



VARIATION IN NITROGEN USE STRATEGIES AND PHOTOSYNTHETIC PATHWAYS AMONG VASCULAR EPIPHYTES IN THE BRAZILIAN CENTRAL AMAZON

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INTRODUCTION

Epiphytes are a conspicuous and characteristic life form in tropical forests throughout the world, accounting for up to 35 percent of the vascular flora in some wet Neotropical forests (Nadkarni 1984). In the Amazon Basin, white - sand vegetations have a canopy that is less dense than the observed in lowland tropical forest, allowing more light to diffuse into the lower extracts. This feature permits a great occurrence of diverse epiphytic groups, including many vascular epiphytes (Takeuchi 1960). Epiphytes and associated dead organic matter constitute a considerable part of the above - ground biomass and nutrient pools in these systems, playing an important role in forest primary production and nutrient cycling (Nadkarni 1984).

However, epiphytic habit implies some physiological constraints as the demand for water and nutrients is often not buffered by layers of soil, as observed in ground - rooted plants (Nadkarni and Matelson 1992, Wania *et al.*, 2002). Beyond this, epiphytes are exposed to a higher light condition, greater extremes of temperature and relative humidity than forest understory vegetation (Ingram and Nadkarni 1993). In order to surpass those limiting environmental factors and live under such exposed and drier habitats, epiphytes have evolved many morphological and physiological strategies to better tap these sources (Benzing 1990) and to deal with water loss (Zotz and Ziegler 1997; Hietz *et al.*, 1999).

The use of the natural abundance of stable light isotopes has been widely used in ecosystem and plant ecology research. While the natural abundance of ¹⁵N is a useful indicator of the sources and pathways of nitrogen (Hogberg 1997), the $\delta^{13}\text{C}$ values of leaves are widely used to identify the pathway of photosynthesis and to estimate the water - use efficiency (WUE) of plants, reflecting the interplay among all aspects of plant carbon and water relations (Holtum and Winter 2005). Previous studies have pointed differences in foliar $\delta^{15}\text{N}$ values of epiphytes and their host trees (Stewart

et al., 1995), as well as among epiphytic groups according to the differential use of N sources. Differences in epiphyte $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were also observed when comparing systematic and ecological groups of epiphytes along an altitudinal gradient (Hietz *et al.*, 1999) and along tree canopy (Wania *et al.*, 2002). Epiphyte $\delta^{15}\text{N}$ N variation was not only attributed to differences on N - source use by epiphytes of different strata but also to differences on isotope discrimination during N acquisition and intraplant variation; while differences in $\delta^{13}\text{C}$ were related to the lower water availability and/or light incidence experienced by epiphytes of higher strata.

OBJECTIVES

In this study, we measured the foliar content of nitrogen and the relative abundances of ¹³C and ¹⁵N ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of different systematic groups of vascular epiphytes (Araceae, Bromeliaceae and Orchidaceae) of a white - sand vegetation in Central Brazilian Amazon in order to test if epiphytes from different families had distinct strategies related to N use and photosynthetic pathways. We also wanted to look for variation within families, as well as for differences on the nutritional status between epiphytes and host tree species.

MATERIAL AND METHODS

2.1 - Study site

The study was carried out at the *Reserva Biológica de Campina*-INPA, situated 60 km north of the city of Manaus, AM, Brazil (02° 35'S, 60° 02'W). The Reserve covers an area of 900 ha and is formed by three main vegetation types: *campina* (dense sclerophyllous shrub, 4-10 - m high, generally forming a sparse cover over bare sand), *campinarana* (dense sclerophyllous forest, with trees 10-20 - m high) and dense *terra - firme* (lowland tropical forest). *Campina* and

campinarana grows in highly weathered sandy soils (Hydromorphic Spodosols). Climate is tropical, with mean annual temperature of 26°C and air humidity ranging from 85 - 88%. The annual precipitation in the region averages 2200–2400 mm, with 2 - 3 months with less than 100 mm of rainfall (Sombroek 2001).

