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^{15}N natural abundance of vascular rainforest epiphytes: implications for nitrogen source and acquisition

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ABSTRACT

The foliar natural abundance of ^{15}N was analysed to compare the potential nitrogen sources of vascular rainforest epiphytes and associated soil-rooted trees. Leaves of epiphytes collected from six rainforest communities in Brazil, Australia and the Solomon Islands were depleted in ^{15}N relative to the trees at each site. Epiphyte $\delta^{15}N$ was as low as –6·4‰, while trees were generally enriched in ^{15}N (0·7 to 3·5‰). These results indicate either that epiphytes use nitrogen sources depleted in ^{15}N or that discrimination against ^{15}N is an intrinsic function of epiphyte physiology. At three sites, epiphytes could be grouped into those having both low $\delta^{15}N$ and low leaf-nitrogen content and those possessing both high $\delta^{15}N$ and high leaf-nitrogen content. The second group had $\delta^{15}N$ values in the range sometimes attributable to N_2 fixation (–2 to 0‰). There was no correlation between growth form and $\delta^{15}N$. It is concluded that epiphytes may utilize ^{15}N-depleted nitrogen from atmospheric deposition and N_2 fixation.

Key-words: atmospheric deposition; N_2 fixation; natural abundance ^{15}N; nitrogen sources; rainforest; vascular epiphytes.

INTRODUCTION

The natural abundance of ^{15}N has been used to determine preferential sources of inorganic nitrogen for plants, including both soil and atmospheric sources (Virginia & Delwiche 1982; Pate, Stewart & Unkovich 1993). In the majority of reported instances, soil nitrogen was enriched relative to N_2 (Shearer & Kohl 1989) due to kinetic fractionation during soil nitrogen cycling (Handley & Raven 1992). The $\delta^{15}N$ values of plants are a function of both preferential use of isotopically distinct nitrogen sources and assimilatory fractionation. $\delta^{15}N$ values recorded for terrestrial plants from various ecosystems range from –6 to +15‰ (Virginia *et al.* 1989; Popp 1993) with low values of –7‰ reported for plants growing in very young volcanic soils greatly depleted in ^{15}N (Vitousek, Shearer & Kohl 1989).

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There appear to be no ^{15}N data for epiphytes, although epiphytes are extremely diverse and can represent a large proportion of the total biomass of some ecosystems. In tropical rainforests, epiphyte weight has been estimated to be as much as 35–50% of tree leaf biomass (Edwards & Grubb 1977; Tanner 1977). Plants from many plant families grow epiphytically and exhibit numerous adaptations to their growth environment. Adaptations for nutrient acquisition include trash-basket (*Asplenium*) and tank growth forms (Bromeliaceae), and leaf features such as trichomes (*Tillandsia*) and velamen (Orchidaceae) (Benzing & Renfrow 1974; Benzing 1990).

Potential nitrogen sources for epiphytes must be of either atmospheric or canopy origin once seed reserves have been depleted. The probable nitrogen sources are three major nitrogen pools: (i) canopy-derived nitrogen, (ii) atmospheric deposition, and (iii) nitrogen derived from atmospheric N_2 fixation. Canopy-derived nitrogen includes both solubles leached from canopy leaves, stems or epiphytes and litter, and gaseous NH_3 released by leaves (Parton *et al.* 1988). Atmospherically derived nitrogen includes exogenous dry and wet deposition and N_2 fixation; N_2-fixing microorganisms have been reported on the leaf surfaces of epiphytes (Sengupta *et al.* 1981; Brighigna *et al.* 1992). Another possible nitrogen source is nitrogen derived from animals, either directly as excreta and dead biomass or through insect symbioses such as ant-plants, where host plants are thought to receive nutrients from their ant partners (Huxley 1980).

Soil nitrogen is the major source of nitrogen for non-N_2-fixing terrestrial plants; here we report investigations into the nitrogen sources of epiphytes. Our $\delta^{15}N$ data for a range of vascular rainforest epiphytes show that they contain isotopically light nitrogen. We discuss the implications of this with respect to the nitrogen sources utilized by epiphytes.

