Received: 9 January 2025 | Accepted: 27 June 2025

DOI: 10.1111/1365-2745.70131

BRITISH ECOLOGICAL SOCIETY Journal of Ecology

RESEARCH ARTICLE

Group-consistent but species-specific nitrogen isotope fractionation between leaves and roots in tropical vascular epiphytes

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 32171554, 32471641 and 32271698; Yunnan Revitalization Talent Support Program, Grant/Award Number: YNWR-QNBJ-2020-067; 14th Five-Year Plan of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Grant/Award Number: E3ZKFF2B01; Candidates of the Young and Middle-Aged Academic Leaders of Yunnan Province, Grant/Award Number: 202305AC160024

Handling Editor: Gabriela Bielefeld Nardoto

Abstract

1. Epiphytes are believed to exhibit minimal internal nitrogen (N) isotopic composition ($\delta^{15}N$) variation due to severe N limitation in canopy habitats. However, the lack of empirical evidence leaves this question open, complicating the interpretation of foliar $\delta^{15}N$ in understanding the N economy of epiphytes.
2. Here, the variation in $\delta^{15}N$ between leaves and roots ($\Delta^{15}N_{leaf-root}$) of 24 epiphyte species from the tropical Xishuangbanna region of southwest China was calculated. Subsequent linear regression and variance decomposition were performed to identify the underlying driving factors.
3. A small $\Delta^{15}N_{leaf-root}$ (+0.43‰) was observed in epiphytes, which exhibited relatively consistent values across groups (−0.20‰ to 1.78‰) but markedly species-specific values (−2.13‰ to 2.15‰), independently of phylogeny, reflecting the combined effects of external N sources and N acquisition strategies of epiphytes. In particular, positive $\Delta^{15}N_{leaf-root}$ values were primarily correlated with leaf N and root carbon (C), while negative $\Delta^{15}N_{leaf-root}$ values were associated with leaf C isotopic composition ($\delta^{13}C$), indicating that distinct ^{15}N fractionation progresses were at work.
4. *Synthesis*. These results demonstrate for the first time that $\Delta^{15}N_{leaf-root}$ is minimal yet noteworthy within epiphytes compared with soil-rooted plants, reflecting the more severe N stress in the canopy habitats, and suggest that foliar $\delta^{15}N$—when accounting for ^{15}N fractionation—can provide valuable insights into the N dynamics of tropical epiphytes.

KEYWORDS
^{15}N natural abundance ($\delta^{15}N$), carbon isotope composition ($\delta^{13}C$), ecophysiology, forest canopy, nitrogen isotope fractionation, nutrient limitation, vascular epiphyte, water deficit

1 | INTRODUCTION

The forest canopy represents a structurally complex and ecologically critical subsystem of the forest, and its biodiversity is unique. Vascular epiphytes are a striking component of canopies in tropical rainforests and humid montane forests due to their high diversity, comprising more than 31,000 species—approximately 10% of the Earth's vascular plant species (Zotz et al., 2021). They contribute substantially to ecosystem diversity, production and nutrient cycles and provide appreciable nutrient and energy sources to associated organisms (Coxson & Nadkarni, 1995; Hölscher et al., 2004). However, the forest canopy is a challenging habitat for epiphytes due to the removal of terrestrial resource pools. As a result, epiphytes are often considered limited by a low and intermittent supply of water and nutrients, such as nitrogen (N) and phosphorus (P) (Benzing, 1990; Laube & Zotz, 2003). In particular, despite the presence of various potential N sources for epiphytes in the forest canopy, such as atmospheric deposition, litter decomposition, animal faeces and free-living N fixation (Leroy et al., 2016; Zotz, 2016), canopy N supply and forms appear highly spatiotemporally heterogeneous, and their fluxes and relative contributions to epiphytes remain highly uncertain.

Inadequate information regarding N sources and utilisation strategies of epiphytes limits our understanding of the mechanisms underlying their high diversity of species in canopy sub-ecosystems (Cardelús & Mack, 2010). Numerous studies, including a limited focus specifically on epiphytes, show that natural variation in N isotopic composition ($\delta^{15}N$) is increasingly recognised as a powerful tool for elucidating plant N dynamics, as available N sources typically exhibit distinct isotopic signatures (Cardelús & Mack, 2010; Evans, 2001; Houlton et al., 2007; Stewart et al., 1995; Wania et al., 2002; Yoneyama, 1996). However, variations in foliar $\delta^{15}N$ are not solely attributed to the differentiation of N sources. ^{15}N discrimination during internal N absorption, assimilation and distribution within the plant could also result in deviations in $\delta^{15}N$ between plants and their N sources, as well as between leaves and roots, thus complicating the interpretation of foliar $\delta^{15}N$ (Evans, 2001; Hietz & Wanek, 2003; Wania et al., 2002). For example, internal ^{15}N fractionation in terrestrial plants leads to approximately 2‰ differences between roots and shoots in various ecosystems (Dawson et al., 2002; Högberg, 1997; Houlton et al., 2007; Kitayama & Iwamoto, 2001; Shearer & Kohl, 1986), including tropical rainforests (Hu et al., 2022). Even in low-N ecosystems, isotopic fractionation can still occur due to reduced N demand, driven by other environmental factors that are more limiting than N (Hobbie & Colpaert, 2003; McKee et al., 2002; Wania et al., 2002). In particular, the average leaf $\delta^{15}N$ and its variability are generally lower in epiphytes than in terrestrial plants (Craine et al., 2018; Hietz et al., 2022). Therefore, the degree of enrichment caused by ^{15}N fractionation poses a challenge to the accuracy of determining N sources and interpreting N cycling dynamics in epiphytes, thus reducing the reliability of studies based on natural leaf ^{15}N abundance.

Despite increasing interest in ^{15}N fractionation of epiphytes, accurate data remain scarce, as it is particularly time-consuming to reach the canopy and difficult to obtain sufficient representative samples (Barker & Pinard, 2001). The high heterogeneity and complexity of canopy environments, along with the considerable diversity and uncertainty of N sources, further exacerbate the difficulty of this type of research. In general, ^{15}N fractionation in epiphytes is assumed to be negligible, based on a widely accepted premise that canopy environments exhibit very low N availability on an area-based scale (Cardelús & Mack, 2010; Stewart et al., 1995). However, Wanek and Zotz (2011) reported that the field-collected leaves of epiphytic bromeliads exhibited approximately 5‰ ^{15}N fractionation relative to their primary N sources derived from tank water and debris. Additionally, the $\delta^{15}N$ between epiphyte leaves and roots ($\delta^{15}N_{leaf}-\delta^{15}N_{root}=-2.3\pm0.3‰$) also differed significantly in a lowland rainforest of Costa Rica (Wania et al., 2002). Although these few case studies seem to contradict the traditional assumption, the nearly complete lack of pertinent data prevents us from unambiguously assessing fractionation between N sources and plant organs, as well as between different organs, thus hindering the ability to test this assumption. Whether ^{15}N fractionation occurs within epiphytes remains an open question.

Understanding the mechanisms controlling ^{15}N fractionation between the leaf and root of epiphytes can shed light on their internal N metabolic processes (Tcherkez & Hodges, 2008). However, interpreting the underlying causes and processes is not straightforward due to the potentially different strategies for N utilisation in epiphytes compared with terrestrial plants. Studies on terrestrial plants have shown that external N concentration and plant N acquisition strategies (related to N assimilation, reallocation and organ-specific N loss) can affect intra-plant $\delta^{15}N$ variation (Evans, 2001; Kolb & Evans, 2003). Although the lack of $\delta^{15}N$ data is much more aggravated in the case of epiphyte roots compared with leaves, and few studies have characterised the factors affecting isotopic fractionation, however, insights can still be drawn from research on terrestrial plants and the leaf $\delta^{15}N$ signals of epiphytes, which are related to environmental conditions (e.g. ecosystem, level and form of N deposition), habitat preferences (e.g. growth position and substrate) and ecophysiological characteristics (e.g. taxonomic group, species identity, metabolic pathway and morphological structure) (Cardelús & Mack, 2010; Díaz-Álvarez et al., 2016; Hietz et al., 1999; Stewart et al., 1995; Su et al., 2023; Wania et al., 2002). Therefore, it is reasonable to speculate that the ^{15}N fractionation between the leaves and roots of epiphytes is also affected by these factors.

