\text{N}_{\text{Vascular epiphytes}}$  was mainly explained by  $\delta^{15}\text{N}_{\text{Phorophyte leaves}}$  (29.26 %) and  $\delta^{15}\text{N}_{\text{Ground soil}}$  (26.75 %).  $\delta^{15}\text{N}_{\text{Cyanolichens}}$  were also influenced significantly by  $\delta^{15}\text{N-NH}_4^+$  derived from ground soil and throughfall (Table S1). Similar explaining patterns were observed for N of epiphytes. The results of the optimal models again highlighted the importance of



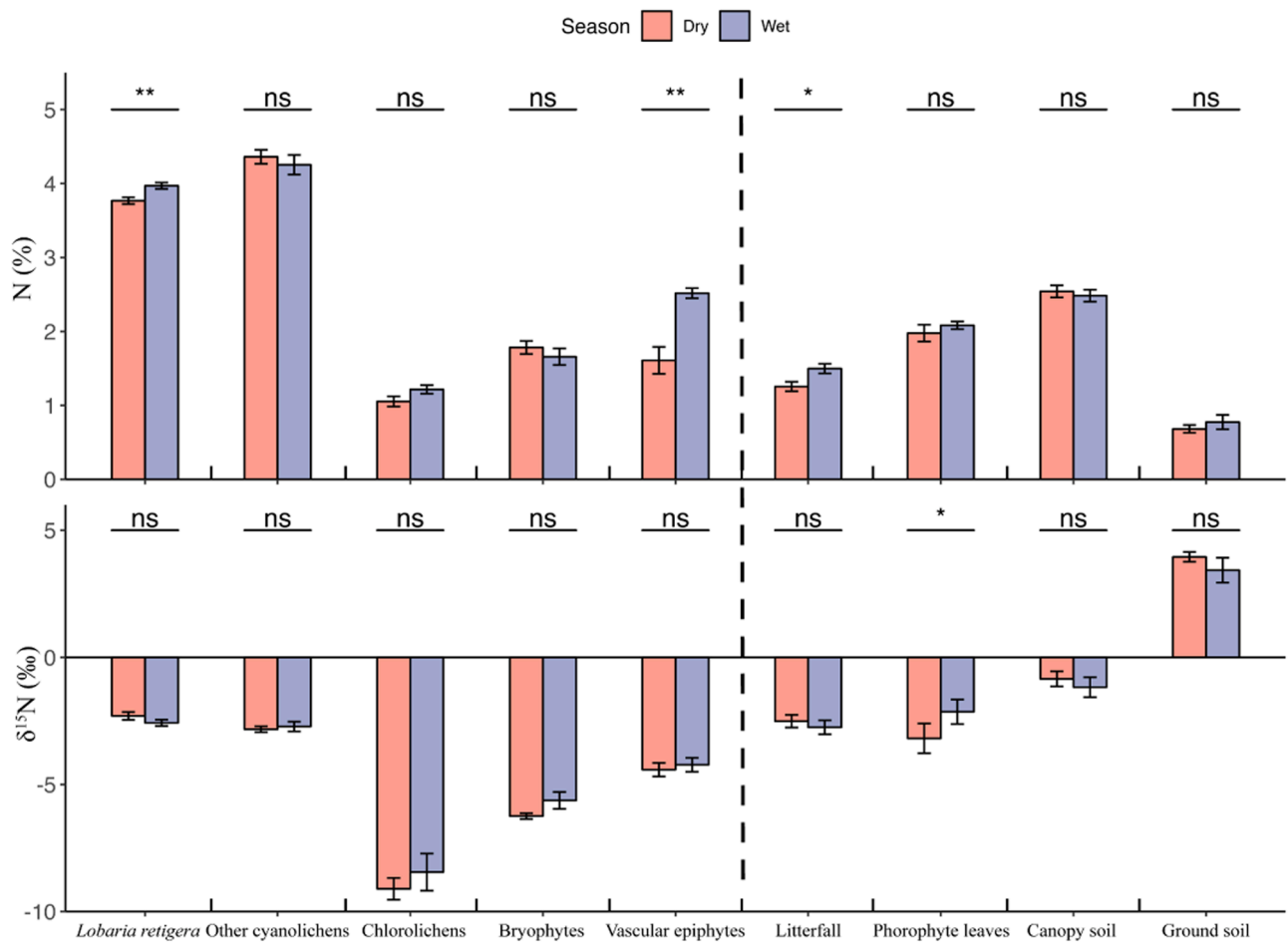


Fig. 1. Barplot of N and δ<sup>15</sup>N (mean ± standard error, n = 5) of plants and substrates in the dry and wet season (ns not significant, \*p < 0.05, \*\*p < 0.01).

