Annals of Botany **112**: 919–926, 2013
doi:10.1093/aob/mct147, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

Mutualistic ants contribute to tank-bromeliad nutrition

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Received: 5 March 2013 Returned for revision: 16 April 2013 Accepted: 3 May 2013 Published electronically: 16 July 2013

- *Background and Aims* Epiphytism imposes physiological constraints resulting from the lack of access to the nutrient sources available to ground-rooted plants. A conspicuous adaptation in response to that lack is the phytotelm (plant-held waters) of tank-bromeliad species that are often nutrient-rich. Associations with terrestrial invertebrates also result in higher plant nutrient acquisition. Assuming that tank-bromeliads rely on reservoir-assisted nutrition, it was hypothesized that the dual association with mutualistic ants and the phytotelm food web provides greater nutritional benefits to the plant compared with those bromeliads involved in only one of these two associations.
- *Methods* Quantitative (water volume, amount of fine particulate organic matter, predator/prey ratio, algal density) and qualitative variables (ant-association and photosynthetic pathways) were compared for eight tank- and one tankless-bromeliad morphospecies from French Guiana. An analysis was also made of which of these variables affect nitrogen acquisition (leaf N and δ^{15}N).
- *Key Results* All variables were significantly different between tank-bromeliad species. Leaf N concentrations and leaf δ^{15}N were both positively correlated with the presence of mutualistic ants. The amount of fine particulate organic matter and predator/prey ratio had a positive and negative effect on leaf δ^{15}N, respectively. Water volume was positively correlated with leaf N concentration whereas algal density was negatively correlated. Finally, the photosynthetic pathway (C_3 vs. CAM) was positively correlated with leaf N concentration with a slightly higher N concentration for C_3-Tillandsioideae compared with CAM-Bromelioideae.
- *Conclusions* The study suggests that some of the differences in N nutrition between bromeliad species can be explained by the presence of mutualistic ants. From a nutritional standpoint, it is more advantageous for a bromeliad to use myrmecotrophy via its roots than to use carnivory via its tank. The results highlight a gap in our knowledge of the reciprocal interactions between bromeliads and the various trophic levels (from bacteria to large metazoan predators) that intervene in reservoir-assisted nutrition.

Key words: Algae, ants, Bromeliaceae, δ^{15}N, food webs, French Guiana, Formicinae, mutualistic interactions, nitrogen, phytotelmata, stable isotopes, tank bromeliad.

INTRODUCTION

Epiphytes are keystone species in tropical rainforests because they provide food and/or habitat resources to different organisms not found elsewhere and because they play a major role in the nutrient cycles in canopy ecosystems (Coxson and Nadkarni, 1995). However, epiphytism imposes physiological constraints resulting from the lack of access to the nutrient sources available to ground-rooted plants. Hence, many epiphytes are characterized by morphological and functional adaptations – such as litter-trapping leaf arrangements (i.e. *Asplenium* 'trash-baskets'); rainwater retention (e.g. tank-forming bromeliads); absorbent leaf trichomes (i.e. *Tillandsia* spp. bromeliads); velamen radicum in aerial roots (i.e. Orchidaceae); and slippery, waxy walls (e.g. insectivorous pitfall plants such as *Brocchinia reducta* and *Catopsis berteroniana*) – that facilitate access to nutrient acquisition (Benzing, 1990; Lüttge, 2008). In addition, many epiphytes are involved in complex associations with animals, particularly ants, that provide them with nutritional benefits (Janzen, 1974; Huxley, 1978; Beattie, 1989; Gay, 1993; Treseder *et al.*, 1995; Watkins *et al.*, 2008; Leroy *et al.*, 2012). One may thus expect that multiple associations with animals would result in higher nutrient acquisition compared with those with fewer interactions either through *direct* (i.e. animal mediated) or *indirect* (i.e. plant-trait mediated) interactions. This question is highly relevant to broadening our understanding of the mechanisms that foster biological diversity in the species-rich Tropics where plant–animal interactions are pervasive (Vazquez *et al.*, 2009).