Vascular epiphytes are highly diverse in tropical and subtropical forests of southwestern China; for example, more than 400 species have been recorded in the tropical Xishuangbanna region alone (Wu et al., 2016). In this study, we used different methods (single-rope climbing, free climbing, and pole pruners) to access the canopies and collect leaf and root samples of vascular epiphytes in this region to test ^{15}N fractionation. Given that external N sources for epiphytes are variable and difficult to determine and collect comprehensively, this study focused solely on evaluating internal ^{15}N fractionation within epiphytes. Our specific objectives are: (1) to confirm whether internal ^{15}N fractionation occurs between the roots and leaves of vascular epiphytes and (2) to characterise the characteristics and influential factors

if fractionation occurs. We hypothesise that (1) ^{15}N fractionation occurs between the roots and leaves of epiphytes, and (2) this fractionation is influenced by environmental conditions, habitat preferences and ecophysiological characteristics (e.g. taxonomic group, species identity and metabolic pathway) of epiphytes, reflecting the variability in N acquisition and internal utilisation strategies of epiphytes.

2 | MATERIALS AND METHODS

2.1 | Study site

This study was carried out in Xishuangbanna Prefecture, Yunnan Province, southwestern China. Xishuangbanna has the largest expanse of tropical rainforest in China, characterised by its relatively northern latitude, higher elevation and drier climate compared with typical tropical rainforests (Zhang & Cao, 1995). The mean annual temperature is 21.5°C and the mean annual precipitation is 1550 mm; of this, 85% occurs during the rainy season (May–October). The dry season (November–April) includes a foggy subseason (November–February) and a brief but hot subseason (March–April). During the foggy subseason, a high frequency of fog occurs from midnight until morning (Mo et al., 2022).

Vascular epiphytes were collected from four sites in the Xishuangbanna region, characterised by different levels of anthropogenic disturbance. These comprised (1) Menglun Town (Town); (2) the Xishuangbanna Tropical Botanical Garden of Chinese Academy of Sciences (XTBG); (3) a fragmented tropical seasonal rainforest along China's National Highway 213 (G213); and (4) a fragmented limestone forest in Yinchang Upper Village, Jinuo Ethnic Township (Yinchang). The Town (547–608 m, 101°21′–101°25′ E, 21°93′–21°95′ N) experiences high human disturbance owing to dense residential development and frequent traffic activity; epiphytes were sampled from ornamental palms and roadside trees. The XTBG (551–698 m, 101°25–101°28′ E, 21°90′–21°92′ N) is a research institution combining natural habitats with moderate human activity. Epiphytes were sampled from four patches within the XTBG: tropical karst forest, tropical rainforest, tropical agroforestry and cultivation area. The G213 (611–917 m, 101°17′–101°22′ E, 21°95′–21°97′ N) lies along a 5 km abandoned segment of National Highway 213 in Menglun District, with minimal disturbance. Epiphytes were sampled from broad-leaved trees, predominantly orchids. The Yinchang (1320–1363 m, 101°23′ E, 21°98′ N) is a mountainous limestone forest with low disturbance, where dominant epiphytes included *Drynaria roosii*, *Oberonia ensiformis*, *Holcoglossum kimballianum* and *Peperomia heyneana*.

2.2 | Sample collection

As fragmented forest patches are distributed mainly on rugged ravines or ridges, and most vascular epiphytes in this region are evergreen species, epiphytes were collected during the dry season (April–May) of 2023 to avoid the high risk associated with canopy climbing during the rainy season. Several methods were used to obtain epiphyte samples from standing trees, including single-rope climbing, free climbing and pole pruners. Our sampling approach attempted to balance the main goals and criteria for epiphyte selection, which were as follows: (1) Healthy, mature individuals growing >1 m above-ground without ground contact were collected. Consequently, hemiepiphytic individuals belonging to *Aeschynanthus*, *Ficus*, *Hoya*, and Araceae were not included in our study. (2) Each sample per epiphyte species must contain sufficient leaf and root material for chemical analysis. Depending on plant size, one to six individuals were pooled per sample. (3) The distance between samples exceeded 50 m at most sites, but was reduced to 5–10 m for certain species in Yinchang, due to the small size of the forest patch and the scarcity of epiphytes. (4) For each sample, information on latitude, longitude, elevation, growth position and height on the host was recorded. Each individual and its substrate microhabitat were also photographed for detailed documentation.

Although we made every effort to collect adequate representations of vascular epiphytes in the studied region, some small or rare species still lacked available root/leaf materials or enough replicates ($n < 3$) to meet experimental requirements and thus were excluded from our study. Finally, a total of 132 samples were chosen, covering 24 species distributed in 10 families, for subsequent analysis. Each species had more than three replicates across the four sites, with corresponding leaf and root subsamples included. In this study, fieldwork was conducted under a framework of long-term institutional collaboration between the XTBG and local authorities, and therefore, no additional permits were required.

2.3 | Chemical analysis

All sample pretreatments were performed in our laboratory at the XTBG. Fresh leaf and root materials were thoroughly washed, oven-dried at 65°C until constant weight was reached, and subsequently ground into fine powder using a ball mill and sieved through a 60-mesh sieve for subsequent analysis.

For each sample, carbon (C) and N concentrations, as well as their $\delta^{13}C$ and $\delta^{15}N$, were determined using an elemental analyser (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 Analyser, Thermo Fisher Scientific Inc., USA). All $\delta^{13}C$ and $\delta^{15}N$ values were expressed in delta notation (‰) relative to internationally accepted standards for C (PeeDee Belemnite, PDB) and N (atmosphere N_2, Atm).

The isotopic composition was calculated as:

$$\delta^{13}C \text{ or } \delta^{15}N\,(‰) = \left(R_{sample} / R_{standard} - 1\right) \times 1000,$$

where R_{sample} and $R_{standard}$ are the isotopic ratios ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in the sample and standard substance, respectively. The analytical precision is 0.1‰ for $\delta^{13}C$ and 0.15‰ for $\delta^{15}N$.

2.4 | Statistical analysis

2.4.1 | Parameter specification

$\delta^{15}N_{leaf}$ indicates the $\delta^{15}N$ in leaf samples and $\delta^{15}N_{root}$ indicates the $\delta^{15}N$ in root samples. $\Delta^{15}N_{leaf-root}$ is defined as $\delta^{15}N_{leaf}-\delta^{15}N_{root}$, indicating ^{15}N fractionation between leaves and roots in epiphytes (Emmerton et al., 2001). A positive $\Delta^{15}N_{leaf-root}$ value indicates that leaves are enriched in ^{15}N compared with roots, whereas a negative value indicates that leaves are depleted in ^{15}N relative to roots.

In addition, $\Delta N_{leaf-root}$ is calculated as an indicator of N allocation strategies in plants. When analysed alongside $\delta^{15}N$ values, it helps elucidate N absorption and utilisation mechanisms (Hu et al., 2022).

The $\delta^{13}C$ represents carbon isotope fractionation during atmospheric CO_2 fixation. This parameter can indicate water use efficiency or water availability, with higher $\delta^{13}C$ values reflecting greater water use efficiency (Eskov et al., 2023; Hultine et al., 2018).

