

The influence of life form on carbon and nitrogen relationships in tropical rainforest ferns

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Abstract Tropical ferns are characterized by a high diversity of plant life forms, yet there have been few large-scale studies on the functional ecology of these different forms. We examined epiphytic, hemiepiphytic, and terrestrial ferns, and asked whether there are differences in the mineral nutrition and water relations across different growth forms of a diverse assemblage of species. We measured specific leaf area, leaf nitrogen concentrations, and natural abundance of the stable isotopes $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of 48 fern species from 36 genera across a wide range of habitats at La Selva Biological Station in Costa Rica. We found that epiphytes were significantly different in all measured variables from hemiepiphytic and terrestrial species, and that terrestrial and soil-rooted hemiepiphytes were indistinguishable in all variables excluding SLW. A multivariate analysis revealed that aspects of N nutrition were the most reliable at separating epiphytic species from other

life forms. Our study demonstrates that the natural abundance of both C and N as well as N relations and leaf morphology are useful when segregating different plant life forms, and that the N cycle of epiphytic and terrestrial habitats function independently from each other.

Keywords Pteridophytes · Epiphyte · Hemiepiphyte · Nitrogen · Carbon

Introduction

Tropical forest ecosystems are characterized by both high species richness and a high diversity of life forms (Richards 2004). While there are numerous and often complex classification systems, all species generally fall into one of three habitat groups or life forms: epiphytic, hemiepiphytic, or terrestrial. There is tremendous variation within each of these groups, not only in terms of species distribution (e.g., understory versus canopy epiphytes, primary versus secondary hemiepiphytes, high-light versus low-light terrestrials, etc.), but also within-group functional ecology (e.g., twig versus canopy soil organic matter-rooted epiphytes) as it relates to nutrient and water relations. The notion of grouping plants based on one or many similar functional attributes (Smith et al. 1997) has been widely applied in tropical ecosystems to aid in the classification of an overwhelming diversity of species. Yet, recent work has demonstrated that several character suites can result in similar fitness within a given functional group (Marks and Lechowicz 2006). Thus, grouping species based on physiognomy alone or a limited suite of morphological characters may obscure much of an individual's species biology.

Decoupling the influence of different life forms on leaf-level traits has been difficult but has been aided by the

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application of stable isotopes, which can reveal many integrated types of information. Studies have examined the natural abundance of the stable isotopes $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ along with leaf nitrogen to reveal the sources of mineral nutrition (Stewart et al. 1995a), water use efficiency (Stewart et al. 1995b; Reich et al. 2003), and photosynthetic pathways (Kluge et al. 1995; Rundel et al. 1979, 1980). The application of $\delta^{15}\text{N}$ has been especially fruitful when examining the mineral nutrition of epiphytes. Data from $\delta^{15}\text{N}$ suggests that some canopy epiphytes take up depleted and likely atmospherically derived forms of N (Cardelús, unpublished data), and that $\delta^{15}\text{N}$ signatures are often correlated with species location in the canopy (Stewart et al. 1995a; Wania et al. 2002). Stable isotopes provide a powerful tool for studying the functional ecology of different plant life forms.

Comparative data on the mineral nutrition of epiphytic and related terrestrial species is limited, and sometimes contradictory. Putz and Holbrook (1989) have shown little to no difference in the nutrient content of terrestrially rooted and canopy-rooted hemiepiphytes, while more recent studies have shown that nutrient economy may change radically with rooting source (e.g., canopy twig versus canopy soil organic matter versus terrestrial soil-rooted) (Hietz et al. 2002; Wanek et al. 2002). Less is known about ferns than other plant groups despite their abundance in tropical rainforest ecosystems. In a recent study on the hemiepiphytic fern *Lomariopsis vestita*, Watkins (2006) has shown that significant ontogenetic shifts occur between epiphytic gametophytes and sporophytes, and terrestrially rooted sporophytes. Such shifts in N nutrition between epiphytic and terrestrial life forms and our ability to record such changes using stable isotopes may aid in the functional grouping of these plants and create a better understanding of canopy and terrestrial nutrient cycling. We have been unable to find a single study that examines C and N relations of soil-rooted and canopy-rooted ferns.

