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Nitrogen isotope ratios shift with plant size in tropical bromeliads

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Abstract We describe an ontogenetic shift in nitrogen (N) isotopic values in two rosette-forming epiphytic bromeliads. Leaf tissue N isotope values of small individuals of two bromeliad species (mean −6.2‰) differed from those of large individuals within each species (mean −0.5‰). Using references for potential N sources, we calculated the relative contribution of autochthonous (soil-derived through leaf litter) and allochthonous (atmospheric deposition) N with a two-member mixing model. Atmospheric sources contributed as much as 77–80% of the N in small individuals, whereas soil-derived N contributed 64–72% (conservative reference value) to 100% (less conservative reference value) of leaf tissue N in large plants. Shifts in N source with increasing plant size may be important aspects of rainforest complexity, an understudied aspect of ecosystem diversity.

Keywords *Guzmania* · *Vriesea* · La Selva · Stable isotopes · $\delta^{15}N$

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Introduction

Epiphytes live in low-nutrient environments (Benzing 2000) and have evolved several modes of nutrient uptake. Epiphytic rosette-forming bromeliads carry out negligible nutrient uptake via holdfast-forming roots (Nadkarni and Primack 1989), so once their minute seed reserves are depleted they depend primarily upon two nitrogen (N) sources: allochthonous (wet and dry atmospheric deposition), and autochthonous (taken up by host trees from the soil, incorporated into leaves, and captured as falling leaf litter).

We predicted that the source of N used by epiphytic bromeliads would shift from being primarily allochthonous when small (i.e., before development of a litter-trapping rosette) to primarily autochthonous as the rosette (and therefore litter capturing efficacy) increases with plant age. We used stable isotopes to characterize tissue N as a function of rosette size in two widespread, neotropical epiphytic bromeliads, *Guzmania monostachya* (L.) Rusby ex Mez and *Vriesea gladioliflora* (H. Wendl.) Antoine. Our approach was based on the assumption that, if small individuals depend on allochthonous N, then their isotopic N values should be similar to those of reference plants known to rely exclusively on atmospheric sources. Likewise, if larger plants rely on autochthonous N, then their isotopic values should be similar to those leaf litter and tissues of non-tank epiphytes known to be dependent upon litter-derived canopy soil for their N.

Materials and methods

This study took place in replicated 10-year-old plantations (*Hyeronima alchorneoides*, Euphorbiaceae) at La Selva Biological Station (10°26′N, 84°01′W; 35 m asl) in Costa Rica. With an average annual rainfall of nearly 4,000 mm, the region is classified as tropical wet forest (Holdridge 1967). The soil is fertile alluvium that sustains N mineralization rates of ~100 kg ha^{-1} year^{-1}(Hiremath and Ewel 2001). The trees had been planted for experimental purposes at high density (2,887 trees ha^{-1}) and periodically thinned lightly to maintain full use of resources while avoiding stand stagnation (Haggar and Ewel 1997).

To determine the relationship between litter-trapping effectiveness and trap area (a surrogate for bromeliad tank area), we constructed incrementally sized (10, 15, 30, and 50 cm in diameter; n=20 per size category), round litter collectors and attached them to host trees 1.5 m above the ground at randomized compass directions. Leaf litter was collected biweekly for 1 year, dried at 70°C to constant mass, and weighed.

Epiphyte samples were collected during December 2000 from the vertical stems of the planted trees. Because epiphyte N isotope values vary as a function of position on the phorophyte in other forests (Wania et al. 2002) all plants were sampled 1–2 m above the ground. We collected the youngest fully expanded leaf (clipped above tank water level to avoid mixing isotopic values of tank water with bromeliad leaf tissue) from developing and mature individuals of two rosette-forming bromeliad species, *G. monostachya* (n=47) and *V. gladioliflora* (n=46). To estimate the isotopic values of allochthonous N, two non-tank-forming bromeliad species assumed to depend entirely upon atmospheric N (Benzing 2000), *Tillandsia anceps* and *T. festucoides*, were sampled (n=5 for each species).