2.2 - Plant sampling

Plant material was sampled during the rainy season (April 2006). We established four 200 - m long to 10 - m wide transects, ranging from open *campina* to *campinarana* vegetation. In each transect, we sampled trees of the seven most frequent species occurring at both *campina* and *campinarana* vegetation: *Aldina heterophylla* Spruce ex Benth. (Leguminosae; sub - family Papilionoidae), *Clusia nemorosa* G. Mey (Clusiaceae), *Matayba opaca* Radlk. (Sapindaceae), *Ouratea spruceana* (Mart.) Engl. (Ochnaceae), *Pagamea duckei* Standley (Rubiaceae), *Pradosia schomburgkiana* (A. DC.) Cronq. subsp. *schomburgkiana* (Sapotaceae), and *Protium heptaphyllum* March. (Burseraceae), summing up 52 trees sampled. We only sampled trees where epiphytes were present. From each tree, we sampled the epiphytes of the families Araceae, Bromeliaceae and Orchidaceae, a total of 66 individuals. For the determination of nitrogen and carbon concentration and their isotopic composition, we collected four to five leaves of each epiphyte and around 10 leaves from its host tree. All samples were constituted of healthy fully expanded leaves.

2.3 - Data analyses

Leaf samples were oven - dried at 65°C until a constant weight was obtained and ground to a fine powder. Sub samples of 1 - 2 mg of organic ground material were sealed in tin capsules and combusted in a Carla Erba elemental analyzer (Milan, Italy) for determination of N and C concentrations. The gas generated from the combustion was purified in a gas chromatographic column and passed directly to the inlet of a gas isotope ratio mass spectrometer (IRMS Delta Plus; Finnigan Mat, San Jose, California, USA). Internal standard (Atropine) was included in every run. From these analyses, we obtained both the nitrogen and carbon isotope ratios ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively) and elemental concentrations (%N and %C). Stable isotope ratios are expressed in a parts - per - thousand (extperthousand) in “delta” notation: $\delta^{15}\text{N}$ or $\delta^{13}\text{C} = (\text{Rsample}/\text{Rstandard} - 1) \times 1000$; where Rsample and Rstandard are the ratios of the heavy to the light isotope in the samples and the standard. The international standards for N and C are the atmospheric air and PDB, respectively.

2.4 - Data processing

We compared the epiphytes belonging to each of the three families in relation to C and N contents, and respective C:N ratios, as well as their N and C isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). The same analysis was made among *taxa* of each epiphyte family, in order to check variations within the families sampled. We also made comparisons between individuals of *A. heterophylla*, the tree species with the highest number of individuals and from where the majority of epiphytes were sampled (including aroids, that were absent in other tree canopies), and its dwellers. We tested data distribution using the Kolmogorov - Smirnov one - sample test. Because some data did not follow normal distribution,

the analyses were performed using non - parametric tests. Differences among each epiphyte family and epiphytes and their host tree were tested using a Kruskal - Wallis test to determine statistically significant differences. All statistical analyses were performed using the software STATISTICA, version 6.1 for Windows (StatSoft, Inc. 2004). A probability level of 0.05 was used as a critical level of significance in all tests.

RESULTS AND DISCUSSION

The tree *A. heterophylla* had a higher foliar N concentration ($P < 0.05$) than the aroids, the bromeliads, and the orchids. Consequently, the host had significantly lower C:N ratio ($P < 0.05$) than its dwellers. The tree had similar $\delta^{15}\text{N}$ values to the aroid and the bromeliad dwellers whereas the orchid dwellers had significantly more depleted signatures ($P < 0.05$). Regarding to $\delta^{13}\text{C}$ values, we noticed that the tree and its aroid dwellers had similar signatures while the bromeliad and the orchid dwellers had less depleted values ($P < 0.05$) than their host. Normally epiphytes have more ^{15}N - depleted signatures than their host trees, as a consequence of the lack of access to soil N sources and the acquisition of more ^{15}N - depleted N sources than those acquired by rooted plants (Stewart *et al.*, 1995; Högborg 1997; Hietz *et al.*, 2002). However, differently from previous observations, only orchids had a more negative ^{15}N signatures than trees. This lack of difference may probably be related to N dynamic of white - sand vegetations which are known to be N - poor ecosystems and to have an efficient use of the available N sources (Luizão *et al.*, 2007a, b), having significantly depleted foliar $\delta^{15}\text{N}$ signatures compared to dense *terra - firme* forests (Ometto *et al.*, 2006; Nardoto *et al.*, 2008). Araceae species had the highest foliar nitrogen concentration and the lowest C:N ratio ($P < 0.05$), while those from Bromeliaceae and Orchidaceae had similar average foliar nitrogen concentration and C:N ratio. The orchids had the most depleted $\delta^{15}\text{N}$ values when compared to the aroids and the bromeliads ($P < 0.05$). When making comparisons within each family, we noticed that aroids' and orchids' taxa differed in relation to foliar nitrogen concentrations and C:N ratios ($P < 0.05$), while no internal variation was detected within bromeliads. The differences in N patterns observed between the epiphyte families might be influenced by the variation on the availability of N - source (Gebauer and Meyer 2003, Inselsbacher *et al.*, 2007), as well as by epiphyte microhabitat within canopy (Hietz *et al.*, 2002). As canopy organic matter mostly accumulates over thicker branches, a gradient of nutrient supply from thicker to thinner branches may be expected (Wania *et al.*, 2002). Consequently, epiphytes on thicker branches, such as the aroids here sampled, improve their N supply by accessing more available N sources than epiphytes on thinner ones, such as orchids. However, aroids and bromeliads had similar $\delta^{15}\text{N}$, yet they have distinct life forms. Aroid and bromeliad dwellers have distinct life forms. While the earlier are rooted on canopy soil, the bromeliads obtain their nutrition from water and debris accumulated within their impounding shoots (Bezing 1990, Inselsbacher *et al.*, 2007). The initial discrimination against ^{15}N - enriched nitrogen compounds dur-