Because tropical epiphytic ferns lack access to comparatively larger nutrient and more stable water pools available to terrestrially rooted species, we predict that (1) epiphytic species will show evidence of water stress and thus exhibit higher (less negative) $\delta^{13}\text{C}$ signatures than soil-rooted species, and (2) if epiphytes are utilizing more depleted N pools, $\delta^{15}\text{N}$ signatures will be lower than soil-rooted hemiepiphytic and terrestrial species. We also predict that from a functional nutrient standpoint, hemiepiphytes that are rooted in forest floor soil will utilize these larger N pools and will be indistinguishable from terrestrial species. In order to address our hypotheses, we incorporate natural abundances of the stable isotopes $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, leaf N concentrations, and leaf morphological measurements to better understand how life form influences mineral nutrition, water use, leaf attributes, and photosynthetic pathways.

Ferns as a study system

Our study system is limited to pteridophytes, specifically the ferns. Ferns are most diverse in tropical forests and in lowland forest like Costa Rica, they often make up a considerable component of canopy cover (Cardelús 2007) and species diversity (Cardelús et al. 2006; Watkins et al. 2006). The ferns also pose an intriguing model system for the study of life form diversity on carbon and nutrient relations. The group is a monophyletic lineage of vascular plants with diverse life forms that have radiated into numerous habitats. The clade itself consists of 10,000–12,000 species and is thus small relative to the seed-bearing vascular plants. What the group lacks in species richness it makes up for in its morphological and functional diversity. Terrestrial, hemiepiphytic, and epiphytic growth habits are common in the group, which provides a powerful phylogenetically controlled comparative tool. Additional variation within each of these growth forms (xeric to mesic terrestrial species, primary and secondary hemiepiphytes, trunk, twig, and epiphytic epiphytes, etc.). Fern diversity peaks in lowland tropical rainforests, thus providing a biogeographic control where several species from different functional groups can be examined from the same forest.

Methods

Study site and species

This study was conducted at La Selva Biological Station in Heredia Province, Costa Rica. The site is a 1,400-ha tropical wet forest that has a mean annual rainfall of approximately 4,000 mm, with peaks of precipitation in June–July and November–December, and a drier period in January–April (McDade et al. 1994). Mean monthly rainfall nevertheless never falls below 150 mm in any month during the dry season based on long-term station meteorological records.

A total of 48 fern species from 36 genera were sampled across a wide range of habitats at La Selva (Table 1). Each species was categorized as being from a high- or low-light habitat based on the habitat in which it was most characteristically found. To assess light environments, a digital, hemispherical photograph was taken <0.5 m above one individual of each species with a Nikon Coolpix 950 digital camera (Melville, NY, USA) with a fisheye lens attachment. Images were then analyzed using Gap Light Analyzer software (Frazer et al. 1999) to estimate the percentage of total transmittance. Low-light environments were defined as those where the percentage of total transmittance was less than 25%. High-light environments were defined as those that exhibited >25%. Leaf

Table 1 Species, life form, natural light level, mean specific leaf weight (SLW), natural abundance $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures, percent nitrogen (mass-based) and leaf nitrogen concentration (area-based) for 48 fern species collected at La Selva Biological Station in Costa Rica