MATERIALS AND METHODS

Study sites

Leaf material of vascular rainforest epiphytes and associated tree species was collected from four tropical and two subtropical rainforests. Sites in Brazil were situated in São Paulo State and comprised a tropical gallery forest (Brotas,

22°15'S, 48°07'W, altitude 640 m) and a tropical semideciduous rainforest (St Genebra, 22°49'S, 47°06'W, altitude 669 m). Sites in Australia were a tropical lowland rainforest in north-east Australia (Cape Tribulation, 16°25'S, 145°E) and two subtropical rainforests in eastern Australia near Brisbane (Lamington National Park, 28°14'S, 152°30'E, altitude 900 m; Mt Glorious/Mt Nebo, 27°20'S, 153°E, altitude 640 m). Samples from the Solomon Islands were collected from tropical rainforests on the islands of Kolombangara and New Georgia (8°S, 157°00'E and 157°30'E).

Plant material

Mature leaves were collected from epiphytes and from soil-rooted trees. The Brazilian epiphytes (n=18; for details see Table 1) included members of the families Bromeliaceae, Orchidaceae, Peperomiaceae, Polypodiaceae and Cactaceae. The Australian epiphytes (n=63) were members of the Aspleniaceae, Polypodiaceae, Orchidaceae, Peperomiaceae, Pandaceae and Adiantaceae. The epiphytes (n=10) collected in the Solomon Islands were members of the Orchidaceae and Rubiaceae. Leaves from

Table 1. Foliar $\delta^{15}N$ and % nitrogen (±SD) of epiphytes (n = number of samples; number of species in parentheses) and trees (pooled) from six rainforest sites

		n	%N	$\delta^{15}N$(‰)
Brazil (Brotas), tropical gallery forest				
Epiphytes	Cactaceae	1	2·4	0·3
	Polypodiaceae	2 (2)	1·7 (0·8)	−1·2 (1·5)
	Peperomiaceae	2 (2)	1·9 (0·8)	−2·3 (1·6)
	Bromeliaceae	2 (2)	0·9 (0·0)	−5·2 (0·1)
	Orchidaceae	3 (3)	0·8 (0·3)	−2·1 (0·6)
Total epiphytes		**10 (10)**	**1·8 (1·3)**	**−2·3 (2·1)**
Soil-grown trees		**26 (26)**	**2·6 (0·7)**	**2·6 (1·5)**
Brazil (St Genebra), tropical semideciduous forest				
Epiphytes	Bromeliaceae	1	1·1	−4·9
	Orchidaceae	1	0·8	−2·6
	Piperaceae	1	2·3	−1·3
	Cactaceae	2 (2)	1·6 (0·4)	−0·5 (4·8)
	Polypodiaceae	3 (3)	2·2 (0·3)	−0·7 (1·8)
Total epiphytes		**8 (8)**	**1·7 (0·6)**	**−1·5 (2·6)**
Soil-grown trees		**18 (18)**	**3·1 (0·8)**	**3·1 (1·2)**
Australia (Cape Tribulation), tropical lowland rainforest				
Epiphytes	Adiantaceae	1	3·6	0·1
	Orchidaceae	1	0·4	−4·0
	Pandaceae	1	1·1	−6·4
	Aspleniaceae	3 (1)	1·3 (0·7)	−2·2 (0·8)
	Polypodiaceae	6 (2)	1·6 (0·7)	−2·5 (1·7)
Total epiphytes		**12 (6)**	**1·2 (0·7)**	**−2·7 (1·8)**
Soil-grown trees		**12 (4)**	**1·5 (0·4)**	**2·3 (2·8)**
Australia (Mt Glorious), subtropical rainforest				
Epiphytes	Orchidaceae	6 (1)	1·0 (0·2)	0·3 (1·3)
	Polypodiaceae	8 (1)	1·2 (0·6)	−0·1 (2·2)
	Aspleniaceae	10 (1)	1·4 (0·5)	−0·3 (1·9)
Total epiphytes		**24 (3)**	**1·2 (0·5)**	**−0·1 (1·8)**
Soil-grown trees		**8 (4)**	**1·6 (0·3)**	**0·7 (1·9)**
Australia (Lamington NP), subtropical rainforest				
Epiphytes	Orchidaceae	2 (2)	1·0 (0·2)	0·6 (0·3)
	Peperomiaceae	2 (1)	1·4 (0·0)	−0·1 (0·6)
	Polypodiaceae	5 (3)	1·3 (0·5)	−0·8 (1·8)
	Aspleniaceae	18 (2)	1·8 (0·6)	0·2 (1·4)
Total epiphytes		**27 (8)**	**1·6 (0·6)**	**0·0 (1·4)**
Soil-grown trees		**19 (12)**	**1·6 (1·1)**	**3·5 (1·2)**
Solomon Islands, tropical rainforest				
Epiphytes	Orchidaceae	7 (6)	1·5 (0·6)	−2·2 (1·3)
	Rubiaceae	3 (2)	1·1 (0·4)	−3·0 (0·5)
Total epiphytes		**10 (8)**	**1·4 (0·6)**	**−2·4 (1·2)**
Soil-grown trees		**27 (25)**	**1·5 (0·6)**	**−1·1 (1·7)**