2.4.2 | Group division

To identify factors influencing internal ^{15}N fractionation in epiphytes, we grouped samples according to experimental designs and plant characteristics: (1) sampling sites: G213, Town, XTBG and Yinchang; (2) vertical positions: canopy and understorey (growing on trunks); (3) taxonomic groups: ferns, orchids and others (including Gesneriaceae, Moraceae and Apocynaceae); (4) photosynthetic pathways: epiphytes can be divided into C_3 ($\delta^{13}C$: −35‰ to −21‰) and Crassulacean acid metabolism (CAM, $\delta^{13}C$: −20‰ to −10‰) according to their leaf $\delta^{13}C$. However, C_4 plants have not been reported in epiphytes (Silvera et al., 2009); and (5) substrate microhabitats (Figure S1): classified according to the primary substrate of each sample as ant-abundant (AA, with visible signs of ant nesting and foraging activities around roots), bare bark (BB), bryophyte cover (BRY) and canopy soil-rich (CS).

2.4.3 | Data analysis

All data analyses were conducted in R 4.2.0 (R Core Team, 2023).

To assess ^{15}N fractionation between leaves and roots in epiphytes, we analysed N, $\delta^{15}N$, and $\Delta^{15}N_{leaf-root}$ at both species and group levels. As the data did not meet the assumptions of normality and homogeneity of variance, the non-parametric Kruskal–Wallis test and Dunn's post hoc test were employed to determine any differences. 95% confidence intervals based on non-parametric bootstrapping were used to assess differences in N concentration and $\delta^{15}N$ between leaves and roots for each species, their deviation from zero and interspecific differences. We also conducted paired *t*-tests to compare the N concentration and $\delta^{15}N$ values of leaves and roots within each group type.

We used simple linear regression to analyse the relationships between epiphyte $\Delta^{15}N_{leaf-root}$ and a single continuous abiotic variable (atmospheric N deposition, altitude, latitude and longitude), and plant trait (C and N concentrations, $\delta^{13}C$ in leaves and roots and their ratios). Atmospheric N deposition data—including NH_4^+-N, NO_3^--N, and dissolved inorganic N (DIN, the sum of NH_4^+-N and NO_3^--N)—were extracted from the open dataset of wet N deposition in China at a spatial resolution of 1 km × 1 km (Jia et al., 2019), and the level of N deposition in this region ranged from 9.88 to 10.22 kg ha^{-1} year^{-1}.

To assess the combined effects of factors on the $\Delta^{15}N_{leaf-root}$ of epiphytes, all recorded variables were used to construct multivariate linear or generalised linear regression models with an identity link function and Gaussian distribution. As we identified different driving factors that influence positive and negative fractionation, and the identities of the species and taxonomic group were aliasing coefficients, we constructed six types of competing models to identify the set of predictors that produced the most reasonable model for explaining the variation of $\Delta^{15}N_{leaf-root}$, $\Delta^{15}N_{leaf-root}>0$, and $\Delta^{15}N_{leaf-root}<0$ within epiphytes: (i) a 'full—species' model (FS, including all predictors but excluding taxonomic group), (ii) a 'full—taxonomic group' model (FG, including all predictors but excluding species), (iii) a 'categorical variables—species' model (CS, including all categorical variables but excluding taxonomic group), (iv) a 'categorical variables—taxonomic group' model (CG, including all categorical variables but excluding species); (v) a 'traits' model (Trait, including all continuous plant traits) and (vi) an 'abiotic' model (Abi, including all abiotic factors). To identify the best predictors, model selections (mentioned as optimal models, derived from 'full—species' and 'full—taxonomic group' models) were further conducted based on the corrected Akaike's information criterion (AICc; ΔAICc <2) using the *dredge* function in the R package *MuMIn* (Bartón, 2022). When constructing each model, all continuous data were standardised to *Z*-scores and high collinearity was checked. When a pair of variables showed a high Pearson correlation coefficient ($|r|\geq 0.7$) (Table S1), one of the variables was removed. Next, the variance inflation factor (VIF) for the continuous variable or the generalised variance inflation factor (GVIF) for categorical variables was calculated. The variable with the highest VIF was removed, followed by the model adjustment. This process was iterated until all variables had a VIF of less than 10 (or $GVIF^{(1/(2\times df))}<5$) (Fox & Monette, 1992). Finally, the *glmm.hp* package was employed to perform variance decomposition on the model to determine the relative contribution of each predictor (Lai et al., 2022). In cases where the explanatory power of a specific factor was found to be negative, the factor was eliminated and the equation was recalculated accordingly. Through these steps, the key factors influencing the variation in leaf–root fractionation in epiphytes were identified and quantified, ensuring the robustness and interpretability of the model.

Lastly, to assess whether the effect of species on $\Delta^{15}N_{leaf-root}$ was based on the phylogeny of epiphytes and to assess the role of phylogeny in plant traits (C, N, $\delta^{13}C$, $\delta^{15}N$, N_{leaf}/N_{root} and C/N ratios in leaves and roots), we constructed a phylogenetic tree using the *V. PhyloMaker* package (Jin & Qian, 2019), calculated the phylogenetic signal with the *phyloSignal* function of the *phyloSignal* package (Keck et al., 2016), and reported Blomberg's *K* values. Blomberg's

K was used to assess the relationship between trait variation and phylogenetic structure. A value of $K=1$ suggests that trait evolution follows a Brownian motion model, reflecting a moderate phylogenetic signal and evolutionary conservatism. $K>1$ indicates a strong phylogenetic signal and greater evolutionary conservatism, while a *K* value close to 0 implies random evolution with a weak phylogenetic signal (Blomberg et al., 2003).

3 | RESULTS

3.1 | Difference in N concentration and $\delta^{15}N$ among epiphyte species

The N concentration varied considerably among 24 epiphyte species, with ranges of 5.99–26.24 mg/g in leaves and 7.44–23.99 mg/g in roots (Figure 1a). The average $\Delta N_{leaf-root}$ in epiphytes was 0.66 mg/g (−6.33–12.04 mg/g) across all species, and significant differences in N concentration between leaves and roots were observed in 17 epiphyte species (Figure 1a).

The $\delta^{15}N$ values ranged from −3.35‰ to 3.76‰ in leaves and from −3.01‰ to 2.71‰ in roots among epiphyte species, of which 11 species showed significant differences in $\delta^{15}N$ between leaves and roots (Figure 1b). The average $\Delta^{15}N_{leaf-root}$ was 0.43‰ (−2.13‰ to 2.15‰, Figure 1b), indicating that the leaves were generally more enriched in ^{15}N than the roots. Moreover, the $\Delta^{15}N_{leaf-root}$ values showed a weak phylogenetic signal ($K=0.11$), similar to other measured traits, including C, N, $\delta^{13}C$, $\delta^{15}N$, N_{leaf}/N_{root}, and C/N ratios for leaves and roots ($K=0.10$–0.43) (Figure 2).

3.2 | Difference in N concentration and $\delta^{15}N$ among epiphyte groups

The N concentration in the leaves and roots of epiphytes differed significantly ($p<0.05$) among most groups (Figure 3a–e), with the exception of sampling sites (Figure 3a). Epiphyte groups with higher leaf and root N concentration often exhibited significantly higher values of $\Delta N_{leaf-root}$ ($p<0.05$) (Figure S2a–e), indicating that increased internal N storage promoted epiphytes to allocate more N to their leaves. Specifically, ferns had the highest N concentration in both leaves (15.23 ± 6.43 mg/g) and roots (13.22 ± 4.26 mg/g), while orchids had the lowest values (leaf N 9.87 ± 3.05 mg/g, and root N 9.93 ± 2.39 mg/g) among taxonomic groups (Figure 3c). The C_3 plants exhibited a significantly higher N concentration than CAM plants in both leaves and roots (Figure 3d). The epiphytes growing in canopy soil had the highest leaf N concentration (16.10 ± 6.21 mg/g) compared with those on other substrates (Figure 3e).