Species	Life form	Light level	SLW (g cm ⁻²)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	%N (mg g ⁻¹)	Leaf N (g m ⁻²)
<i>Adiantum latifolium</i> Lam.	Terrestrial	Low	31	-34.9	2.6	33.6	1.04
<i>Adiantum obliquum</i> Willd.	Terrestrial	Low	-	-33.9	3.0	28.4	-
<i>Alsophila cuspidata</i>	Terrestrial	High	28	-31.0	2.1	32.6	0.91
<i>Anetium citrifolium</i> (L.) Splitg.	Epiphyte	Low	33	-32.9	0.9	29.3	0.97
<i>Asplenium serratum</i> L.	Epiphyte	Low	24	-31.0	2.4	36.1	0.87
<i>Asplenium auritum</i> Sw. sensu lato	Epiphyte	High	55	-32.1	0.7	15.7	0.86
<i>Asplenium serra</i> Langds. and Fischer	Epiphyte	High	55	-29.2	4.0	25.8	1.42
<i>Blechnum</i> sp1	Terrestrial	Low	26	-33.6	3.2	20.3	0.52
<i>Bolbitis nicotianifolia</i> (Sw.) Alston	Hemiepiphyte	Low	37	-34.4	2.6	35.8	1.32
<i>Campyloneurum brevifolium</i> (Lodd. ex Link) Link	Epiphyte	High	87	-30.2	1.4	18.4	1.6
<i>Colchlidium serrulatum</i> (Sw.) Bishop	Epiphyte	High	112	-35.3	2.4	15.4	1.72
<i>Cyathea multiflora</i> J. Sm.	Terrestrial	High	37	-33.0	1.9	24.7	0.91
<i>Cyclopeltis semicordata</i> (Sw.) J. Sm.	Terrestrial	Low	32	-34.1	1.3	31.8	1.02
<i>Danaea wendlandii</i> Reichenb. F.	Terrestrial	Low	32	-34.8	2.4	25.1	0.8
<i>Dicranoglossum panamense</i> (C. Chr.) L.D. Gomez	Epiphyte	Low	52	-31.4	-1.0	18.2	0.95
<i>Didymochlaena truncatula</i> (Sw.) J. Sm	Terrestrial	Low	32	-35.1	2.4	28.8	0.92
<i>Diplazium macrophyllum</i> Desv.	Terrestrial	Low	34	-	-	-	-
<i>Diplazium striatastrum</i> Lellinger	Terrestrial	Low	32	-33.9	2.2	37.6	1.2
<i>Elaphoglossum herminieri</i> (Bory ex Fei) T. Moor	Epiphyte	High	202	-30.4	0.9	9	1.82
<i>Elaphoglossum latifolium</i> (Sw.) J. Sm.	Epiphyte	High	108	-	1.6	13.5	1.46
<i>Elaphoglossum peltatum</i> (Sw.) Urban	Epiphyte	Low	73	-33.4	-0.7	23	1.68
<i>Hemiontis palmata</i> L.	Terrestrial	High	34	-33.9	2.5	27.8	0.94
<i>Hymenophyllum</i> sp.	Epiphyte	Low	-	-28.4	0.1	12.9	-
<i>Lomariopsis japurensis</i> (Mort.) J. Sm.	Hemiepiphyte	Low	34	-34.2	1	33.3	1.13
<i>Lomariopsis vestita</i> E. Fourn.	Hemiepiphyte	Low	38	-35	2.3	34.6	1.31
<i>Lonchitis hirsuta</i> L.	Terrestrial	Low	18	-34.7	0.4	34.6	0.62
<i>Microgramma lycopodioides</i> (L.) Copel.	Epiphyte	High	81	-28.6	-0.1	13.4	1.08
<i>Microgramma reptans</i> (Cav.) A.R. Sm.	Epiphyte	High	51	-29	2	12.3	0.63
<i>Nephrolepis rivularis</i> (Vahl) Mett. ex Krug.	Epiphyte	High	43	-30.1	0.4	12.5	0.54
<i>Oleandra articulata</i> (Sw.) C. Presl	Epiphyte	High	42	-30.2	1.4	15.6	0.65
<i>Olfersia cervina</i> (L.) Kunze	Hemiepiphyte	Low	48	-34.7	3.2	25.4	1.22
<i>Ophioglossum reticulatum</i> L.	Terrestrial	High	26	-31.9	-	59.8	1.55
<i>Phlebodium pseudoaureum</i> (Cav.) Lellinger	Epiphyte	High	22	-30.6	2.5	28.3	0.62
<i>Pleopeltis</i> sp.	Epiphyte	High	84	-31.1	-0.6	12.7	1.07
<i>Polybotrya caudata</i> Kunze	Hemiepiphyte	Low	41	-34.6	3.3	22.5	0.92
<i>Polypodium triseriale</i> Sw.	Epiphyte	High	51	-29.4	0.8	20.1	1.03
<i>Polytaenium ensiforme</i> (Hook.) Benedict	Epiphyte	Low	54	-32.5	2.1	23	1.24
<i>Saccoloma inaequale</i> (Kunze) Mett.	Terrestrial	Low	-	-35.2	1.3	36.2	-
<i>Salpichlaena volubilis</i> (Kaulf.) J. Sm.	Terrestrial	Low	37	-34	2.9	25.1	0.93
<i>Tectaria draconifolia</i> (D.C. Eaton) Copel.	Terrestrial	Low	31	-34.1	2	33	1.02
<i>Thelypteris</i> (<i>Goniopteris</i>) <i>poiteana</i> (Bory) Proctor	Terrestrial	Low	36	-34.2	1.9	28.5	1.03
<i>Thelypteris</i> (<i>meniscium</i>) <i>lingulata</i>	Terrestrial	Low	29	-34.6	2.2	27.1	0.79

tissue from four to five individuals was collected for each species. Leaf area and mass was measured on fresh samples and specific leaf weight (SLW, cm² g⁻¹) was calculated using these data.