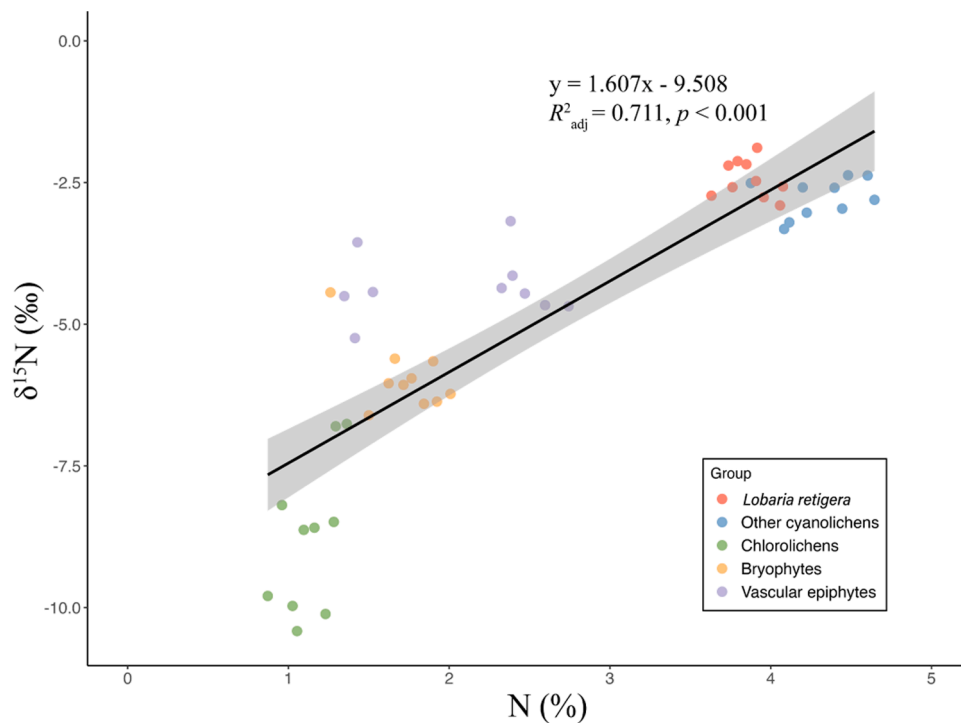
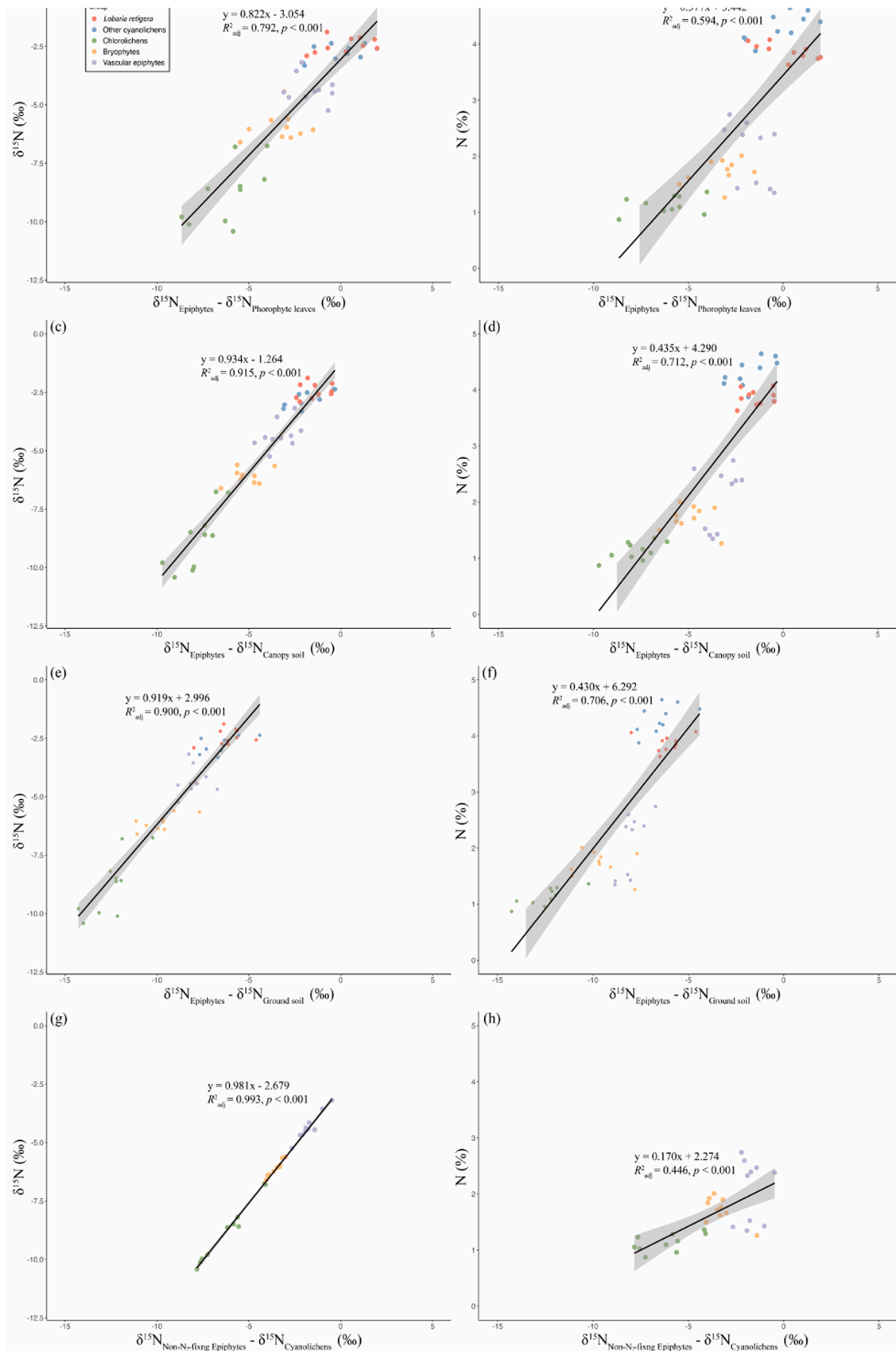


Fig. 2. Epiphyte δ<sup>15</sup>N as a function of epiphyte N concentration (n = 50). General linear model and its R<sup>2</sup> and p-values are shown. Grey areas represent the 95 % confidence intervals.