Both leaf and root $\delta^{15}N$ of epiphytes differed significantly (Kruskal–Wallis $\chi^2=11.0$–35.2 in leaf, Kruskal–Wallis $\chi^2=7.81$–26.1 in root, $p<0.05$) in most groups (Figure 3f–j). Leaf $\delta^{15}N$ (Figure 3f–j) showed patterns similar to those of leaf N (Figure 3a–e) for most groups, reflecting that epiphytes with a higher N concentration primarily absorbed ^{15}N-enriched N sources. In particular, the mean values of $\Delta^{15}N_{leaf-root}$ fluctuated around 0, ranging from −0.20 ± 1.63‰ (orchids) to 1.77 ± 1.08‰ (Town) (Figure 3k–o), and did not show significant differences among groups, except for those between the sampling site of G213 and Town (Kruskal–Wallis $\chi^2=15.8$, $p<0.01$) (Figure 3k), and between the taxonomic group of orchids and ferns (Kruskal–Wallis $\chi^2=7.85$, $p<0.05$) (Figure 3m). Similar to leaf N, epiphyte groups with higher leaf $\delta^{15}N$ values (Figure 3f–j) often exhibited higher $\Delta^{15}N_{leaf-root}$ values (Figure 3k–o).

3.3 | Influencing factors on $\Delta^{15}N_{leaf-root}$

Across all datasets, the Abi model accounted for only 1.47%–2.80% (R^2) of variation (Figure 4a–c; Figure S3o–q), indicating that the epiphytes originated from a homogeneous macroenvironment. By contrast, other models applied to the full dataset explained 11.85% to 45.68% of total variation in $\Delta^{15}N_{leaf-root}$ (Figure 4a)—higher than those for the positive subset (7.62%–35.76%, Figure 4b) but lower than those for the negative subset (23.60%–45.02%, Figure 4c). More importantly, species identity consistently explained more variation in $\Delta^{15}N_{leaf-root}$ (16.32%–25.48% in FS models; 21.96%–33.25% in CS models) than taxonomic groups (0.80%–4.43% in FG models; 0.97%–4.56% in CG models) across all datasets (Figure 4a–c), indicating that the species, rather than the taxonomic groups, consistently exerted the main influence. Additionally, NO_3^--N deposition (0.60%–2.80%) exerted a consistently greater, though weak, influence than NH_4^+-N deposition (0.18%–1.65%) across three datasets (Figure 4a–c).

The CS and CG models had consistently lower R^2 values than full models within each dataset (Figure 4a–c). Of them, taxonomic groups, sampling sites, photosynthetic pathways and substrates better explained negative $\Delta^{15}N_{leaf-root}$ than positive values, whereas vertical positions showed the opposite trend (Figure 4b,c). Trait models composed of continuous variables performed better for negative $\Delta^{15}N_{leaf-root}$ (Figure 4c), with N_{leaf} (7.37%–10.37%) and C_{root} (3.06%–4.63%) being the most important for the positive subset (Figure 4b; Figure S4), and $\delta^{13}C_{leaf}$ (10.54%–21.27%, Figure 4c; Figure S4) dominating the negative subset. These results suggested that distinct ^{15}N enrichment progresses were at work in the two subsets. Simple linear regression analysis (Figure S3) and the optimal models (Figure S4) further corroborated these findings.

4 | DISCUSSION

4.1 | Variation of $\delta^{15}N$ between leaves and roots

Our first hypothesis was supported: Epiphytes exhibited ^{15}N fractionation between leaves and roots (mean $\Delta^{15}N_{leaf-root}=+0.43‰$, range: −2.13‰ to +2.15‰) across all species. This mean is lower than the 2.0‰ commonly observed in soil-rooted plants (Dawson et al., 2002; Högberg, 1997; Houlton et al., 2007; Shearer &

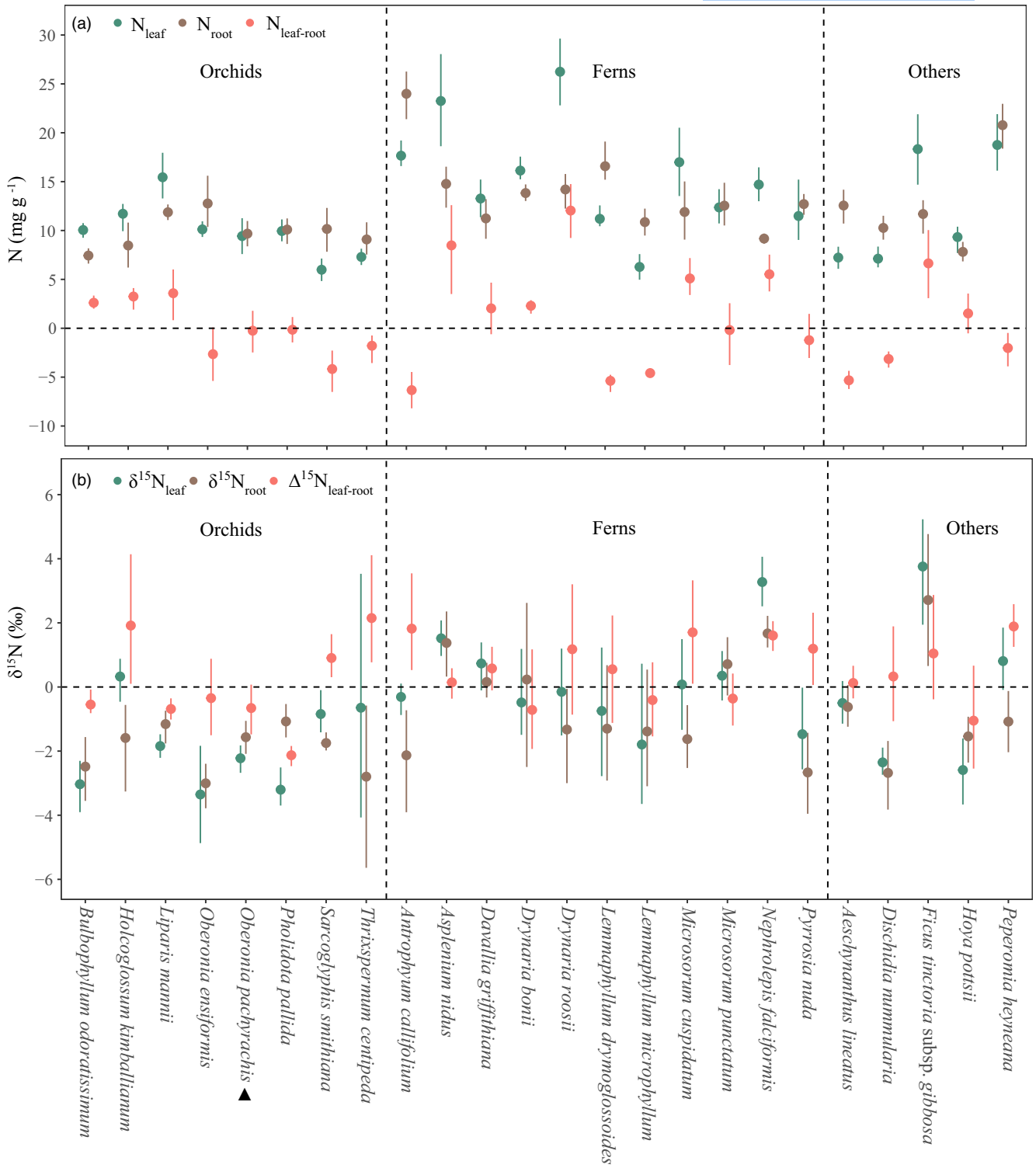

FIGURE 1 N profiles of investigated epiphytic species from Xishuangbanna, SW China. (a) Leaf (N_{leaf}) and root (N_{root}) N concentrations with their difference ($\Delta N_{leaf-root}$); (b) N isotopic composition in leaves ($\delta^{15}N_{leaf}$) and roots ($\delta^{15}N_{root}$) with isotopic discrimination ($\Delta^{15}N_{leaf-root}$). Values represent means ±95% confidence interval. If the confidence intervals do not overlap, this indicates a statistically significant difference between organs/species, while if the confidence interval does not include zero, this indicates a significant deviation from 0. The triangle (▲) denotes the C_3-CAM intermediate species.