Sample preparation

Air-dried and pulverized tissues from each leaf sample were analyzed for total C and N, and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios,

Table 1 continued

Species	Life form	Light level	SLW (g cm ⁻²)	δ ¹³ C (‰)	δ ¹⁵ N (‰)	%N (mg g ⁻¹)	Leaf N (g m ⁻²)
<i>Thelypteris</i> sp.	Terrestrial	Low	45	-32.2	1.7	51.5	2.32
<i>Trichomanes collariatum</i> Bosch.	Epiphyte	Low	29	-32.5	0.7	21.5	0.62
<i>Trichomanes diversifrons</i> (Bory) Mett. ex Sadeb.	Terrestrial	Low	35	-34.1	0.3	18.1	0.63
<i>Trichomanes godmanii</i> Hook.	Epiphyte	Low	31	-31.8	3.2	2.1	0.65
<i>Vittaria lineata</i> (L.) J. Sm.	Epiphyte	High	142	-30.2	1.3	15.3	2.17
<i>Vittaria stipitata</i> Kunze	Epiphyte	High	117	-30.9	0.2	7.1	0.83

Empty cells represent incomplete data

at the University of Arkansas Stable Isotope Laboratory on a Finnigan MATT Delta IRMS isotope ratio mass spectrometer operated in automatic trapping mode after combustion of samples in an elemental analyzer (Carlo Erba Instrumentation, Milan, Italy). The reference CO₂ was calibrated against standard Pee Dee belemnite (PDB). The measurement precision was better than 0.2‰ for C and 0.4‰ for N.

Statistical analysis

Analysis of variance (ANOVA) followed by post hoc Tukey tests were run to determine differences between groups for each variable measured. Multivariate ANOVA (MANOVA) and canonical discriminant function analysis were employed to identify significant relationships among epiphytic, hemiepiphytic, and terrestrial species using the combined data set of δ¹⁵N, δ¹³C, SLW, and leaf N. All statistical analyses were run using the program JMP (SAS institute 2005).

Results

Specific leaf weight varied significantly between terrestrial, hemiepiphytic, and epiphytic growth forms (Fig. 1A). Epiphytes had significantly lower SLW compared to terrestrial species, while hemiepiphytic species were intermediate, but did not differ significantly from epiphytic species (Fig. 1A; $F = 7.01$, $P = 0.002$). Epiphytes from high-light environments had significantly lower SLW compared to high light terrestrial species (Fig. 1B; $F = 4.16$, $P = 0.0482$), whereas low-light epiphytes and terrestrial species did not differ (Fig. 1B; $F = 2.54$, $P = 0.101$). Epiphytic species had a greater range of values of SLW than either of the other two groups (Fig. 1A). Species at the low extremes of the SLW spectrum occurred strictly as epiphytes, and this relationship was partially influenced by light (Fig. 1B). When epiphytes and terrestrial species were separated on the basis of their characteristic light environment, high-light epiphytes had significantly lower values of SLW than low-light epiphytic

species (Fig. 1B; $F = 4.26$, $p = 0.0431$), and from either high- or low-light terrestrial species (Fig. 1B). The SLW of terrestrial species was not significantly different between high- and low-light environments (Fig. 1B; $F = 0.073$, $p = 0.79$). All hemiepiphytic species encountered in this study were in low-light habitats, and for this reason they have been excluded from light level comparisons.

Epiphytic species were more enriched in δ¹³C with a mean carbon isotope signature of $-31.0 \pm 1.7‰$ relative to both terrestrial and hemiepiphytic species (mean of -33.8 ± 1.1 and $-34.6 \pm 0.3‰$, respectively; Fig. 1C; $F = 40.03$, $P < 0.000$). There was no evidence of ecologically significant CAM activity in any of the epiphytic species because all fell in the range expected of C₃ plants. As with SLW, hemiepiphytic and terrestrial ferns were not significantly different from each other in δ¹³C (Fig. 1C). Habitat light regime was an important factor influencing δ¹³C both within and between light levels when epiphytes were compared to terrestrial species (Fig. 1D). High-light epiphytic species had significantly higher values than high-light terrestrial species ($F = 15.56$, $P < 0.000$), as did low-light epiphytes compared to low-light terrestrial species ($F = 19.15$, $P < 0.000$). High-light epiphytes had significantly higher values than low-light epiphytes ($F = 8.48$, $P < 0.000$), and the same pattern existed between high- and low-light terrestrial species ($F = 11.89$, $P < 0.000$).