**Fig. 3.** Epiphyte  $\delta^{15}\text{N}$  or N% as a function of the enrichment factors for epiphyte groups ( $n = 50$  in a-f,  $n = 30$  in g and h). General linear models and their  $R^2$  and  $p$ -values are shown. Grey areas represent the 95 % confidence intervals.  $\delta^{15}\text{N}_{\text{Cyanolichens}}$  was the  $\delta^{15}\text{N}$  average of other cyanolichens and *Lobaria retigera*.

**Table 2**

Results of full linear (mixed) models and variance decomposition analysis for N and  $\delta^{15}\text{N}$  of non- $\text{N}_2$ -fixing epiphytes. In linear mixed models,  $R^2\text{m}$  represents the proportion of all fixed effects that explain the response variables in total, and  $R^2\text{c}$  represents the proportion of all fixed and random effects.  $R^2_{\text{adj}}$  represents adjusted  $R^2$  for linear models. The percentage of explained for each factor to the  $R^2\text{m}$  or  $R^2_{\text{adj}}$  are shown, and “-” represents this factor is not included in the selected model.

		Non- $\text{N}_2$ -fixing epiphytes							
		$\delta^{15}\text{N}_{\text{Non-N}_2\text{-fixing epiphytes}}$	$\delta^{15}\text{N}_{\text{Chlorolichens}}$	$\delta^{15}\text{N}_{\text{Bryophytes}}$	$\delta^{15}\text{N}_{\text{Vascular epiphytes}}$	N <sub>Non-N<sub>2</sub>-fixing epiphytes</sub>	N <sub>Chlorolichens</sub>	N <sub>Bryophytes</sub>	N <sub>Vascular epiphytes</sub>
Random effects	Group	-	-	-	-	Group	-	-	-
$R^2\text{c}$	0.8828	-	-	-	-	0.7051	-	-	-
$R^2\text{m}$	0.0452	-	-	-	-	0.1277	-	-	-
$R^2_{\text{adj}}$	-	0.8020	0.8838	0.9713	-	-	0.6676	0.9165	0.7430
Relative contribution to $R^2\text{m}$ or $R^2_{\text{adj}}$ (%)									
$\delta^{15}\text{N}_{\text{Cyanolichens}}$	17.70	2.68	51.77	6.62	4.62	7.09	35.32	-	-
$\delta^{15}\text{N}_{\text{Phorophyte leaves}}$	12.61	20.11	0.87	29.26	10.89	19.47	-	-	17.78
$\delta^{15}\text{N}_{\text{Canopy soil}}$	20.35	20.68	-	14.06	9.09	18.32	1.74	8.03	-
$\delta^{15}\text{N}_{\text{Ground soil}}$	11.06	3.11	26.67	26.75	26.02	17.57	1.69	23.45	-
$\delta^{15}\text{N-NH}_4^+$	3.98	-	5.16	10.63	-	-	-	-	-
$\delta^{15}\text{N-NO}_3^-$	-	-	11.05	-	-	-	26.35	-	-
$\delta^{15}\text{N-NH}_4^+$	7.08	-	4.38	5.08	2.51	-	0.35	13.01	-
$\delta^{15}\text{N-NO}_3^-$	-	32.56	-	7.60	9.80	22.98	25.09	11.95	-
$\delta^{15}\text{N-NH}_4^+$	-	-	0.10	-	-	14.57	-	-	-
$\delta^{15}\text{N-NO}_3^-$	27.22	20.86	-	-	37.07	-	9.46	25.78	-

abovementioned N sources (Table S2).

The analysis of all measured qualitative and quantitative factors using random forest regression revealed that approximately 78 % of the deviance for  $\delta^{15}\text{N}$  and 55 % for N of non- $\text{N}_2$ -fixing epiphytes could be explained (Table S3). Once again, epiphyte group merged as the most important predictor variable. The results were similar to those presented in Table 2, but with lower explanatory rates for each epiphyte group, implying that the combination of too many predictors may have led to confounding effects.

When the SEMs were employed to disentangle the direct and indirect effects of N sources on N and  $\delta^{15}\text{N}$  of epiphytes, the models explained 71–98 % of the variance in  $\delta^{15}\text{N}$  and 53–90 % in N for non- $\text{N}_2$ -fixing epiphytes, while the SEMs explained 47 % in  $\delta^{15}\text{N}$  and 81 % in N for cyanolichens. Interestingly, only phorophyte leaves exhibited a significant increase in  $\delta^{15}\text{N-NO}_3^-$  values of stemflow.

Regarding the  $\delta^{15}\text{N}$  values, the SEM analysis showed that  $\delta^{15}\text{N}_{\text{Cyanolichens}}$  was more affected by  $\text{NH}_4^+$  of throughfall (Fig. 4a).  $\delta^{15}\text{N}_{\text{Chlorolichens}}$  was more directly and negatively affected by  $\text{NO}_3^-$  in both throughfall and stemflow but was directly and positively affected by phorophyte leaves and ground soil (Fig. 4b).  $\delta^{15}\text{N}_{\text{Bryophytes}}$  was directly and negatively affected by cyanolichens and ground soil (Fig. 4c).  $\delta^{15}\text{N}_{\text{Vascular epiphytes}}$  was directly influenced by various N sources, with cyanolichens, phorophyte leaves and ground soil having positive effects, while canopy soil had a negative effect (Fig. 4d). However, stemflow and throughfall (nitrate N) had weak negative links to both  $\delta^{15}\text{N}_{\text{Bryophytes}}$  and  $\delta^{15}\text{N}_{\text{Vascular epiphytes}}$ . Similar SEMs were observed for N of each epiphyte group (Fig. 4e-h).