Kohl, 1986). In the same area of Xishuangbanna, for example, the internal leaf–root ^{15}N fractionation was 1.9‰ in soil-rooted native plants (Hu et al., 2022). Given that studies on soil-rooted plant species show that external N concentration and plant N acquisition strategies can affect intra-plant $\delta^{15}N$ variation (Evans, 2001; Kolb & Evans, 2003), the low $\Delta^{15}N_{leaf-root}$ in epiphytes may be related to the N accessibility, N form, uptake organ (leaf vs. root), or the assimilatory pathway (Stewart et al., 1995).

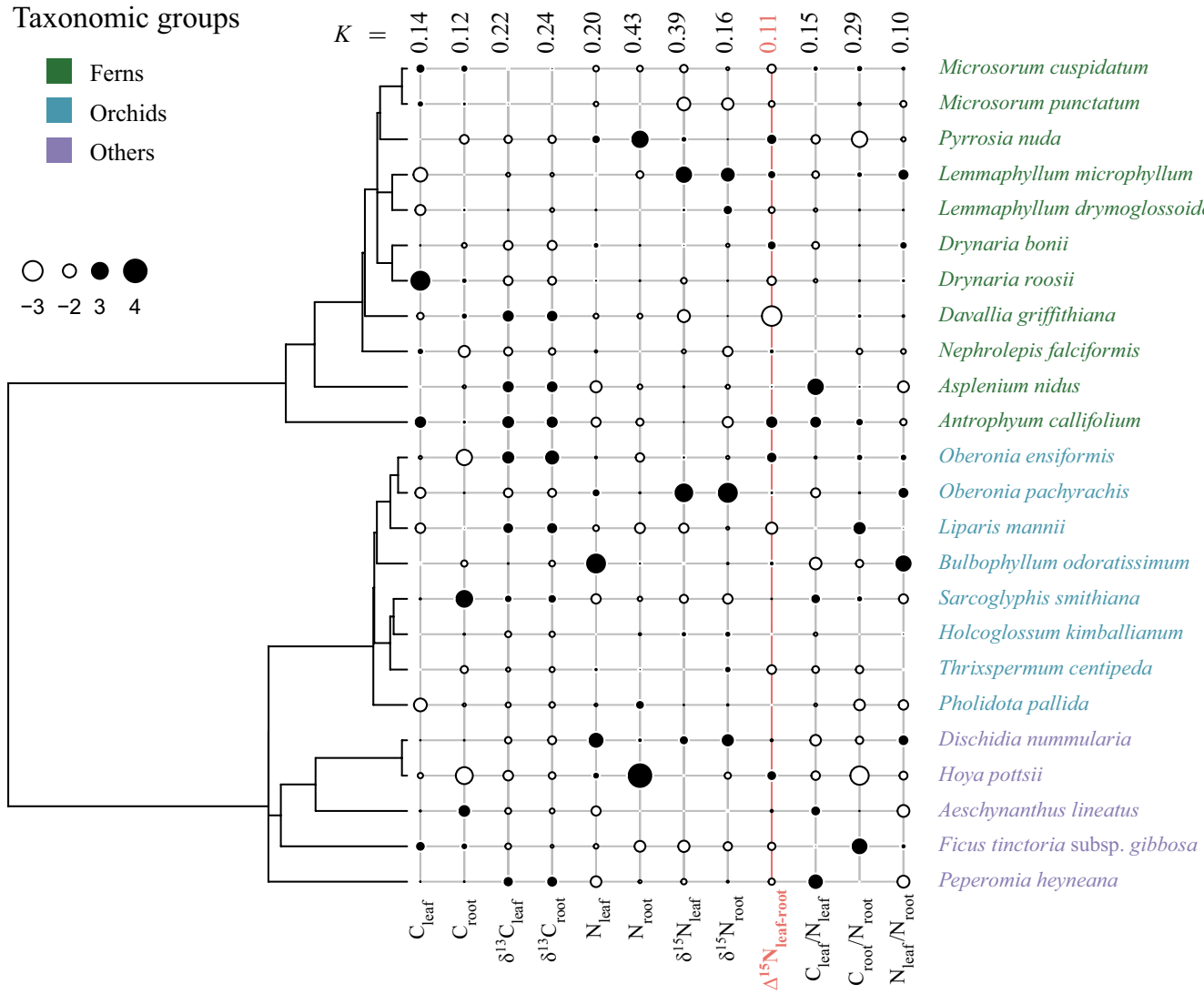

FIGURE 2 Phylogenetic signal (Blomberg's *K*) in $\Delta^{15}N_{leaf-root}$ and associated leaf/root traits (C, N, $\delta^{13}C$, $\delta^{15}N$, N_{leaf}/N_{root} and C/N ratios) of epiphytes. The upper panel shows species-level data, with Blomberg's *K* values presented above each trait column. Circles represent standardised trait values.

The intra-plant $\delta^{15}N$ variation is thought to be primarily caused by translocation of unassimilated inorganic N from roots to leaves (Comstock, 2001; Evans, 2001; Kalcsits et al., 2014). Enzymes involved in N assimilation, such as nitrate reductase and glutamine synthetase, discriminate against ^{15}N, resulting in an ^{15}N-enriched unassimilated pool relative to the assimilated inorganic N within the root cytoplasm (Comstock, 2001; Evans et al., 1996; Evans, 2001; Kalcsits et al., 2014). Once this enriched pool is transported to leaves and fully assimilated, leaves become enriched in ^{15}N relative to roots (Evans et al., 1996; Kolb & Evans, 2002; Yoneyama & Kaneko, 1989). Consistent with this, we observed that increasing leaf N relative to root N in epiphytes corresponded to more positive $\Delta^{15}N_{leaf-root}$ values (Figure S3c). Furthermore, ammonium is typically assimilated in roots and distributed as organic N to leaves, minimising $\delta^{15}N$ variation between organs (Evans et al., 1996; Evans, 2001). Conversely, nitrate assimilation can occur in both roots and leaves and often drives significant isotopic differences between organs (Evans, 2001; Haynes, 1986). Indeed, our regression analysis revealed no correlation between $\Delta^{15}N_{leaf-root}$ and NH_4^+-N deposition (Figure S3p), but a significant positive correlation with NO_3^--N (Figure S3q). Thus, intra-plant $\delta^{15}N$ variation in epiphytes may reflect differences in nitrate mobilisation and assimilation capacities between leaves and roots.