Nitrogen-specific leaf weight (g N m⁻²) was not significantly different between any of the three life forms ($F = 0.3662$, $P = 0.696$) (Fig. 2A). When compared on a mass basis (mg N g⁻¹ leaf mass), epiphytic species exhibited significantly lower leaf N concentrations (17.4 ± 7.7 mg g⁻¹) when compared to terrestrial (31.8 mg g⁻¹ ± 9.9) and hemiepiphytic ferns (30.3 ± 5.97 mg g⁻¹) (Fig. 2B) ($F = 15.00$, $P < 0.0001$) (Fig. 2B).

Epiphytic ferns were significantly depleted in δ¹⁵N ($1.2 \pm 1.3‰$) when compared to terrestrial ($2.0 \pm 0.8‰$) and hemiepiphytic species ($2.5 \pm 0.9‰$), and hemiepiphytic and terrestrial species did not differ significantly in their δ¹⁵N signatures (Fig. 2C; $F = 5.49$, $P = 0.008$). There was no significant effect of light regime on δ¹⁵N signatures of either epiphytic or terrestrial species ($F = 6.54$, $P = 0.4229$, data

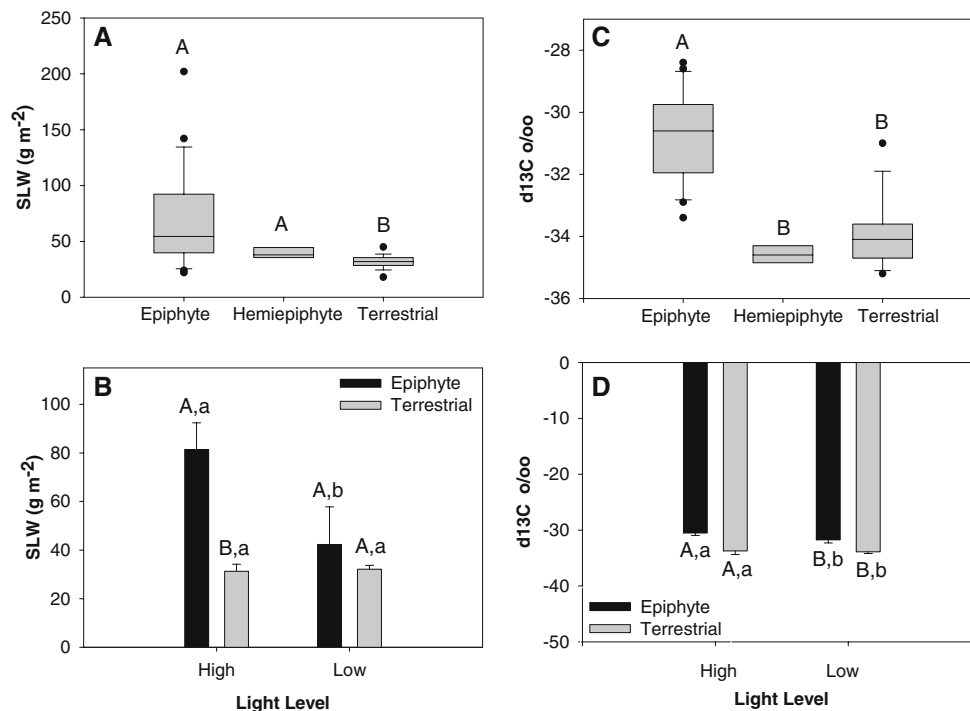


Fig. 1A–D The influence of life form on specific leaf weight (SLW) and the natural abundance of $\delta^{13}\text{C}$ from 48 tropical fern species from La Selva Biological Station, Costa Rica. **A** Variation in SLW as it relates to plant life form and **B** from different habitat light regimes. **C** The influence of plant life form on $\delta^{13}\text{C}$ signatures. **D** Variation in $\delta^{13}\text{C}$ as it relates to epiphytic and terrestrial species from different habitat light regimes. For **A** and **C**, the boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the

boundary of the box farthest from zero indicates the 75th percentile. *Error bars* above and below the box indicate the 90th and 10th percentiles; points beyond *error bars* are outliers. Differences between means were determined by a post hoc Tukey test: *capital letters* refer to within categorical comparisons (epiphyte–terrestrial) and *lower case letters* refer to between categorical comparisons (high–low). Levels sharing the same letter are not significantly different

not shown), although the range of variation within epiphytic species was greater than that in the terrestrial species.