## 4. Discussion

### 4.1. Variation in $\delta^{15}\text{N}$ and N of epiphytes in subtropical forests

We observed that the  $\delta^{15}\text{N}$  of the studied epiphytes ranged from  $-10.42\text{‰}$  to  $-1.89\text{‰}$  and N ranged from 0.87 % to 4.65 % among different groups. Only the N of *Lobaria retigera* and vascular epiphytes showed significant differences between dry and wet seasons, partially supporting our first hypothesis that N of epiphytes differed significantly among groups and between seasons in subtropical forests. These N concentrations were within the range of values previously reported in this area (Huang et al., 2019; Li et al., 2014; Hu et al., 2023). However, the  $\delta^{15}\text{N}$  of epiphytic bryophytes was from  $-6.61\text{‰}$  to  $-4.44\text{‰}$ , which was lower than the ranges reported for other bryophytes associated with cyanobacteria in primary forests ( $\delta^{15}\text{N} -3.31\text{‰}$  in Fan, 2022;  $\delta^{15}\text{N} -3.27\text{‰}$  in Song et al., 2016). This difference may be due to the lower abundance of  $\text{N}_2$ -fixing bryophytes in the secondary patches within

primary forests (Song et al., 2011).

In the present study, our results of non-parametric test, linear mixed models and random forest regression suggested that functional group was the most important factor that could affect  $\delta^{15}\text{N}$  and N of epiphytes in a subtropical forest, in line with limited information available on tropical forests (Hietz et al., 2002; Stewart et al., 1995; Wania et al., 2002; Cardelús and Mack, 2010). In Europe beech forests,  $\delta^{15}\text{N}$  of cyanolichens ( $-5.46\text{‰}$  to  $-1.44\text{‰}$ ), tripartite lichens ( $-4.12\text{‰}$  to  $-2.76\text{‰}$ ) and chlorolichens ( $-15.04\text{‰}$  to  $-6.86\text{‰}$ ) is indicative of  $\text{N}_2$ -fixing capability (Hurtado et al., 2020). Meanwhile, in Costa Rica,  $\delta^{15}\text{N}$  of epiphytic ferns ( $-5.6$  to  $1.11\text{‰}$ ), orchids ( $-3.33$  to  $1.08\text{‰}$ ) and bromeliads ( $-3.38\text{‰}$  to  $-1.90\text{‰}$ ) changes over a wide range in tropical wet forests (Cardelús and Mack, 2010), and epiphytes rooting in canopy soil and on bare branches exhibit varying  $\delta^{15}\text{N}$  and N concentrations in montane cloud forest (Hietz et al., 2002). The significant differences in N and  $\delta^{15}\text{N}$  among various epiphyte groups are mainly due to their diverse ecophysiological features (e.g., morphological structure, metabolic pathway, life history), habitat preference (N source, light and moisture availability) and environmental conditions (e.g., elevation, N deposition, climate) (Cardelús and Mack, 2010; Hietz et al., 2002; Stewart et al., 1995; Wania et al., 2002). However, in our study, there were no significant differences in the aforementioned factors between the sites (Li et al., 2013), and the confounding effects of microhabitat heterogeneity were also minimized by sampling in as similar trunk habitats as possible at local level, indicating that the differences in N and  $\delta^{15}\text{N}$  among functional groups were likely to reflect the influence of N sources (see the next section). Indeed, we found that epiphytes had substantially different  $\delta^{15}\text{N}$  values, suggesting that epiphyte species that coexist under severe nutrient limitations may utilize different nutrient sources, leading to significant differences in the isotopic and nutritional composition within epiphyte communities, even within the same habitat (Chapin et al., 1993; Kielland, 1994; Schulze et al., 1994).

There was slight seasonal variation in  $\delta^{15}\text{N}$  and N among different epiphyte groups, except for the N of *L. retigera* and vascular epiphytes. *L. retigera* exhibited higher N concentration during the wet season, which could be attributed to a combination of its increased nitrogenase activity, accumulated biomass, and exogenous N input (Liu et al., 2018). Deciduous vascular epiphytes were sampled only during the wet season, resulting in a higher leaf N concentration and composition variation of sampled epiphyte species, which influenced mixed leaf N and led to a higher leaf N in wet than dry season for vascular epiphytes (Hu et al., 2023; Huang et al., 2019). However, most epiphytes showed a common, albeit weaker, increase in  $\delta^{15}\text{N}$  during the wet season, which could be due to seasonal increase in nitrogenase activity of  $\text{N}_2$ -fixers resulting in a higher N pool originating from these species (Liu et al., 2018; Fan et al.,