To determine whether stemflow and throughfall contributed appreciably to N inputs to epiphytes at our site, we collected samples from five randomly located samplers for stemflow, throughfall, and rainfall using protocols described by Hiremath (1999). Samples were collected for two rainfall events in December 2000. Samples were filtered and immediately analyzed for NH_4 and NO_3 concentration (Alpkem 1986), after which concentrations in rainwater were subtracted from those in stemflow and throughfall to estimate net inputs.

The reference isotopic value for autochthonous N was obtained in two ways. First, fresh leaf litter was collected daily for 3 weeks using elevated litter traps, dried to constant mass, then pooled to produce weekly litter samples. For a less conservative estimate of autochthonous N, mature leaf tissue was collected from four species of epiphyte that root in decomposing leaf litter and are incapable of trapping litter. Three of those species were epiphytic ferns (*Campyloneuron brevifolium*, *Phlebodium pseudoaureum*, and *Polypodium triseriale*) and the fourth was a fern ally (*Lycopodium linearifolium*) (n=5 for each species).

Leaf tissue was cleaned before drying to constant mass at 70°C, ground to a fine powder in a ball grinder, and weighed into tin capsules for continuous flow isotope ratio mass spectrometry (Center for Stable Isotope Biogeochemistry, UC Berkeley and the University of Arkansas Stable Isotope Laboratory, Fayetteville). Samples were analyzed for δ^{15}N values and N content by mass. Nitrogen isotope values are expressed in parts per thousand (Ehleringer and Rundel 1988):

$$\delta X_{\text{standard}} = \left(R_{\text{sample}}/R_{\text{standard}} - 1\right) \times 1000$$

where $\delta X_{\text{standard}}$ is the isotope ratio relative to a standard, and R_{sample} and R_{standard} are the absolute isotope ratios of the sample and standard, respectively. The isotopic value for δ^{15}N was calculated relative to the atmospheric N standard ($^{15}N/^{14}N_{\text{atmosphere}}$= 0.0036765). We used curvilinear regression to test the relationships between plant size and δ^{15}N value for *G. monostachya* and *V. gladioliflora*.

To determine the relative contribution of allochthonous and autochthonous N to individual bromeliads as a function of plant size, the following two-ended mixing model was used (Robinson 2001):

$$X_{\text{tracer}} = \frac{\delta^{15}N_{\text{sample}} - \delta^{15}N_{\text{background}}}{\delta^{15}N_{\text{tracer}} - \delta^{15}N_{\text{background}}}$$

where X_{tracer} is the fraction of N from the tracer (i.e., allochthonous) source in the sample relative to the fraction of N from the background (i.e., authochthonous) source. The model assumes that the fractionation of N isotopes during uptake is similar for all sampled individuals.

Results

The amount of N trapped by litter baskets in the form of fresh leaf litter increased linearly as a function of trapping area (r^2=0.74, P<0.0001). Small baskets trapped ≤10 mg N (0.037 g mean dry leaf litter) every 2 weeks, whereas large baskets trapped up to 100 mg N (6.93 g mean dry leaf litter) in that same time period. We found no significant spatial variation in litter inputs (MS=67,806, df=16, F=1.02, P=0.44), nor did we find significant temporal variation in litter inputs (MS=5.76, df=23, F=0.556, P=0.95).

Mean NO_3 and NH_4 concentrations were 0.08–0.17 and 0.11 mg l^{-1} in rainfall, respectively. Net NO_3 and NH_4 concentrations in stemflow were −0.08 to +0.04 and −0.06 to −0.01 mg l^{-1}, respectively, while NO_3 and NH_4 concentrations in throughfall were −0.09 to +0.05 and −0.07 to −0.06 mg l^{-1}, respectively.

Mean mass-based N content of *G. monostachya* leaves did not differ from that of *V. gladioliflora* (Table 1; t= −1.04, df=45, P=0.30). Leaf mass per area was 0.014 ±0.3 g m^{-2} in *G. monostachya* and 0.0135±0.2 g m^{-2} in *V. gladioliflora.* Mean area-based N content was 0.07±0.02 g N m^{-2} in *G. monostachya*, and 0.07±0.02 g N m^{-2} in *V. gladioliflora.* There were no strong correlations between N content and δ^{15}N value in either *G. monostachya* or *V. gladioliflora* (r^2=0.001 and 0.17, and P=0.514 and 0.003, respectively).