N availability largely determines the degree of intra-plant ^{15}N fractionation (Kohl & Shearer, 1980; Kolb & Evans, 2003). In this study, the mean leaf N concentration in epiphytes was 13.04 mg/g, consistent with values reported by Hietz et al. (2022) for 2882 vascular epiphyte species (mean 12.9 mg/g). This represents a 45% decrease compared with terrestrial plants, indicating low-N conditions for epiphytes. Studies show that isotopic fractionation during N-compound synthesis, degradation, and transport is reduced under N scarcity compared to abundance (Dijkstra et al., 2003; Kohl & Shearer, 1980). Our analysis supports this, as $\Delta^{15}N_{leaf-root}$ decreased with declining N_{leaf} (Figure S3a) and N_{root} (Figure S3b). Further

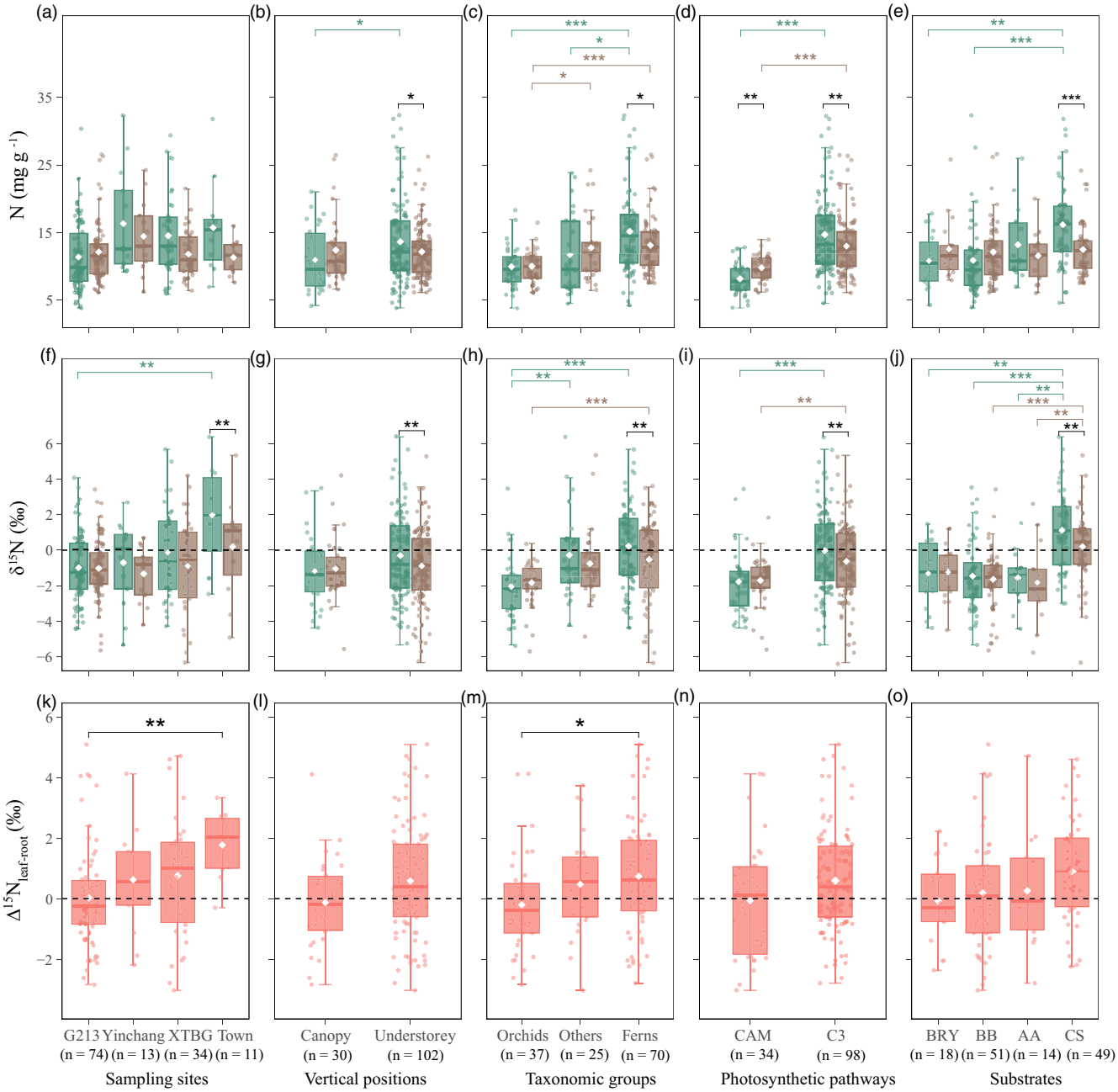

FIGURE 3 N profiles of epiphytes across group divisions. Panels (a–e) and (f–j) present N concentration and $\delta^{15}N$ values in leaves and roots (green and brown boxes respectively), while panels (k–o) show $\Delta^{15}N_{leaf-root}$ values (white diamonds indicate means). Significant differences between groups (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) are marked, with factors ordered by ascending $\Delta^{15}N_{leaf-root}$ values. Sample size (*n*) are indicated.

evidence of the N scarcity in the canopy is reflected in the N_{leaf}/N_{root} ratio ≈1 across all epiphytes (Figure 1a)—substantially lower than soil-rooted plants (>2) (Dijkstra et al., 2003; Hu et al., 2022), indicating that epiphytes allocate more N to roots. This aligns with resource allocation theory predicting increased root investment under infertility to optimise nutrient uptake (Bazzaz & Grace, 1997; Wright et al., 2004). From another point of view, homogeneous N distribution/assimilation between organs may further weaken internal $\delta^{15}N$ differences within epiphytes (Hu et al., 2022; Kolb & Evans, 2002). Thus, it is reasonable to infer that the minimal variation in $\delta^{15}N$ within epiphytes is mainly caused by the more severe canopy N stress than in terrestrial habitats.

Besides low-N stress conditions, the minimal variance of $\delta^{15}N$ within epiphytes might relate to the way they uptake N from the environment (Emmerton et al., 2001; Takahashi et al., 2022). Root-absorbed N is transported to other organs (including leaves, shoots, and twigs; Nair et al., 2016; Wang et al., 2021), whereas leaf-absorbed N rarely moves to other parts (Ferraretto et al., 2022; Wang et al., 2024). Unlike terrestrial plants that acquire most nutrients through roots, vascular epiphytes may absorb N via both

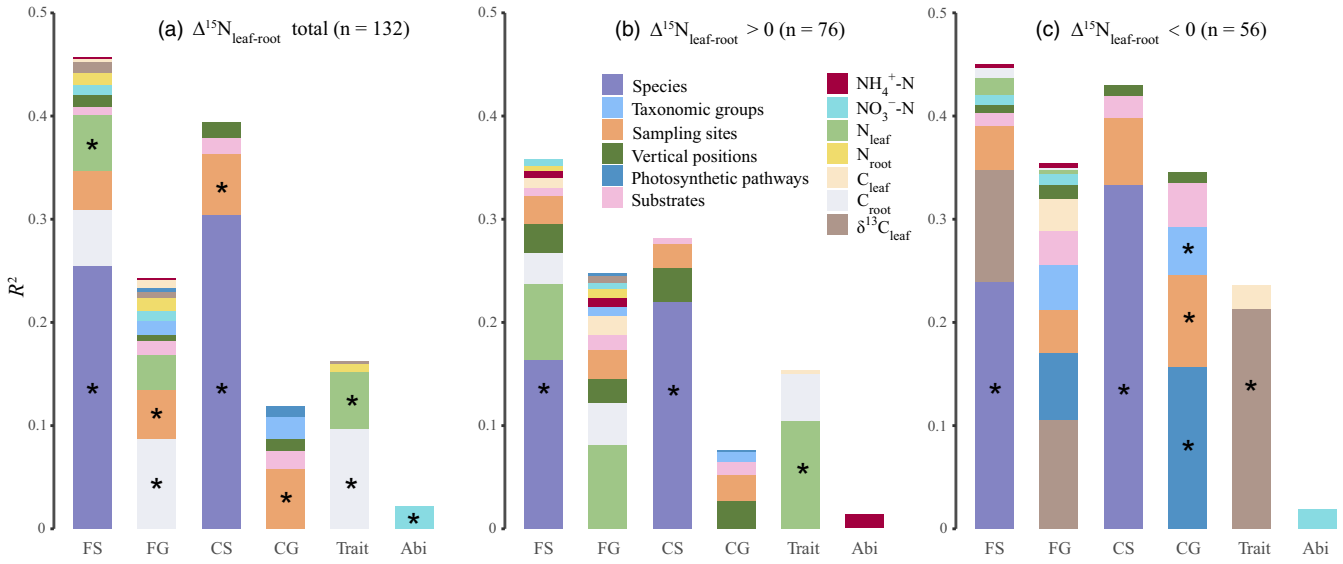

FIGURE 4 Results of generalised or general linear models and variance decomposition analysis for the $\Delta^{15}N_{leaf-root}$ in epiphytes. (a) Analysis of total samples, (b) Subset with $\Delta^{15}N_{leaf-root} > 0$, (c) Subset with $\Delta^{15}N_{leaf-root} < 0$. Asterisks (*) indicate predictors with significant coefficients ($p < 0.05$), while R^2 values represent individual fixed effects from variance partitioning analysis.