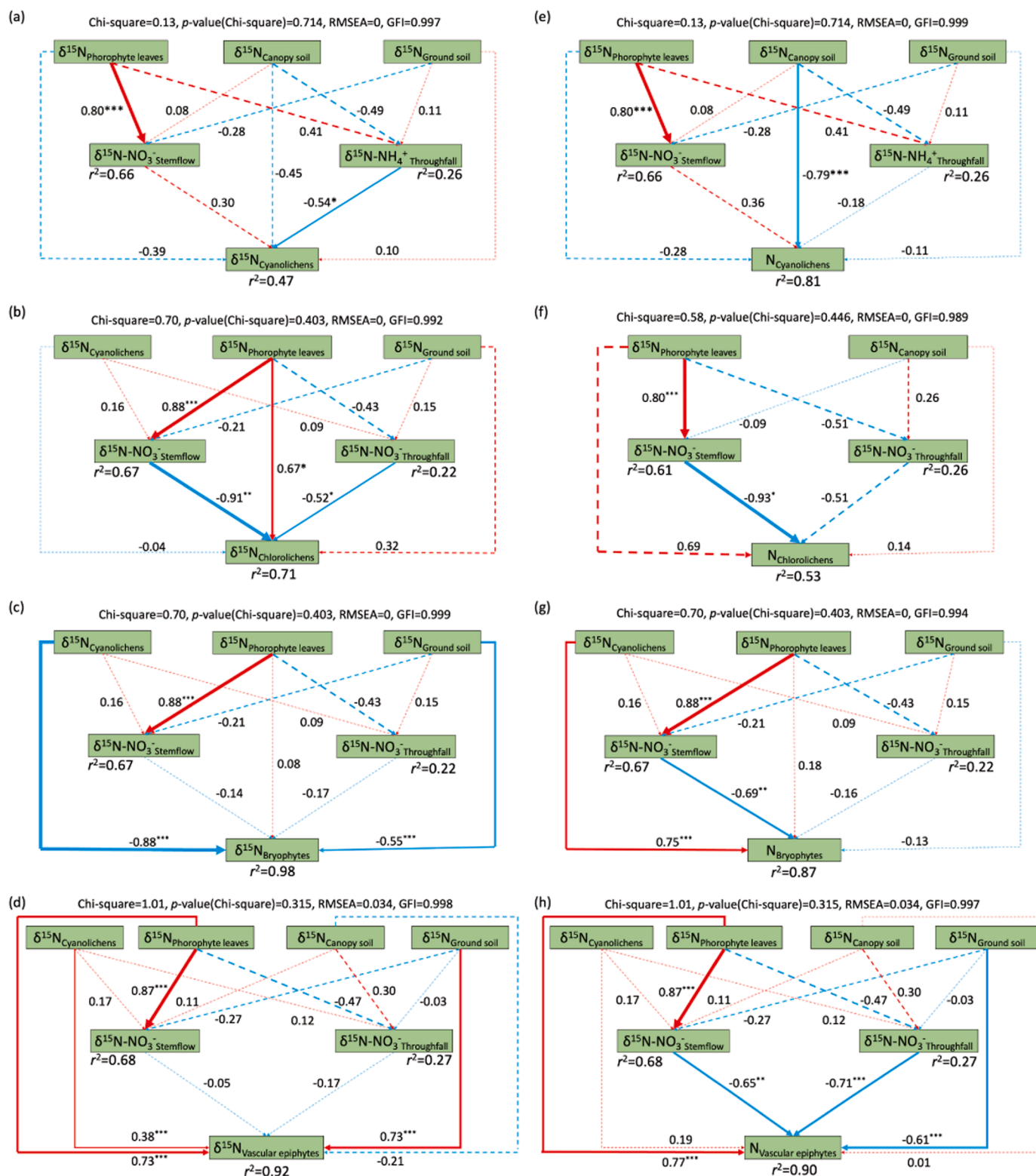


Fig. 4. Structural equation models (SEMs) of the influences of N sources on N and  $\delta^{15}\text{N}$  of epiphytes. Numbers adjacent to arrows were standardized path coefficients, indicating the effect size of the relationship. Red lines represent positive correlations between two variables, while blue lines represent negative correlation. Solid line and dotted line represent significant and non-significant pathways. The proportion of variance explained is given as  $r^2$ .

2022; Rzepczynska et al., 2022).

Compared with the data from phorophyte leaves in our current and previous studies (Li et al., 2014), our study further supports the idea that non- $\text{N}_2$ -fixing epiphytes have lower N and  $\delta^{15}\text{N}$  than terrestrial plants (Hietz et al., 2022). In contrast, cyanolichens, a nearly ubiquitous

epiphyte group in primary and secondary forests, have higher N and  $\delta^{15}\text{N}$  and have been assumed to be an important N resource for non- $\text{N}_2$ -fixing epiphytes (Li et al., 2013, 2014). Moreover, in our study area, non- $\text{N}_2$ -fixing epiphytes are strongly nutrient limited (Huang et al., 2019; Zhang et al., 2021), we therefore suggest that both phorophytes



and N<sub>2</sub>-fixing epiphytes are important N resources for canopy plants in subtropical forests with low N deposition in southwest China.