ing microbial decomposition of accumulated canopy litter could lead to ^{15}N - enrichment of nitrogen sources inside tank water (Hietz & Wanek 2003). In contrast, nitrogen compounds derived from rainwater usually have negative $\delta^{15}\text{N}$ values (Clarck & Nadkarni 1990, Fukuzaki & Hayasaka 2009). Based on our results, we are not able to quantify the contribution of these sources for the bromeliad nitrogen nutrition. According to related literature, it is likely that the bromeliads may rely on the mineralization of canopy litter within tank shoots as a major source of nitrogen (Clark & Nadkarni 1990, Benzing 1990, Inselsbacher *et al.*, 2007).

As a consequence, they only have access to the nutrients in the water running over the surface (Hietz *et al.*, 1999), and from atmospheric deposition, sources that are proportionally more ^{15}N - depleted (< -3 extperthousand) (Benzing 2000 Fukuzaki & Hayasaka 2009). Moreover, lowland rainforest - orchids are commonly associated with mycorrhiza (Lesica & Antibus 1990). This association enable a more efficient water and nutrient assimilation (Wania *et al.*, 2002, Geabuer & Meyer 2003, Midgley *et al.*, 2005), although symbionts deliver N compounds isotopically depleted (Högberg 1997). Thus, the isotopic signatures up to 2 extperthousand more depleted found on orchids may reflect a high reliance on nitrogen sources derived from atmospheric deposition and symbiotic association.

In relation to $\delta^{13}\text{C}$ signatures, we observed that, except for the aroids and one orchid genus (*Encyclia*), the majority of epiphytes sampled exhibit CAM - photosynthetic pathway. It is common that a large number of vascular epiphytes use the water - conserving CAM - pathway of photosynthesis to survive to such drought and light - exposure conditions (Benzing 1990; Medina *et al.*, 1977), as observed in this study. The CO_2 - concentrating strategy of the CAM photosynthetic pathway (Hietz *et al.*, 1999) allows these plants to have lower transpiration rates and higher water use efficiency (WUE) than C_3 - and C_4 - plants under comparable conditions (Zotz & Winter 1994, Cushman 2001). CAM expression greatly varies within epiphyte groups, and internal variations are related to variation of environmental conditions (i.e., air humidity, light exposure) (Hietz *et al.*, 1999).

CONCLUSION

The values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ observed herein indicate that epiphytes develop many strategies to face the limiting conditions of their environment. Epiphyte $\delta^{15}\text{N}$ values showed the variation within families in relation to N resource used by these plants, being this source related with habitat quality and epiphyte life form. Orchid's $\delta^{15}\text{N}$ indicates the use of depleted N sources from precipitation, as well as the use of source derived from symbiotic associations. The $\delta^{15}\text{N}$ values of aroid and bromeliad dwellers indicate that, despite having distinct life forms, these groups may access similar N sources. The N derived from the decomposition of organic matter in canopy soil, as well as within tanks seems to be the major source for these two epiphytic groups. Regarding the photosynthetic pathway, $\delta^{13}\text{C}$ showed that both CAM and C_3 - pathways are present among these epiphytes.

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