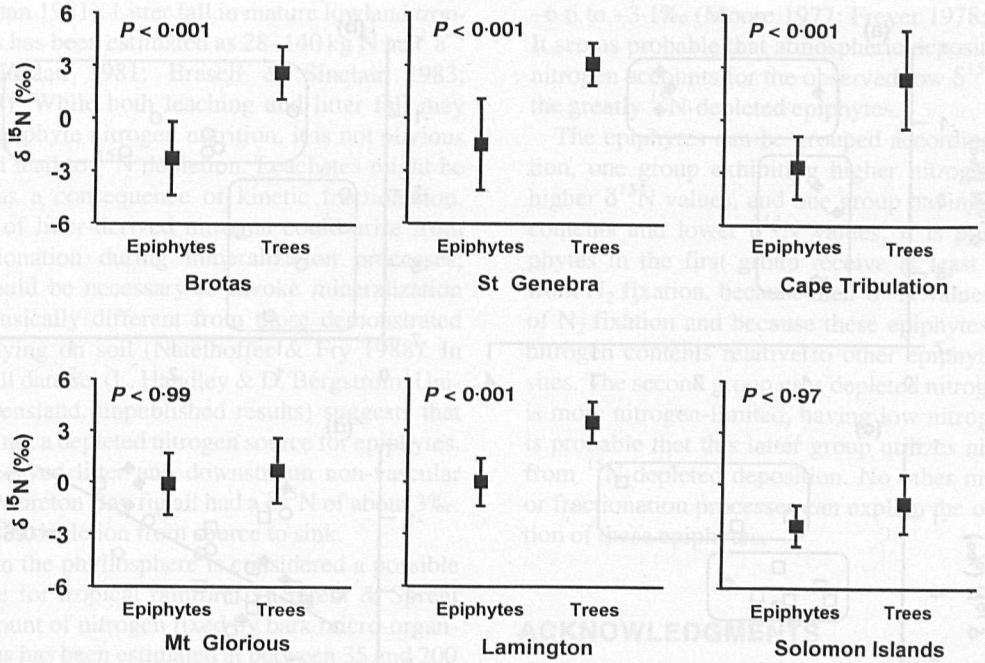

Figure 1. Foliar $\delta^{15}N$ (±SD) values for epiphytes and trees from six rainforest sites.

non-N_2-fixing trees were collected from trees in the vicinity of the epiphyte samples and comprised a large range of species and families representing the dominant members of each forest community.

Nitrogen isotope analysis

The leaf samples were oven-dried at 60 °C on the day of collection; samples from the Solomon Islands and Cape Tribulation were air-dried in the field and oven-dried after approximately 1 week. The samples were ground to a fine powder in a vibratory ball mill (Retsch MM-2, Haan, Germany) and the nitrogen content of the leaf material was determined by automated combustion. Duplicate samples of approximately 120 μg nitrogen were analysed for ^{15}N using a continuous-flow isotope ratio mass spectrometer (CF-IRMS, Tracer Mass, Europa Scientific, Crewe, UK) set to the single nitrogen mode. The precision of the instrument, based on multiple analysis (n=133) of a laboratory standard (*Eucalyptus crebra* leaves), is 0·21‰ SD.

Statistics

The data were analysed using STATISTICA (Statsoft, Tulsa, Oklahoma). Significant differences were determined by ANOVA followed by Scheffé's post-hoc test. The Kruskal–Wallis ANOVA by ranks was used for comparison of the $\delta^{15}N$ values and nitrogen contents of epiphytes at each site.