#### 4.2. Potential N sources of epiphytes in subtropical forests

The distinct N isotopic compositions reflect local N sources of epiphytes (Hietz et al., 2002; Wania et al., 2002). However, this isotopic signature is also influenced by the absorption, transport and regulation of N associated with habitat variability (Handley and Raven, 1992; Robinson, 2001; Evans, 2001). Lichens and bryophytes can take up nutrients over their entire surface area, and no significant fractionation is assumed to occur during N absorption (Gordon et al., 2001; Bragazza et al., 2005). Strong N limitation can lead to isotopic discrimination during root uptake decreases or even disappears regardless of the isotopic form of available N for vascular epiphytes (McKee et al., 2002; Clarkson et al., 2005; Huang et al., 2019; Zhang et al., 2021). Our sampling schemes reduced microhabitat-heterogeneity-driven variation as a possible cause for the magnitude of epiphyte  $\delta^{15}\text{N}$ , and the results seem to point to N-source-driven variation as the main causal factor (Michelsen et al., 1996). In fact, we found a close relationship between  $\delta^{15}\text{N}$  and N across epiphytes groups (Fig. 1) in our study, which was similar to the results obtained by Querejeta et al. (2018). This implies that stored N of epiphytes contains a significant amount of unique information on N sources. Our results from random forest models further suggest that many factors, such as  $\delta^{13}\text{C}$  and C content, have very little effect on the  $\delta^{15}\text{N}$  of epiphytes. We therefore suggest that various N sources are involved in the acquisition of N by epiphytes, providing partial support for our second hypothesis that there is differentiation in the N sources of epiphytes.

Several studies have demonstrated that atmospheric wet and dry deposition (Benzing, 1990; Gotsch et al., 2016; Eskov et al., 2019), canopy soil (Coxson and Nadkarni, 1995; Cardelús et al., 2009), foliar leaching and decomposition of trees and epiphytes (Hietz et al., 2002), and biological nitrogen fixation (Forman, 1975; Freiberg, 1998; Stewart et al., 1995) are all potential N sources for epiphytes (Eskov et al., 2019; Wania et al., 2002). Our analysis of  $\delta^{15}\text{N}$  enrichment factors in subtropical forests confirms the involvement of various N sources in nutrients of epiphytes, leading to differentiation in N utilization among different epiphyte groups.

Atmospheric wet and dry deposition is the primary N source for epiphytes (Eskov et al., 2019; Hietz et al., 1999, 2002; Stewart et al., 1995). Low  $\delta^{15}\text{N}$  values for epiphytes were often found in forest ecosystems, especially in unpolluted forests, which have been suggested to result from  $^{15}\text{N}$ -depleted bulk precipitation (Hietz et al., 2002; Kohls et al., 1994). The bulk precipitation (depleted in  $\text{NH}_4^+$  and enriched in  $\text{NO}_3^-$ ) was only collected in open land near the Ailao ecological station and was thus not a one-to-one match for throughfall and stemflow (Fig. S4). However, when bulk precipitation converted to throughfall and stemflow, we found that  $\delta^{15}\text{N}$  of throughfall and stemflow increased ( $> 0\text{‰}$ ), which was related to canopy-derived N such as N<sub>2</sub>-fixers, phorophytes, canopy soil and ground soil (e.g., wind-blown dust) (Fig. 4), as reported by Liu et al. (2002) and Wania et al. (2002). Moreover, our SEM results showed that phorophytes should be the dominant factor positively correlated with  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$ -N and negatively correlated with  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$ -N of throughfall and stemflow. Although data regarding organic N in precipitation were not available in our study, Song et al. (2016) found organic N was one of the main N sources for epiphytes, especially trunk epiphytes. Our results also support the idea that epiphytes rely more on atmospheric or within-canopy-derived N than soil-derived sources (Watkins et al., 2007).

We also found all trunk epiphytes were more heavily influenced by  $\text{NO}_3^-$ -N rather than  $\text{NH}_4^+$ -N in precipitation, which does not support the notion that epiphytes have a clear preference for  $\text{NH}_4^+$ -N (Inselsbacher et al., 2007). This likely reflects a combination of the N uptake characteristics of epiphytes as well as the effects of N forms. Vascular epiphytes can take up nutrients through both their leaf and root, even

mainly over their leaf surface (Inselsbacher et al., 2007), while lichen and bryophytes can absorb nutrients across their entire surface area (Benzing, 1990; Hietz et al., 1999). Additionally,  $\text{NO}_3^-$ -N can be absorbed through both roots and leaves, whereas  $\text{NH}_4^+$ -N must be assimilated in the roots of vascular plants (Wang and Macko, 2011; Uscola et al., 2014). Dahlman et al. (2004) further emphasize that ammonium absorption is largely passive, relative to amino acid and nitrate absorption for lichens. This finding is supported by Hu and Liu (2022), who found terrestrial vascular plants preferred  $\text{NO}_3^-$ -N over reduced N in subtropical forests of southwestern China, despite the latter being more abundant than  $\text{NO}_3^-$ -N in deposition (Liu et al., 2002). The authors speculate that anthropogenic N pollution has enhanced the utilization of  $\text{NO}_3^-$ -N by vascular plants (Hu and Liu, 2022). Similarly, Houlton et al. (2007) found that plants almost exclusively use nitrate as N source (80 %) in mature, undisturbed tropical forests with an average annual precipitation of 2000–3500 mm in Hawaii. However, since nitrate must be reduced to ammonia before it can be assimilated, which requires more energy cost than ammonium assimilation (Wang and Macko, 2011), the ecological origins and significance of epiphyte N uptake preferences remain in need of further research.