Both *G. monostachya* and *V. gladioliflora* underwent a dramatic shift in the N isotopic value as a function of age and size. Small plants of both species had values as low as −6.6‰, whereas the mean value of larger plants was as high as −0.8‰ in *G. monostachya* and +0.6‰ in *V.*

Table 1 . Mean nitrogen content and δ^{15}N values for *Guzmania monostachya*, *Vriesea gladioliflora*, plants dependent upon allochthonous N (two*Tillandsia* species), plants dependent upon N from decomposed litter (ferns and fern ally: *Campyloneuron brevifolium*, *Phlebodium pseudoaureum*, *Polypodium triseriale*, and *Lycopodium linearifolium*), and fresh litter. *N.A.* not available

	N content		δ^{15}N (‰)
	(mg g^{-1})	(g m^{-2})	
G. monostachya	0.9±0.3	0.07±0.02	[a]
V. gladioliflora	0.9±0.2	0.07±0.02	[a]
Tillandsia spp.	0.7±0.2	N.A.	−6.4±2.0
Ferns and fern allies	1.5±0.4	N.A.	+0.9±1.6
Fresh litter	1.8±0.5	N.A.	+2.5±0.7

[a]See Fig. 1 for δ^{15}N values of *G. monostachya* and *V. gladioliflora*

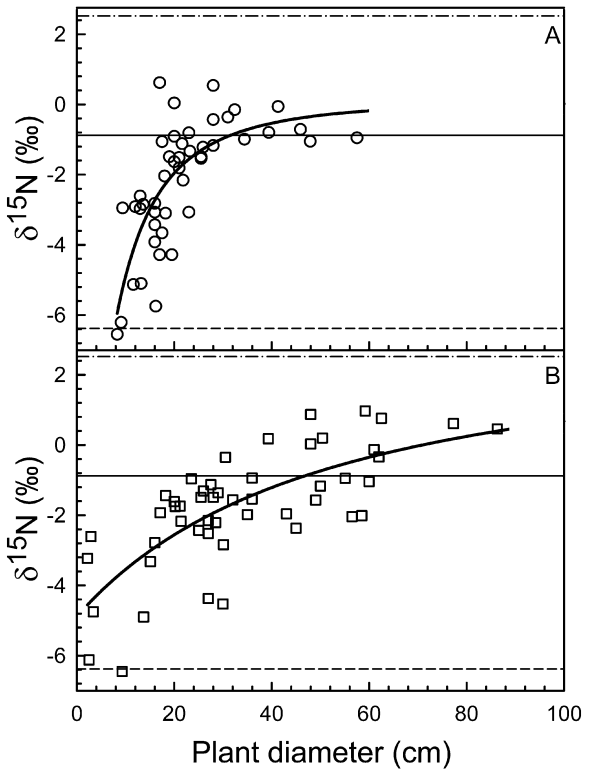

Fig. 1A, B Nitrogen isotope values as a function of plant size. **A** *Guzmania monostachya* (r^2=0.57, P<0.0001) and **B** *Vriesea gladioliflora* (r^2=0.54, P<0.0001). Reference lines for the δ^{15}N values for the potential N sources: bromeliads known to depend on atmospheric N, *Tillandsia anceps* and *T. festucoides* (*dashed horizontal line*, n=5); plants with roots accessing decomposed litter, *Lycopodium linearifolium*, *Campyloneuron brevifolium*, *Phlebodium pseudoaureum*, *Polypodium triseriale* (*solid horizontal line*, n=5); and fresh leaf litter (*dot-dashed line*, n=18). All potential sources differed from one another (*df*=2, MS=159.3, F=112.2, P<0.0001 and Bonferroni post hoc analyses P<0.001)

gladioliflora (Fig. 1). The mean N isotopic value of the two *Tillandsia* species that were selected to approximate that of allochthonous N differed from that of fresh litter (Table 1), as did the mean value of the ferns and fern allies.