RESULTS

Epiphytes representing a range of morphological structures and nutrient acquisition strategies exhibited both low $\delta^{15}N$ and low leaf-nitrogen contents relative to associated tree species (Table 1). Mean $\delta^{15}N$ values for epiphytes from the six sites (total n=91) were depleted when compared with associated tree species (Fig. 1). Epiphytes at all sites, except Lamington National Park, exhibited lower leaf-nitrogen content than trees, indicating possible nitrogen limitation in the epiphyte growth environment. These results are similar to those found previously for species growing epiphytically when compared with those growing terrestrially (Ball *et al.* 1991). At the Brazilian sites, epiphyte $\delta^{15}N$ was –2·3‰ at Brotas and –1·5‰ at St Genebra, ranging from –5·3 to 0·0‰ and –4·9 to 2·9‰, respectively. Corresponding mean $\delta^{15}N$ values for tree species were 2·6‰ (Brotas) and 3·1‰ (St Genebra). The mean $\delta^{15}N$ of epiphytes from tropical rainforest at Cape Tribulation was –2·7‰, ranging from –6·4‰ to 0·1‰, and a mean value of 2·3‰ was found for associated trees. Epiphytes from the subtropical rainforests were not as greatly depleted in ^{15}N as epiphytes from tropical forests, with mean values of –0·1‰ at Mt Glorious and 0·0‰ at Lamington National Park, ranging from –4·5 to 2·6‰ and –2·3 to 2·4‰ respectively. The corresponding mean $\delta^{15}N$ values for trees were 0·7‰ (Mt Glorious) and 3·5‰ (Lamington NP). Epiphytes from the Solomon Islands had a mean $\delta^{15}N$ value of –2·4‰, ranging from –3·5 to –0·1‰, and trees had an average value of –1·1‰. At all sites the $\delta^{15}N$ of epiphytes was more depleted than the $\delta^{15}N$ of leaves from associated