Epiphytic N<sub>2</sub> fixers, such as cyanolichens and bryophytes, play a crucial role in supplying N to tropical and temperate rainforests, and even compensating for N lost from these local ecosystems (Lindo and Whiteley, 2011; Markham and Fernández Otárola, 2021). Also, release of nitrogenous compounds from lichen thalli could be a normal feature, independent of death and decay (Millbank, 1985). Although it has been suggested that epiphytic N<sub>2</sub> fixers supply N to epiphytes (Eskov et al., 2019; Forman, 1975; Wania et al., 2002), the lack of convincing experimental data has led to widespread underestimation and neglect of the importance of biological nitrogen fixation to epiphytes. In our sites, most trees harbored abundant cyanolichens (N 4.09 % and  $\delta^{15}\text{N}$   $-2.61\text{‰}$ , this study) and/or bryophytes associated with cyanobacteria ( $\delta^{15}\text{N}$   $-3.31\text{‰}$  in Fan, 2022; N 1.86 % in Li et al., 2014;  $\delta^{15}\text{N}$   $-3.27\text{‰}$  in Song et al., 2016). Notably, in this study, the  $\delta^{15}\text{N}$  value and N concentration of bryophytes were strongly influenced by fixed N, which resulted not only from cyanolichens but also from symbiotic or free-living N<sub>2</sub>-fixing microorganisms (Song et al., 2016). In addition, Liu et al. (2002) found that bryophytes living on trunks could cause N accumulation in this forest area.

The N and  $\delta^{15}\text{N}$  of canopy soil in our study were 2.51 % and  $-1.01\text{‰}$ , respectively, which was in accordance with that of Wang et al. (2008) in the same area and Wania et al. (2002) in tropical wet forests in Costa Rica. Canopy soil is originated from organic matter consisting of decomposing epiphytes, leaf litter, invertebrates, fungi and microorganisms (Hietz et al., 2002). However, it should be noted that its inorganic N concentration was very low or undetectable in our study, indicating that the bulk N in canopy soil was dominated by organic N as a major component, which may not be immediately available to epiphytes. Also, canopy soil is distributed extremely unevenly, and most epiphytes tend to grow on bare bark in many forests (Coxson and Nadkarni, 1995; Cardelús et al., 2009; Lesica and Antibus, 1990). As a result, its significance to epiphytes (e.g., chlorolichens) may be overestimated, particularly on young trees and in secondary forests, where canopy soils are either absent or poorly developed (Hietz et al., 2002).

There is limited empirical evidence demonstrating that epiphytes, with the exception of hemiepiphytes, directly benefit from ground soil N, and the ground soil is believed to only influence epiphyte N via phorophytes (Benner and Vitousek, 2007). In terrestrial ecosystems, soil is the largest N pool and is characteristic of low N and high  $\delta^{15}\text{N}$ , such as 0.73 % N and 3.69%  $\delta^{15}\text{N}$  for soil in our study areas, which was consistent with those in Wang et al. (2008) and Lu et al. (2017). In the Ailao Mountains, wind-blown dust is often observed on cryptogam cover and leaf surface during the dry season (Li, personal observation). We speculated that ground soil could directly contribute to the N of epiphytes, which was supported by regression analysis. Accordingly, our results in conjunction with findings of Eskov et al. (2019), suggested that

epiphytes directly derived at least part of N from the phorophytes, primarily from tree leaves, which represented soil-derived N (Hietz et al., 2002). Compared to most epiphytes, phorophyte leaves have a large N pool (N 2.03 % and  $\delta^{15}\text{N}$  -2.66‰), which was also in line with previous studies in subtropical China (Fang et al., 2013). Epiphytes could catch the leaf litter and/or utilize leachates from phorophytes (Hietz et al., 2002). On the other hand, forest canopies could affect the chemistry of throughfall through canopy exchange processes (Benner and Vitousek, 2007; Boelter et al., 2014; Eskov et al., 2019; Wania et al., 2002).

#### 4.3. The N utilization differentiation of epiphytes in subtropical forests

Through the combined application of variance decomposition, random forest regression and the SEMs, group type proved to be the most significant factor affecting the  $\delta^{15}\text{N}$  and N of epiphytes, that is, different epiphytes have adopted diverse N utilization strategies in our study area. Our SEM pathway network further revealed that the N sources of epiphytes were influenced by a combination of direct and indirect effects. For example, the impact of phorophytes (leaves and litters) on epiphyte N was primarily an indirect effect resulting from phorophytes' influence on stemflow and throughfall.