Plants of the family Bromeliaceae, possessing both CAM and C_3 photosynthetic pathways, dominate the vascular flora in Neotropical forests (Benzing, 1990) and most of them (i.e. all of the members of the Bromelioideae and Tillandsioideae subfamilies) absorb water and nutrients through specialized leaf trichomes (Benzing, 1976). According to Nadkarni and Primack (1989) and Winkler and Zotz (2009), their mechanical roots are used to maintain the plant's position and do not play a significant role in plant nutrition. A conspicuous adaptation to improve nutrient acquisition by bromeliads is the phytotelm ('plant-held water'). Bromeliad leaves are often tightly interlocking and form rosettes, creating tanks that collect rainwater and debris (Benzing, 2000; Kitching, 2000). These tanks provide a habitat for specialized aquatic organisms, the so-called inquilines. Most major taxa are involved, including bacteria, algae, prokaryotes, protists, micro- and macro-invertebrates, and vertebrates (Frank and Lounibos, 1983; Richardson, 1999; Carrias *et al.*, 2001; Brouard *et al.*, 2012; Dunthorn *et al.*, 2012). The detritus that enter the tank (mostly leaf litter) constitutes the main source of nutrients for the aquatic food web. Invertebrate chewers reduce the incoming litter. Nitrogen and other nutrients are then made available to the plant through the bacterial decomposition of the small detritus and faecal pellets of aquatic metazoans (Ngai and Srivastava, 2006). In sun-exposed areas, algae can grow in the phytotelm. They may then represent a higher trophic resource than leaf litter (McNeely *et al.*, 2007); while constituting an important food source for filter-feeding invertebrates, algae may also compete with the plant for nitrogen (Brouard *et al.*, 2011). Other *direct* interactions with the terrestrial or amphibious animals inhabiting bromeliads may also constitute an important source of nutrients for tank-forming bromeliads. For example, bromeliad-associated spiders (Romero *et al.*, 2006, 2008; Gonçalves *et al.*, 2011) and treefrogs (Romero *et al.*, 2010) release faeces that are washed into the plant's pools and collect at the leaf bases where they provide a source of nutrients for aquatic decomposers and for the bromeliad itself. In summary, tank-bromeliads can be considered 'assisted saprophytes' (Benzing, 2000).

Recent studies have highlighted much more complex, *indirect* interactions between bromeliads and terrestrial animals. Mutualistic ants influence the vegetative traits of their associated bromeliads by determining the distribution of seedlings along gradients of incident light, thereby affecting the taxonomic composition and complexity of the aquatic food web contained in the phytotelmata, and, subsequently, the nitrogen flux to the plant's leaves (Leroy *et al.*, 2009). Assuming that tank-bromeliads rely on reservoir-assisted nutrition, we hypothesized that the dual association with mutualistic ants and the phytotelm food web provides greater nutritional benefits to the plant compared with those bromeliads involved in only one of the two associations (i.e. ants only or phytotelm food web only).

To test this hypothesis, we assessed nutrient stress and nitrogen sources using leaf N concentrations and leaf δ^{15}N, respectively. We then analysed the relationship between leaf δ^{15}N values and nitrogen concentrations with quantitative (i.e. water volume, amount of fine particulate organic matter, predator/prey ratio and algal density) and qualitative (i.e. association with ants and photosynthetic pathways) variables for eight tank- and one tankless-bromeliad morphospecies associated with ants and/or phytotelm food webs.

MATERIALS AND METHODS

Study site

This study was conducted in French Guiana at two sites: (1) the Nouragues Natural Reserve in the primary rainforest around the Nouragues research station (04 °05′16·4″N, 52 °40′49·3″W, elevation 100 m a.s.l.), and (2) the primary rainforest and pioneer growths near the Petit Saut field station, Sinnamary (05 °03′30·0″N, 52 °58′34·6″W, elevation 80 m a.s.l.). In both areas, the climate is tropical moist, with 3000/3500 mm of annual precipitation distributed over 280 d. There is a major drop in rainfall between September and November (dry season) and another shorter and more irregular dry period in March. The maximum monthly temperatures average 33·5 °C and the monthly minimum is 20·3 °C.

Bromeliad species

We sampled eight tank-bromeliad species spanning a broad range of environmental conditions at the two sites (Table 1). Around the Nouragues station, *Guzmania lingulata* Mez, *Vriesea pleiosticha* (Griseb.) Gouda and *Aechmea bromeliifolia* Baker are situated in the rainforest understorey at 130 m a.s.l., while *Catopsis berteroniana* (Schultes f.) Mez and *Aechmea aquilega* (Salisb.) Griseb. are restricted to the inselberg (420 m a.s.l.; above the tree-line). Near the Petit Saut station, *Vriesea splendens* (Brongn.) Lem. grows in the understorey at 60 m a.s.l., while *Aechmea mertensii* Schult.f. (Rudge) Baker is found in pioneer growths. Additionally, we also sampled *Streptocalyx longifolius* (Rudge) Baker, a tankless species found in pioneer growths.