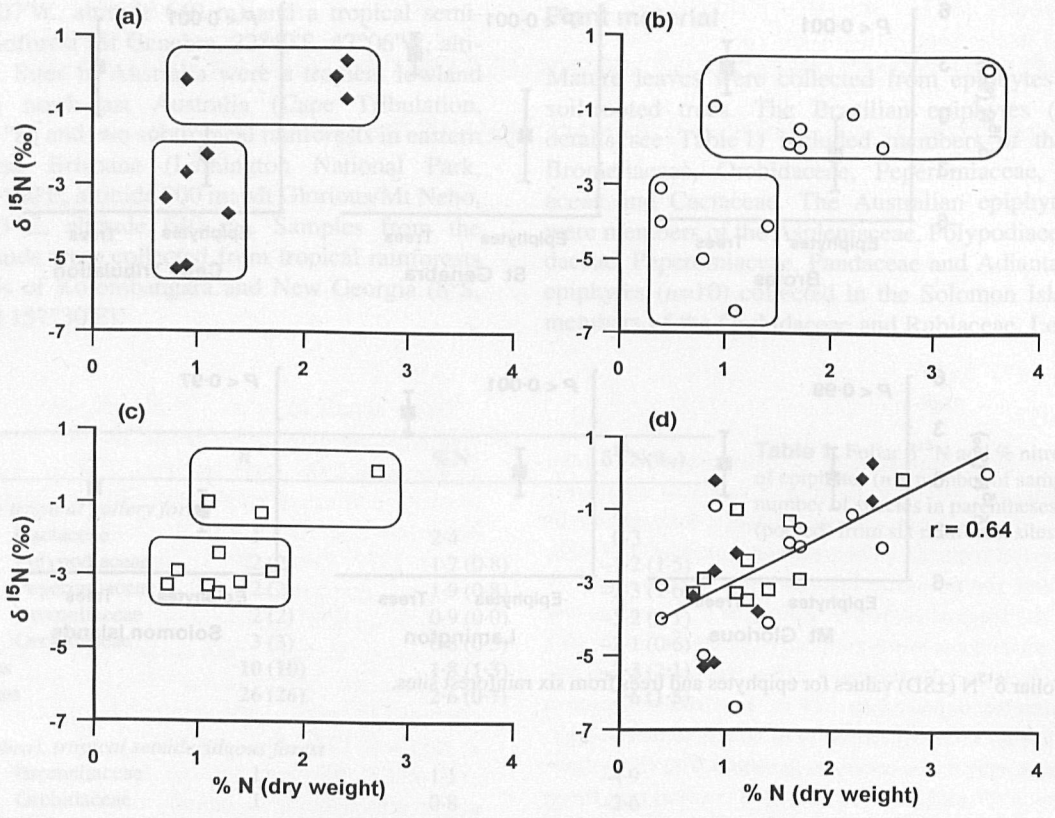

Figure 2. Foliar $\delta^{15}N$ values and leaf nitrogen contents (% dry weight) for epiphytes from three rainforests: Brotas (a), Cape Tribulation (b) and the Solomon Islands (c) indicating groupings of epiphytes with both low $\delta^{15}N$ and %N or both high $\delta^{15}N$ and high %N. Groupings were determined according to Kruskal–Wallis ANOVA by ranks. The data are pooled in (d) and the regression of $\delta^{15}N$ on %N is shown.

trees. At the Mt Glorious and Solomon Islands sites these differences were not statistically significant, although the same trend existed.

A strongly positive relationship ($r=0{\cdot}64$; $\delta^{15}N=1{\cdot}51x-4{\cdot}62$ at $P<0{\cdot}01$) between $\delta^{15}N$ value and nitrogen content was found for epiphytes from Brotas, Cape Tribulation and the Solomon Islands (Fig. 2d). At these sites, epiphytes could be divided into two significantly different groups: a relatively ^{15}N-enriched group and a relatively depleted one (Figs 2a–c).

There was no correlation between growth form (litter-collecting species versus non-collecting species) and $\delta^{15}N$. Litter-collecting epiphytes from the Australian sites (*Asplenium* and *Platycerium*; $\delta^{15}N=-2{\cdot}4$ to $0{\cdot}1‰$) did not differ in their $\delta^{15}N$ values from non-litter-collecting epiphytes (*Dendrobium, Adiantum, Peperomia, Freycinetia* and *Dictymia*; $\delta^{15}N=-3{\cdot}5$ to $0{\cdot}1‰$).

DISCUSSION

At five sites epiphytes were greatly depleted in ^{15}N. The reason for this must be either that ^{15}N depletion of epiphytes is an intrinsic function of physiology, or that it arises from the nitrogen source(s) that epiphytes use. The low $\delta^{15}N$ signatures of trees from the Solomon Islands are in accordance with ^{15}N depletions recorded for young pleistocene soils of volcanic origin (Vitousek *et al.* 1989). Here, most epiphytes were not significantly more depleted in ^{15}N than tree species; only the epiphytic ant plants (*Hydnophytum* and *Mrymecodia*) were more depleted (>1‰) than the tree species, which might reflect the ^{15}N depletion of animal excreta (Wada, Mizutani & Minagawa 1991).

Discrimination against ^{15}N during nitrogen uptake and nitrogen assimilation would result in ^{15}N depletion in the plant. This could be related to nitrogen form, the fractionation of leaf-absorbed versus root-absorbed nitrogen, different assimilatory pathways, or mycorrhizal status. NH_4^+-grown plants are reported to be depleted in ^{15}N relative to source nitrogen (Yoneyama *et al.* 1991). There is evidence that non-mycorrhizal plants discriminate against ^{15}N less than do those with VA- or ecto-mycorrhizal associations (Handley *et al.* 1993; Pate *et al.* 1993). However, there is no reason to suppose *a priori* that epiphytes and trees in the same ecosystem exhibit differential discrimination against ^{15}N related to either nitrogen source or mycorrhizal status.

Possible nitrogen sources for epiphytes are (i) canopy-derived nitrogen, (ii) nitrogen derived from N_2 fixation, and (iii) nitrogen derived from atmospheric deposition. In tropical rainforests the leaching of mineral nutrients from the canopy can be substantial, with 4 to 80 kg N ha^{-1} a^{-1} reported (Bernhard-Reversat 1975; Jordan *et al.* 1980;