The two-ended mixing model also showed that *G. monostachya* and *V. gladioliflora* underwent a dramatic shift in the source of N used as a function of plant age and size. On average, atmospheric sources accounted for 80% and 77% of the foliar N in smaller individuals, whereas litter-derived sources accounted for 20% and 23% in *G. monostachya* and *V. gladioliflora*, respectively. In contrast, atmospheric sources accounted for 37% and 28% of the foliar N in larger individuals, whereas litter-derived sources (conservative estimate) accounted for 63% (*G. monostachya*) and 72% (*V. gladioliflora*). In both *G. monostachya* and *V. gladioliflora*, the model predicted that 100% of the N in large individuals is autochthonous (i.e., decomposed litter).

Discussion

The N isotopic values and N contents in our study are within the range of those reported for epiphytes and leaf litter in Costa Rican lowland wet and mid-elevation cloud forests (Hietz et al. 2002; Wania et al. 2002), and in other tropical forests (Stewart et al. 1995).

The dramatic shift in N isotopic values as a function of size observed in these two bromeliad species supports the prediction that N sources shift as plants age and grow. Small bromeliads that have not developed tanks depend upon atmospheric inputs, giving them isotopic values similar to those of atmosphere-dependent *Tillandsia* species, whose N isotopic value was reflective of atmospheric N (Heaton 1986). We suggest the δ^{15}N values of larger plants reflect the integration of the isotopic composition of leaf litter (2.5‰), possible discrimination during net N uptake, and the −2 to −3‰ shift that occurs during leaf decomposition (Nadelhoffer and Fry 1988).

An alternative interpretation of these data is that slow-growing plants rely on different N sources than do fast-growing plants, a possibility we can test with growth data. Rates of leaf production by *V. gladioliflora* are approximately double those of *G. monostachya* for most months during the year (A. Reich, unpublished data), and because the two species exhibit similar shifts in N isotopic values as a function of plant size, this alternative hypothesis can be ruled out.

Canopy leaching is an unlikely source of nitrogen for these epiphytes. First, the N concentration in rainfall is extremely low (Eklund et al. 1997). Furthermore, the tree crowns at our site are net N sinks, not N sources (Hiremath and Ewel 2001).

What about possible N sources other than the atmosphere and leaf litter? Although fixation of diatomic N by microbes in the water-holding rosettes may be an additional N source in some locales, it is low or absent in the bromeliads at our site (Bentley and Carpenter 1984). Animals (e.g., insects, spiders, and frogs) are known to associate with bromeliad tanks (Benzing 1986; Richardson 1999; Maple 2002), and we do not discount the possibility that animal immigrations, excretions, and decomposition contribute to bromeliad nutrition in some habitats. In our monospecific stands of *H. alchorneoides*, the frog *Dendrobates pumilio* deposits its tadpoles and nutritive eggs in the tanks of larger individuals of *G. monostachya*, but tadpoles are found in fewer than 10% of the plants (Maple 2002). Although we did not measure the N isotopic values of animal inputs, we suspect that they are at best modest N sources for the bromeliads in our plantations.

We documented a difference between the isotopic values of fresh leaf litter and those plants that derive N from decomposed litter. A 2–4‰ shift has been documented during litter decomposition (Evans 2001) and is reflected here in our less conservative autochthonous reference tissues (ferns). The less conservative estimate of the relative input from autochthonous N is analogous to accounting for decomposition by subtracting approxi-

mately 2‰ from fresh litter isotope values. The isotopic N values documented in *G. monostachya* and *V. gladioliflora* support the notion that larger bromeliads are more dependent on autochthonous N than are smaller ones.