In order to determine variables that were most important in the separation of the different functional groups, we ran a discriminant function analysis of all the data ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, SLW, leaf N). This multivariate analysis demonstrated that the epiphytic species were significantly different from terrestrial and hemiepiphytic species (Pillai's trace = 0.8312, $F = 7.468$, $df = 8$, $P < 0.001$) based on the MANOVA (Fig. 3). The 95% confidence intervals of terrestrial and hemiepiphytic ferns overlapped, suggesting that these groups were not significantly different (Fig. 3). The first discriminant function explained 94.56% of the between-group variance; the second explained 5.4%. $\delta^{15}\text{N}$ and percent leaf N contributed most to the first function, and $\delta^{13}\text{C}$ the second; SLW was the least useful character when separating the groups.

Discussion

A number of studies have demonstrated the importance of edaphic characteristics in shaping the distributions of

terrestrial ferns (Tuomisto et al. 2003 and references therein), whereas aspects tied to water relations seem to play a larger role with epiphytic species (Hietz and Briones 1998). Epiphytic and terrestrial habitats vary widely in many dimensions, including water, nutrients, and light. Watkins et al. (2006) have demonstrated that, in the case of ferns, reciprocal establishment of species between the two habitats is exceptionally rare. This is perhaps not surprising given that the direction and intensity of selection can be radically different between these two habitats. Here we examine a suite of ecophysiological variables directly related to whole plant physiology and compare differences among these variables to different fern life forms.

As a functionally based leaf trait, SLW is important to plant growth, as it is directly related to light capture efficiency and is thus related to the carbon fixation strategy of a leaf (Reich 1991; Reich et al. 1999; Wright et al. 2002). This carbon fixation strategy is confounded by the influence of both water and nutrient stress on SLW. Species of hot and dry habitats are thought to have evolved thick leaves to cope with the effects of frequent and prolonged drought (Nelson et al. 2002). Nutrient stress has also been

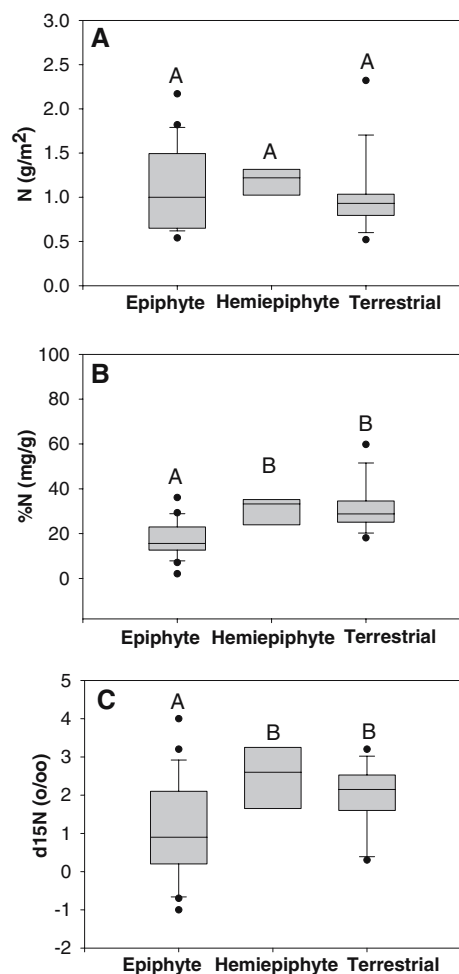


Fig. 2A–C The relationship of **A** area-based leaf nitrogen, **B** mass-based leaf nitrogen, and **C** natural abundance of $\delta^{15}\text{N}$ isotopes in epiphytic, hemiepiphytic, and terrestrial fern species from La Selva Biological Station, Costa Rica. The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. *Error bars* above and below the box indicate the 90th and 10th percentiles; points beyond *error bars* are outliers. Differences between means were determined by a post hoc Tukey test; levels sharing the same letter are not significantly different

employed to explain differences in leaf thickness between nutrient-poor and nutrient-rich sites (Chapin 1980; Wright et al. 2002). The investment in thick, long-lived leaves maximizes a plant's investment in sites where nutrients are scarce. Epiphytic species clearly live in an environment that is exposed to frequent, and in some cases, extended periods of drought relative to terrestrial species (Freiberg and Turton 2007) as well as nutrient-poor compared to forest floor soils (Cardelús and Mack, unpublished data). The combination of drought, nutrient poverty, and the intrinsically heterogeneous nature of canopy systems are likely explanations for the high variation in both SLW and $\delta^{13}\text{C}$ signatures in epiphytic ferns (Figs. 1A, 2C), and their differences from terrestrial species. It is possible that there