Cyanolichens primarily obtain N through  $\text{N}_2$ -fixation, which is an energy-intensive process requiring 16 ATP to convert  $\text{N}_2$  to 2  $\text{NH}_3$  molecules, however, exogenous N was still important for the growth of cyanolichens in forests (Table S1). The SEM pathway network explained 47 % of the variance in cyanolichen  $\delta^{15}\text{N}$  (Fig. 4a). Cyanolichens were found to prefer  $\text{NH}_4^+$ -N, which was in accordance with laboratory studies (Dahlman et al., 2004; Inselsbacher et al., 2007). However, the significant importance of ground soil for cyanolichens was only detected in the multivariate LRMs (Table S1) and was not evident in the SEM. Nonetheless, cyanolichens commonly occur on lower trunks, and their growth appears to benefit from soil N (Li et al., 2015), which is, however, in need of further research. Compared to cyanolichens, the SEM was able to explain 71 % variance in  $\delta^{15}\text{N}$  for chlorolichens. Notably,  $\text{NO}_3^-$ -N in stemflow and throughfall had a greater effect on chlorolichens, while the proportion of overall variance explained by the linear model reached 80.2 %, among which precipitation explained 53.42 % of its  $R_{\text{adj}}^2$  (Table 2). This suggests that chlorolichens are most directly dependent on nutrients from atmospheric dry and wet deposition and obtained the majority of their N from rainwater (Dahlman et al., 2004; Hyvärinen and Crittenden, 1998). Additionally, the reported  $\delta^{15}\text{N}$  for chlorolichens was in the range of  $\text{NH}_4^+$ -N reported for bulk precipitation (Fig. S4), although  $\text{NH}_4^+$ -N was not a significant variable in this case. Cyanolichens had a negligible contribution to the N of chlorolichens, while ground soil and canopy soil also explained 17.9 % of the variance in chlorolichen  $\delta^{15}\text{N}$  (Table S2), but their effect on lichens is not well understood. For epiphytic bryophytes, the proportion of overall variance explained in  $\delta^{15}\text{N}$  and N reached 87–98 % by the linear models and SEMs. They were largely and directly affected by N fixation, ground soil, and  $\text{NO}_3^-$ -N of stemflow. In contrast, the phorophytes indirectly influenced bryophytes via stemflow and throughfall and had a negligible and direct contribution to them (Fig. 4c, 4g). Bryophyte-cyanobacteria symbiosis could also provide a source of N (Deane-Coe, 2015). However, canopy soil had little influence on epiphytic bryophytes, likely due to the lack of accumulated soil on trunks (Hietz et al., 2002). Vascular epiphytes, on the other hand, almost acquired N from all sources (Eskov et al., 2019; Wania et al., 2002), with the SEMs explaining 90–92 % of the variance in  $\delta^{15}\text{N}$  and N (Fig. 4d, 4h). Ground soil and phorophytes were more closely related to  $\delta^{15}\text{N}$  and N of vascular epiphytes while precipitation was related to N (Table 2 and S2) (Hietz et al., 2002; Stewart et al., 1995). This was confirmed by the fact that abundant ferns and seed plants occurred on lower trunks (Xu and Liu, 2005). Canopy soil was also less important for vascular epiphytes. All in all, our results also fit the niche complementarity hypothesis, which suggests that plant species occupy distinct ecological niches and utilize resources in complementary ways, allowing different species and growth forms to coexist

(Kahmen et al., 2006).

#### 4.4. Limitations of the study

Although our study provides the first evidence for the differentiation of N sources among the main epiphyte groups in Chinese subtropical forests, there are significant limitations to this study. The use of  $^{15}\text{N}$  natural abundance analyses may have led to overestimations of the importance of N sources. The overlapping isotopic values of various potential N sources make it difficult to identify N sources based only on the natural abundance of  $^{15}\text{N}$  (Wania et al., 2002). Rainwater also varies greatly across months and is susceptible to contamination from bird faeces, insect debris, leaf litter and bark wounds, and N would be lost due to collection-funnel adhesion and microbial assimilation, which may result in inaccurate and unreliable  $\delta^{15}\text{N}$  data. The uncertainty in N supply of epiphytes often limits the interpretability of the results. Clearly, carefully controlled experiments using  $^{15}\text{N}$  labeling methods are needed to accurately explore the potential N sources of epiphytes and quantify the nutrients sharing among different epiphytes under field conditions.

## 5. Conclusion

Our study has shed light on N sources of co-occurring epiphytes in Chinese subtropical forests and has confirmed that atmospheric or within-canopy-derived N is more important than soil-derived N for epiphyte growth. Enrichment factor analyses have also suggested that different potential N sources contribute to epiphyte N acquisition to varying degrees. Throughfall and stemflow were found to have an important impact on N and  $\delta^{15}\text{N}$  of epiphytes, and other N sources (e.g., biological nitrogen fixation, phorophytes, canopy soil and ground soil) could alter the chemistry of throughfall and stemflow and thereby affect N acquisition of epiphytes. Linear mixed models and random forest regression suggested that functional group was the main factor affecting N and  $\delta^{15}\text{N}$  of epiphytes, reflecting differences in N acquisition, uptake and utilization strategies among the different groups. Cyanolichens mainly acquired N through  $\text{N}_2$ -fixation, while chlorolichens relied more on rainwater N. Bryophytes obtained considerable N from N fixation and ground soil, while vascular epiphytes acquired N from a wide range of sources, including ground soil, phorophytes and precipitation.

## Author contributions

For this research article: Conceptualization and methodology, S.L., and T.S.; Samples collecting, T.S., C.Z., W.S., and Z-F.L.; Experiment assistance, Z-Y.L.; Data curation and formal analysis, T.S., S.L., and T.H.; Funding acquisition, S.L.; Visualization, T.S.; Writing—original draft, review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

## CRedit authorship contribution statement

**Tian-Hao Su:** Conceptualization, Methodology, Software, Samples collecting, Data curation, Formal analysis, Writing- original draft. **Chun-Yan Zhou:** Samples collecting, Writing- original draft. **Wei Sun:** Samples collecting, Writing- original draft. **Tao Jia:** Writing- original draft. **Tao Hu:** Data curation, Formal analysis, Writing- original draft. **Zhong-Fa Li:** Samples collecting, Writing- original draft. **Xiao-Yan Pu:** Writing- original draft. **Zhi-Yun Li:** Experiment assistance. **Su Li:** Conceptualization, Methodology, Data curation, Formal analysis, Funding acquisition, Writing- original draft. **All authors:** Writing- Reviewing and Editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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