leaves/shoots and roots, or even mainly through leaves/shoots (Takahashi et al., 2022), as demonstrated in epiphytic bromeliads (Endres & Mercier, 2001; Inselsbacher et al., 2007). Moreover, Endres and Mercier (2001) propose that epiphytic bromeliads preferentially absorb organic N through leaves, while their terrestrial counterparts primarily utilise inorganic N from the soil, potentially explaining the divergence in internal ^{15}N fractionation between groups (Emmerton et al., 2001). In particular, we observed that many epiphytes hosted abundant epiphyllous mosses, lichens, algae and cyanobacteria (Figure S5), most known to fix N (Campbell et al., 2010; Lindo & Whiteley, 2011). If epiphyte leaves actively or passively take up fixed N from these organisms, it could weaken the ^{15}N variation between leaves and roots, owing to enriched-^{15}N of fixed N and rapid transport and synthesis processes (Kohl & Shearer, 1980).

4.2 | Potential factors influencing the variation of $\delta^{15}N$ between leaf and root of epiphytes

We also present data that support the second hypothesis. The $\Delta^{15}N_{leaf-root}$ values show marked species-specific variability (Figure 1b), but no differences between treatment groups (Figure 3k–o). Species identity explained the largest proportions of the total variations in $\Delta^{15}N_{leaf-root}$ of epiphytes (Figure 4), but appeared unrelated to their evolutionary history (Figure 2). This finding agrees with previous studies on soil-rooted plants in tropical forests (Townsend et al., 2007; Yin, 2024). The species-specific variability of ^{15}N fractionation might be associated with the high degree of variations in N transport and assimilation capacities in epiphyte leaves or roots (Andrews, 1986; Hu et al., 2022; Kolb & Evans, 2002). In addition, the deviations in ^{15}N fractionation between group and species levels probably reflect the fact that epiphytes have undergone both convergent evolution of independent species and divergent evolution of species with a common ancestor, evolving various strategies of N acquisition and utilisation, when they have no or limited contact with the pedosphere (Benzing, 1990; Benzing & Renfrow, 1974; Lüttge, 2008; Pridgeon, 1987). For example, whereas the orchid *Bulbophyllum odoratissimum* and the fern *Microsorum punctatum* are trash-basket epiphytes, *Microsorum cuspidatum* accumulates canopy soil beneath its rhizomes. Consequently, the inherent diversity and specificity in N acquisition at the species level of epiphytes attenuate treatment-based differences, resulting in the observed convergent fractionation pattern across treatments.

The taxonomic group was determined as a key contributor to the variation in $\Delta^{15}N_{leaf-root}$ (0.80%–4.56%, Figure 4). A study by Su et al. (2023) revealed that the functional group is the main factor influencing N and $\delta^{15}N$ in epiphytes, highlighting different pathways in the acquisition, absorption, and utilisation of N. Therefore, the difference in $\Delta^{15}N_{leaf-root}$ between orchids and ferns may also reflect their disparities in N uptake capacity (Cardelús & Mack, 2010; Hietz et al., 1999). The higher $\Delta^{15}N_{leaf-root}$ observed in ferns probably results from their greater environmental N uptake efficiency, leading to relatively less N limitation (Watkins et al., 2007). In contrast, the reduced fractionation in orchids may reflect their more N-limited condition (Kolb & Evans, 2003).

The sampling site substantially influenced $\Delta^{15}N_{leaf-root}$ and explained 2.36%–8.90% (Figure 4) of the total variations. The data showed that the increase in positive $\Delta^{15}N_{leaf-root}$ values of epiphytes among sampling sites (Figure 3k) was paralleled by the rise in leaf N (Figure 3a) and $\delta^{15}N$ (Figure 3f) and was closely associated with nitrate deposition levels (Table S2). Specifically, the higher N concentration and $\delta^{15}N$ in the leaves and roots of epiphytes in town was probably due to their proximity to streets and exposure to

^{15}N-enriched traffic emissions, as street tree canopies temporarily intercept vehicle exhaust below them (Boltersdorf & Werner, 2014; Delgado et al., 2013; Felix et al., 2016; Morera-Gómez et al., 2024). This further suggests that ^{15}N fractionation in epiphytes is primarily driven by microenvironmental N levels rather than local N availability, particularly regarding nitrate input (Evans, 2001). Similar results were reported by Díaz-Álvarez et al. (2016), who found higher $\Delta^{15}N_{leaf-root}$ (~1.98‰) in epiphytic orchids of the city subjected to higher N deposition, compared with near-zero fractionation (~−0.1‰) in oak forests with low N deposition.

We also confirmed that vertical position affected $\Delta^{15}N_{leaf-root}$ of epiphytes (Bergstrom & Tweedie, 1998; Wania et al., 2002), accounting for 0.59%–3.26% (Figure 4) of the total variations. The $\Delta^{15}N_{leaf-root}$ values of understorey epiphytes were generally greater than 0, whereas those of the canopy were lower than 0, accompanied by decreased N concentration and δ^{15}N values in leaf and root. This pattern was observed by Wania et al. (2002) in a lowland rainforest in Costa Rica. Such results can be explained by the varying availability and isotopic signatures of their differentiated N sources along tree height (Bergstrom & Tweedie, 1998), as canopy epiphytes obtain N primarily from atmospheric deposition, while understorey epiphytes access more N from fallen litter, leachates and stemflow (Bergstrom & Tweedie, 1998; Hietz et al., 1999; Wania et al., 2002).

In particular, epiphytes growing on different substrates showed smaller changes in $\Delta^{15}N_{leaf-root}$, despite there being significant differences in leaf and root N. Differentiation in N sources at the microhabitat scale may partly determine the δ^{15}N signatures of epiphytes (Hietz et al., 1999; Su et al., 2023), accounting for 0.60%–4.36% (Figure 4) of the variation in $\Delta^{15}N_{leaf-root}$ in this study. Canopy soil, which provides ^{15}N-enriched N, explained the observed higher N and δ^{15}N in this epiphyte group, leading to more positive $\Delta^{15}N_{leaf-root}$ (Hietz et al., 2002; Wania et al., 2002). Epiphytes with animal symbionts/inhabitants acquire ^{15}N-enriched N from animal faeces and carcasses (Gegenbauer et al., 2023), whereas the δ^{15}N value of this group was more negative in our study. This was presumably because epiphytes lacked specialised symbiosis with ants in this region, instead relying on limited nutrients in soil transported by ants from terrestrial habitats (Watkins et al., 2007). Epiphytes growing on bryophytes could obtain N from bryophyte-cyanobacteria associations (Lindo & Whiteley, 2011), while those growing on bare bark may also obtain fixed N from abundant free-living diazotrophs on bark in tropical forests (Brighigna et al., 1992). Thus, we suggest that the prevalence of relatively ^{15}N-enriched N sources from soil or biological fixation reduces the influence of substrate type on leaf–root ^{15}N fractionation in tropical epiphytes. Moreover, individuals within the same sample often grew on various substrate mosaics, but were classified by predominant microhabitat, which might have masked their influence.