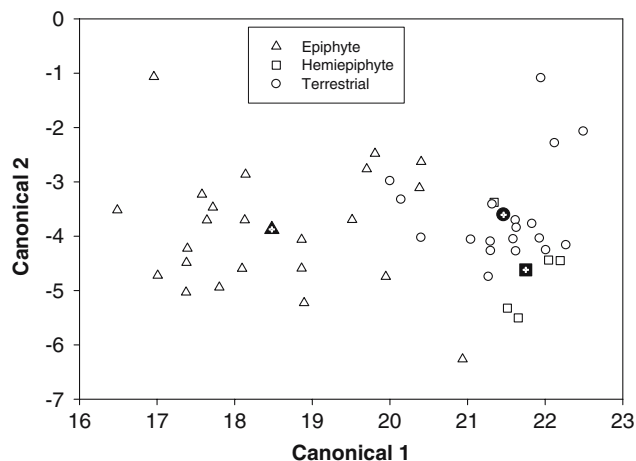


Fig. 3 Discriminant function analysis of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, specific leaf weight ($\text{cm}^2 \text{g}^{-1}$), and leaf N (mg g^{-1}) in epiphytic, hemiepiphytic, and terrestrial fern species from La Selva Biological Station, Costa Rica. *Shapes marked in bold* are means of respective life forms. This analysis demonstrated that epiphytic species were significantly different from terrestrial and hemiepiphytic species (MANOVA: Pillai's trace = 0.8312, $F = 7.468$, $df = 8$, $P < 0.0001$). The 95% confidence intervals of terrestrial and hemiepiphytic overlapped, suggesting that these groups were not significantly different. The first discriminant function explained 94.56% of the between-group variance; the second explains 5.4%. $\delta^{15}\text{N}$ and percent leaf N contributed most to the first function, and ^{13}C the second; SLW was not a useful character in any of the functions

are some intrinsic differences in SLW between hemiepiphyte and terrestrial species; however, the observed pattern is most likely due to low sample size driven by low hemiepiphytic species availability at La Selva.

The data support our original hypothesis that since they lack terrestrial connections, epiphytic ferns rely wholly on atmospheric- and within-canopy-derived sources of water. Leaf $\delta^{13}\text{C}$ values are closely tied to whole plant water use efficiency (WUE), and plants that use water more efficiently have been shown to exhibit less negative $\delta^{13}\text{C}$ values. Stewart et al. (1995b) report ranges of $\delta^{13}\text{C}$ values of epiphytes between -27 to -34‰ to be typical of those in wet tropical forests. The values for epiphytic ferns in this study were significantly more enriched than those for either hemiepiphytic or terrestrial species (-31.0 ± 1.4 , -34.6 ± 0.3 , and $-33.8 \pm 1.1\text{‰}$, respectively). All species were in the range of those reported for other epiphytes and terrestrial species in tropical forests (Stewart et al. 1995b; Zotz et al. 2001).

In the case of ferns, life form is clearly a robust indicator of carbon isotope discrimination as related to WUE. Hemiepiphytic and terrestrial species were neither significantly different in C isotope discrimination nor in any other trait measured in this study, excluding SLW. This similarity may well relate to the fact that all the mature hemiepiphytic ferns at this site retain a primary root connection with the

soil and reflect water relations and nutrient strategies of strictly terrestrial species.

Total leaf nitrogen was lower for epiphytic than terrestrial species on a mass basis but not significantly different when compared on an area basis (Fig. 2A,B). Differential nitrogen packaging in thicker leaves of the epiphytes could result in a dilution effect when area-based measurements are used. It is possible that differences in nitrogen content may not be due to nutrient poverty in canopy soil organic matters but rather differences in nitrogen packaging and/or faster growth rates in terrestrial than epiphytic species. While we did not measure soil nutrients from our plots, canopy soil organic matter at La Selva is significantly higher in bulk N but lower in plant-available N when compared to terrestrial soils (Cardelús, unpublished data). Thus, it is more likely that our results support the notion of nutrient poverty alone or a combination of poverty and some dilution effect.

The sources and concentration of plant-available nitrogen vary significantly in some forests between canopy and terrestrial substrates (Vance and Nadkarni 1990), which can influence biological processes that occur in these habitats. Variation in leaf $\delta^{15}\text{N}$ signatures can often reflect utilization of different N sources; however, there are several mechanisms that control foliar nitrogen isotope compositions, thus $\delta^{15}\text{N}$ signatures likely reflect a series of integrated fractionation events. Epiphytes with lower $\delta^{15}\text{N}$ signatures are predicted to obtain nitrogen N from depleted atmospheric sources (precipitation, fog), whereas those with more enriched values obtain nitrogen from host tree matter, canopy soil organic matter organic matter, or from N fixation (Bergstrom and Tweedie 1998; Hietz et al. 1999; Stewart et al. 1995a).