The use of N isotope values has increased our understanding of how lifeform complexity contributes to the diversity of ecological function in ecosystems in French Guyana (Guehl et al. 1998) and in Costa Rican cloud forests (Hietz et al. 2002). The ontogenetic shifts in N isotopic values in the neotropical bromeliads we studied might be mirrored in palaeotropical taxa whose foliage forms tanks or baskets, such as some ferns in the genera *Asplenium* and *Platycerium*. In addition to the functional diversity among lifeforms in tropical forests (Ewel and Bigelow 1996), a size-dependent shift in source of N used by tank-forming epiphytes adds a temporal and developmental dimension to the functioning of these complex ecosystems.

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References

Alpkem (1986) RFA methodology. Alpkem Corporation, Clackamas, Ore.

Bentley BL, Carpenter EJ (1984) Direct transfer of newly-fixed nitrogen from free-living epiphyllous microorganisms to their host plant. Oecologia 63:52–56

Benzing D (1986) In: Juniper B, Southwood T (eds) Insects and the plant surface. Arnold, London, pp235–256

Benzing D (2000) Bromeliaceae. Cambridge University Press, Cambridge

Ehleringer J, Rundel PW (1988) Stable isotopes: history, units, and instrumentation. In: Rundel PW, Ehleringer J, Nagy K (eds) Stable isotopes in ecological research, vol 68. Springer, Berlin Heidelberg New York, pp1–15

Eklund TJ, McDowell WH, Pringle CM (1997) Seasonal variation of tropical precipitation chemistry: La Selva, Costa Rica. Atmos Environ 31:3903–3910

Evans RD (2001) Physiological mechanisms influencing plant nitrogen isotope composition. Trends Plant Sci 6:121–126

Ewel JJ, Bigelow S (1996) Plant life-forms and tropical ecosystem functioning. In: Orians G, Dirzo R, Cushman J (eds) Biodiversity and ecosystem processes in tropical forests. Springer, Berlin Heidelberg New York, pp101–126

Guehl JM, Domenach AM, Bereau M, Barigah TS; Casabianca H, Ferhi A, Garbaye J (1998) Functional diversity in an Amazonian rainforest of French Guyana: a dual isotope approach (delta N-15 and delta C-13). Oecologia 116:316–330

Haggar JP, Ewel JJ (1997) Primary productivity and resource partitioning in model tropical ecosystems. Ecology 78:1211–1221

Heaton THE (1986) Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chem Geol Isot Geosci Sect 59:87–102

Hietz P, Wanek W, Wania R, Nadkarni NM (2002) Nitrogen-15 natural abundance in a montane cloud forest canopy as an indicator of nitrogen cycling and epiphyte nutrition. Oecologia 131:350–355

Hiremath AJ (1999) Nutrient use efficiency in simplified tropical ecosystems. University of Forida, Gainesville, Fla.

Hiremath AJ, Ewel JJ (2001) Ecosystem nutrient use efficiency, productivity, and nutrient accrual in model tropical communities. Ecosystems 4:669–682

Holdridge LR (1967) Life zone ecology. Tropical Science Center, San Jose, Costa Rica

Maple M (2002) Maternal effects on offspring fitness in *Dendrobates pumilio*, the strawberry poison frog. University of Kentucky, Lexington

Nadelhoffer K, Fry B (1988) Controls on nitrogen-15 and carbon-13 abundances in forest soil organic matter. Soil Sci Soc Am J 52:1633–1640

Nadkarni NM, Primack RB (1989) The use of gamma spectrometry to measure within-plant nutrient allocation of a tank bromeliad, *Guzmania lingulata*. Selbyana 11:22–25

Richardson BA (1999) The bromeliad microcosm and the assessment of faunal diversity in a Neotropical forest. Biotropica 31:321–336

Robinson D (2001) delta-^{15}N as an integrator of the nitrogen cycle. Trends Ecol Evol 16:153–162

Stewart GR, Schmidt S, Handley LL, Turnbull MH, Erskine PD, Joly CA (1995) N-15 natural-abundance of vascular rain-forest epiphytes—implications for nitrogen-source and acquisition. Plant Cell Environ 18:85–90

Wania R, Hietz P, Wanek W (2002) Natural N-15 abundance of epiphytes depends on the position within the forest canopy: source signals and isotope fractionation. Plant Cell Environ 25:581–589