Furthermore, CAM epiphytes exhibited smaller absolute $\Delta^{15}N_{leaf-root}$, N concentration, and δ^{15}N in both leaves and roots compared with C_3 epiphytes. This pattern, consistent with previous studies (Eskov et al., 2023; Guzmán-Jacob et al., 2022; Hietz et al., 1999), was also evident within individual taxonomic groups (Table S3). Contrary to the previous prospect that this pattern was attributable to N source rather than the photosynthetic pathway (Hietz et al., 1999), our results detected as high as 6.49%–15.69% variance of $\Delta^{15}N_{leaf-root}$ for the photosynthetic pathway in the negative $\Delta^{15}N_{leaf-root}$ data subset (Figure 4b). Therefore, we speculate that this variation in $\Delta^{15}N_{leaf-root}$ may reflect their distinct N uptake efficiencies and sensitivities to water deficit (Eskov et al., 2023; Guzmán-Jacob et al., 2022). CAM plants might have low N demand and higher N use efficiencies (Pereira & Cushman, 2019), considered as an evolutionary adaptation for miniaturisation (Eskov et al., 2023). Conversely, C_3 plants exhibit larger and more variable fractionation values, likely reflecting their high N acquisition efficiency and greater susceptibility to water stress (see the next paragraph). Additionally, our results indicate that the relationship between photosynthetic pathways and N economics of epiphytes may be more complex than previously thought, warranting further study.

Interestingly, our analysis revealed that positive $\Delta^{15}N_{leaf-root}$ values of epiphytes were primarily influenced by C_{root} and N_{leaf} (Figure 4b; Figures S3a,e and S4b), whereas negative values were driven by leaf and root δ^{13}C (Figure 4c; Figures S3g,h and S4c). These findings suggest distinct mechanisms driving intra-plant ^{15}N enrichment, reflecting plants' sensitivity to water and nitrogen deficits (Robinson et al., 2000; Wania et al., 2002). Epiphytes with positive $\Delta^{15}N_{leaf-root}$ likely possess relatively higher N uptake capacity or experience greater N availability. Accordingly, they exhibit a higher total N concentration, with greater leaf N allocation to support photosynthesis and growth, and simultaneously reduce root carbon storage (Fraterrigo et al., 2011; Laungani & Knops, 2009). For example, *Nephrolepis falciformis* and *Peperomia heyneana* thrived in humus-rich substrates (Figure S2f). Conversely, epiphytes with negative $\Delta^{15}N_{leaf-root}$ might experience severe water deficit, thereby impairing their internal nutrient transport and causing more N retention in roots (Alam, 1999; Marschner et al., 1996). Water stress may also reduce plant nutrient demand or uptake capacity, promoting preferential and greater N allocation to roots to maintain basic physiological functions and support water acquisition (Alam, 1999). Hence, the low total N and N_{leaf}/N_{root} contributed to negative $\Delta^{15}N_{leaf-root}$ in epiphytes. For example, *Pholidota pallida* and *Liparis mannii*, with negative $\Delta^{15}N_{leaf-root}$ values, are thin-leaf orchids, pointing to their greater susceptibility to water stress (Figure S2f). As noted in Wania et al. (2002), the increasing divergence of δ^{15}N signatures of epiphyte leaves and roots, from lower zones (~−0.98‰) to higher canopy positions (~−4.0‰), reflects greater water deficit in exposed canopy areas. In our study, the smaller negative fractionation may arise from higher water availability in the dry season, owing to limited sunlight exposure with tree height and the supplement of fog-derived water during the foggy subseason (Liu et al., 2021; Wu et al., 2018).

4.3 | Limitations

Although our study provides the first systematic investigation into the underlying causes and processes of internal ^{15}N fractionation in

tropical epiphytes, certain limitations remain. The labour-intensive, time-consuming and costly nature of conducting canopy surveys and standardised sampling has limited our ability to expand the sample size. Furthermore, our study relied primarily on field-collected samples, with interpretations derived from findings related to soil-rooted plants. Therefore, future controlled experiments are required to provide more robust support for our findings and interpretations.

5 | CONCLUSIONS

Our results clearly demonstrate that vascular epiphytes exhibit a smaller but noteworthy leaf–root ^{15}N fractionation compared with soil-rooted plants. The leaf–root ^{15}N fractionation of epiphytes is species-specific, independent of phylogeny, and varies across habitat scales and taxonomic categories, being strongly associated with the availability of external N sources or their acquisition efficiencies. The fractionation values increase with leaf and root N and their ratio, suggesting that internal N transport and assimilation in epiphytes are strongly influenced by N availability, while water deficit also significantly affects negative ^{15}N fractionation. Therefore, the minimal fractionation in epiphytes may reflect N scarcity in canopy environments and their distinct pathways in N acquisition, absorption and utilisation. Overall, our results support the use of natural leaf $\delta^{15}N$, when considering internal ^{15}N fractionation, as a tool to better explore the N economy of epiphytes.

AUTHOR CONTRIBUTIONS

Su Li, Xiao-Fan Na and Chun-Yan Zhou conceived the ideas and designed methodology. Chun-Yan Zhou and Tao Jia collected and analysed data. Tao Wang participated in the field experiments. Yun-Hong Tan and Qiang Liu contributed to species identification. Yu-Xuan Mo and Tian-Hao Su participated in the data analysis. Su Li, Xiao-Fan Na and Chun-Yan Zhou led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

We thank Xian-Meng Shi, Wen-Jun Zhou, Chuang Yuan, Jie Wang, Xue Lu, Ya Lan and Xun Fu for their assistance in conducting the experiment, analysing the data, and optimising the colour of the figures. We also thank the Biogeochemistry Laboratory of the Xishuangbanna Tropical Botanical Garden and the Nature Reserve of Xishuangbanna for facilitating our experimental work.

FUNDING INFORMATION

This study was funded by the National Natural Science Foundation of China (Grant Nos. 32171554, 32471641 and 32271698), the Yunnan Revitalization Talent Support Program (YNWR-QNBJ-2020-067), the 14th Five-Year Plan of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (E3ZKFF2B01), and the Candidates of the Young and Middle-Aged Academic Leaders of Yunnan Province (202305AC160024).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.70131.

DATA AVAILABILITY STATEMENT

Data available from the Figshare database: https://doi.org/10.6084/m9.figshare.29552981.v3 (Zhou et al., 2025).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Diagram showing substrate microhabitats of vascular epiphytes.

Figure S2. N profiles of investigated epiphytic species in Xishuangbanna, SW China.

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Figure S3. Relationships between epiphyte $\Delta^{15}N_{leaf-root}$ and plant traits, atmospheric N deposition, altitude, latitude, longitude.

Figure S4. Results of the optimal models (derived from the full FS and FG models) and variance decomposition analysis for $\Delta^{15}N_{leaf-root}$ in epiphytes.

Figure S5. Epiphytes with moss, lichen, algal and cyanobacterial cover on leaf surfaces.

Table S1. Pearson correlation matrix of epiphyte parameters.

Table S2. Data of atmospheric N deposition at sampling sites.

Table S3. N concentration, $\delta^{15}N$, and $\Delta^{15}N_{leaf-root}$ values in leaves and roots of C_3/CAM epiphytes across taxonomic groups.

How to cite this article: Zhou, C.-Y., Jia, T., Su, T.-H., Tan, Y.-H., Liu, Q., Wang, T., Mo, Y.-X., Hu, H.-X., Pu, X.-Y., Na, X.-F., & Li, S. (2025). Group-consistent but species-specific nitrogen isotope fractionation between leaves and roots in tropical vascular epiphytes. *Journal of Ecology*, *113*, 2846–2859. https://doi.org/10.1111/1365-2745.70131