Wania et al. (2002) examined the $\delta^{15}\text{N}$ signatures of different potential nutrient sources and compared these to foliar $\delta^{15}\text{N}$ signatures. They found that different potential nitrogen sources for epiphytes had overlapping $\delta^{15}\text{N}$ signatures and argued that simple shifts between sources alone could not explain differences in $\delta^{15}\text{N}$ signatures. Hence, they argue that variation in $\delta^{15}\text{N}$ levels results from both different nitrogen discrimination during nitrogen acquisition as well as differing nitrogen sources. Similar results have been reported by Hietz and Wanek (2003) and Hietz et al. (2002), who show that $\delta^{15}\text{N}$ values of all potential sources in their study were more enriched than expected to explain the depleted values in epiphytes. Thus, assigning nitrogen source from $\delta^{15}\text{N}$ values may be confounded by intrinsic plant physiological differences (Evans 2001). However, the differences between $\delta^{15}\text{N}$ signatures of epiphytic and terrestrial soil-rooted species are clearly due to differences in nitrogen sources.

There have been relatively few measurements made of $\delta^{15}\text{N}$ signatures of potential N sources at La Selva. In an

effort to estimate the contribution of allochthonous (wet and dry atmospheric deposition), and autochthonous (derived from host trees from the soil and eventually captured by the epiphytes as falling leaf litter) N sources of epiphytes, Reich et al. (2003) estimated $\delta^{15}\text{N}$ source signatures available to epiphytes at La Selva. They found that epiphytes relying wholly on atmospheric sources could have $\delta^{15}\text{N}$ values lower than -6‰ . Fresh canopy litter, the precursor of canopy soil organic matter, had an average value of $+2.5$ (± 0.7). Foliar values of epiphytes rooted in CSOM and that did not exhibit atmospheric uptake averaged $+0.9$ (± 1.6). This shift in mean $\delta^{15}\text{N}$ values between epiphytic and terrestrial species (Fig. 2C) further supports the prediction that species of canopy habitats rely much more on atmospheric or within-canopy-derived nitrogen sources, while terrestrial rooted organisms derive nitrogen from a more highly fractionated soil pool.

When all of the data were combined, the discriminate function analysis revealed that nitrogen relations, more than carbon or leaf morphology (SLW), were most important variables when separating the life forms. Radiation of terrestrial ferns into canopy habitats presented the group with a unique set of challenges related to water stress and the alteration of nutrient uptake driven by loss of terrestrial root connections. In spite of the repeated convergence of greater WUE and lower SLW in drought-prone habitats, it appears that a major driving force separating epiphytes from terrestrial species are their nitrogen relations. This is perhaps not surprising given that the nitrogen environment between the two habitats is radically different. The ability to separate functional groups based on life form alone is greatest between epiphytic and terrestrial species. Soil-rooted hemiepiphytic species are functional equivalents to terrestrial species and likely function more as vines when mature.

Our study demonstrates that the natural abundance of both C and N as well as N relations and leaf morphology are useful when segregating different plant life forms at different levels. Nitrogen nutrition as measured by both $\delta^{15}\text{N}$ and leaf N are the most useful when separating epiphytic from terrestrial species. To better understand and separate hemiepiphytes from either terrestrial or epiphytic biology, utilization of additional ecophysiological variables is clearly needed. Such measurements must incorporate both epiphytic and terrestrial stages. Our results indicate that the N and water cycles of epiphytic and terrestrial habitats are largely independent from each other. Epiphytic species derive nutrients and water from either atmospheric sources or intrinsic canopy sources (i.e., canopy soil organic matter composed of recycled epiphyte matter and possibly host tree matter), whereas terrestrial species utilize the terrestrial soil pool that contains a greater degree of enriched N and greater water availability. The convergence of thicker leaves in

epiphytic relative to terrestrial species is likely a combination of drought and reduced nutrient availability in epiphytic habitats. Our results aid in understanding factors that influence the distributions of different fern life forms; however, there is considerable variation in gametophyte ecology of epiphytic and terrestrial ferns (Watkins et al. 2007). Future studies need to incorporate examinations of this phase of the life cycle in order to achieve a comprehensive understanding of fern ecology.

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