Herrera & Jordan 1981). Litter fall in mature lowland tropical rainforests has been estimated as 28–140 kg N ha^{-1} a^{-1} (Herrera & Jordan 1981; Brasell & Sinclair 1983; Vitousek 1984). While both leaching and litter fall may contribute to epiphyte nitrogen nutrition, it is not obvious that this would lead to ^{15}N depletion. Leachates might be ^{15}N-depleted as a consequence of kinetic fractionation. ^{15}N depletion of litter-derived nitrogen could arise from $^{15}N/^{14}N$ fractionation during mineralization processes; however it would be necessary to invoke mineralization processes intrinsically different from those demonstrated for tree litter lying on soil (Natelhoffer & Fry 1988). In addition, a small data set (L. Handley & D. Bergstrom, University of Queensland, unpublished results) suggests that canopy litter is not a depleted nitrogen source for epiphytes. Fresh litter, decayed litter and downstream non-vascular epiphytes of a Moreton Bay fig all had a $\delta^{15}N$ of about 3‰. There was no ^{15}N depletion from source to sink.

N_2 fixation in the phyllosphere is considered a possible nitrogen source for tropical rainforests (Sprent & Sprent 1990). The amount of nitrogen fixed by bark micro-organisms and lichens has been estimated at between 35 and 200 kg nitrogen ha^{-1} a^{-1} (Herrera & Jordan 1981), while nitrogen fixed by canopy lichens may account for an input of 1·5 to 8 kg N ha^{-1} a^{-1} (Forman 1975). Several epiphyte species have been found to have N_2-fixing bacterial microflora in their phyllospheres (Sengupta *et al.* 1981; Brighigna *et al.* 1992). There is also evidence that N_2 fixation occurs in the epiphyte root substrate with estimated values of 40 μg N_2 fixed g^{-1} DW a^{-1} (S. Schmidt, University of Queensland, unpublished results). The $\delta^{15}N$ signature of nitrogen fixed via nitrogenase is close to that of N_2, but $\delta^{15}N$ values of whole plant shoots lie in the range of –2 to 0‰ when plants are exclusively dependent on N_2 fixation (Bergersen, Peoples & Turner 1988). It is possible that the epiphytes with higher nitrogen contents and $\delta^{15}N$ values in the range –2 to 0‰ directly acquired nitrogen from N_2 fixation. For those epiphytes with low $\delta^{15}N$ signatures and low nitrogen contents, nitrogen sources other than N_2 fixation seem likely because their $\delta^{15}N$ values are below those generally associated with N_2 fixation (Shearer & Kohl 1989).

The third possible nitrogen source for epiphytes is atmospheric deposition. Dry deposition is reported to supply 25% of the nitrogen required for forest growth, with a further 15% of the requirements being introduced as wet deposition (Lindberg *et al.* 1986). Nitrogen input from precipitation has been found to be in the range of 11–22 kg N ha^{-1} a^{-1} for various tropical forests (Herrera & Jordan 1981; Jordan *et al.* 1982), with the majority of nitrogen being deposited as NH_4^+ rather than NO_3^-. Greater inputs are reported in Europe, where atmospheric deposition accounts for inputs of 20–100 kg nitrogen ha^{-1} a^{-1}, with 50–80% of the nitrogen deposited as NH_4^+ (Pearson & Stewart 1993). $\delta^{15}N$ for NH_3/NH_4^+ in rainfall can be as low as –12‰ (Freyer 1978), and generally shows ^{15}N depletion relative to N_2 (Moore 1977; Heaton 1987; Garten 1992). Similarly, $\delta^{15}N$ values for NO_x range from –6·6 to –3·1‰ (Moore 1977; Freyer 1978; Heaton 1987). It seems probable that atmospheric deposition of depleted nitrogen accounts for the observed low $\delta^{15}N$ signatures of the greatly ^{15}N-depleted epiphytes.

The epiphytes can be grouped according to ^{15}N depletion, one group exhibiting higher nitrogen contents and higher $\delta^{15}N$ values, and one group having lower nitrogen contents and lower $\delta^{15}N$ values. It is possible that epiphytes in the first group receive at least some nitrogen from N_2 fixation, because their $\delta^{15}N$ values are indicative of N_2 fixation and because these epiphytes also had high nitrogen contents relative to other epiphytes at the same sites. The second group uses depleted nitrogen sources and is more nitrogen-limited, having low nitrogen contents. It is probable that this latter group utilizes nitrogen derived from ^{15}N-depleted deposition. No other nitrogen sources or fractionation processes can explain the observed depletion of these epiphytes.

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