While *G. lingulata*, *V. pleiosticha*, *A. bromeliifolia*, *C. berteroniana*, *A. aquilega* and *V. splendens* are typical tank-forming species never associated with ants, *A. mertensii* (tank-forming) and *S. longifolius* (tankless) are obligate ant-garden (AG) bromeliads (Benzing, 2000). AGs are initiated by ants that build arboreal carton nests containing organic material. The ants collect the seeds of selected epiphyte species and incorporate them into the organic material where they then germinate and grow, so that the plant's intertwining roots stabilize the carton walls of the nest and anchor the entire structure to the supporting tree. The full-grown epiphyte provides food rewards to the ants and, in turn, the plants benefit from seed dispersal and protection from defoliating insects (Orivel and Leroy, 2011). In French Guiana, *A. mertensii* only occurs in AGs initiated either by the ants *Camponotus femoratus* Fabr. or *Pachycondyla goeldii* Forel. These two ant species are generalist predators that also feed on extrafloral nectar and homopteran honeydew (Davidson, 1988; Orivel *et al.*, 2000). Ant-mediated dispersion results in two *A. mertensii* morphotypes in relation to incident radiation. Whereas *C. femoratus*-associated *A. mertensii* (i.e. AGs installed in shady areas) have a funnel-like, crateriform shape, those associated with *P. goeldii* (i.e. AGs in sun-exposed areas) have an amphoral, bulbous shape (Leroy *et al.*, 2009; Orivel and Leroy, 2011). Finally, the tankless *S. longifolius* occurs in *C. femoratus*-associated AGs.

All of these bromeliad species belong to obligate C_3 and Crassulacean Acid Metabolism (CAM) photosynthetic pathways (see Table 1). While all of the *Aechmea* sp. morphospecies and *S. longifolius* are CAM-Bromelioideae, the other bromeliads studied are C_3-Tillandsioideae. Because a small number of studies have highlighted a peculiar link between nitrogen metabolism and the CAM photosynthetic pathway in bromeliads (Nievola *et al.*, 2001; Freschi *et al.*, 2010) we assessed its potential influence on leaf N concentrations and leaf δ^{15}N.

Sampling

We sampled only mature bromeliads near the flowering stage of the plant life cycle to avoid bias resulting from ontogenetic

TABLE 1. *Main characteristics of the eight tank-bromeliad morphospecies sampled*

	CAM-Bromelioideae				C_3-Tillandsioideae			
	AA	AB	AM_CF	AM_PG	CB	GL	VP	VS
Ants	No	No	Yes	Yes	No	No	No	No
n	31	26	31	32	29	19	30	26
Height***	$31{\cdot}4 \pm 1{\cdot}5^{a}$	$12{\cdot}9 \pm 1{\cdot}5^{bc}$	$19{\cdot}0 \pm 0{\cdot}8^{d}$	$11{\cdot}6 \pm 0{\cdot}5^{b}$	$21{\cdot}5 \pm 0{\cdot}6^{e}$	$7{\cdot}3 \pm 0{\cdot}4^{f}$	$19{\cdot}2 \pm 3{\cdot}7^{cd}$	$11{\cdot}7 \pm 0{\cdot}6^{b}$
Diam***	$9{\cdot}5 \pm 0{\cdot}5^{a}$	$5{\cdot}8 \pm 0{\cdot}5^{b}$	$6{\cdot}4 \pm 0{\cdot}3^{b}$	$4{\cdot}5 \pm 0{\cdot}2^{c}$	$7{\cdot}1 \pm 0{\cdot}1^{d}$	$2{\cdot}8 \pm 0{\cdot}2^{e}$	$11{\cdot}1 \pm 1{\cdot}7^{a}$	$11{\cdot}3 \pm 0{\cdot}3^{a}$
IR***	$66{\cdot}9 \pm 2{\cdot}8^{a}$	$25{\cdot}7 \pm 0{\cdot}4^{b}$	$25{\cdot}1 \pm 1{\cdot}7^{b}$	$53{\cdot}1 \pm 3{\cdot}4^{c}$	$73{\cdot}3 \pm 2{\cdot}4^{a}$	$15{\cdot}9 \pm 0{\cdot}6^{d}$	$25{\cdot}1 \pm 0{\cdot}3^{b}$	$18{\cdot}7 \pm 0{\cdot}4^{e}$

Shown are photosynthetic pathway (CAM vs. C_3), number of plants studied for each bromeliad species (*n*), mean (± SE) tank height (cm), tank diameter (Diam, cm) and incident radiation (IR, %). Bromeliad species (ID): AA, *Aechmea aquilega*; AB, *Aechmea bromeliifolia*; AM_CF, *Aechmea mertensii* associated with *Camponotus femoratus*; AM_PG, *Aechmea mertensii* associated with *Pachycondyla goeldii*; CB, *Catopsis berteroniana*; GL, *Guzmania lingulata*; VP, *Vriesea pleiosticha*; VS, *Vriesea splendens*.

Asterisks indicate significant differences after the Kruskall-Wallis test (***$P < 0{\cdot}001$). Values marked with the same letter are not significantly different (Bonferroni corrected Mann–Whitney pairwise comparisons, $P < 0{\cdot}05$).

gradients. Sampling was carried out in April 2008 at the Nouragues research station and in March 2009 and 2010 at Petit Saut. There were no significant inter-annual differences in precipitation during these sampling periods (Kruskal–Wallis test: $H = 1{\cdot}708$, $P = 0{\cdot}425$). Moreover, to prevent any climatic effect on water volume, we sampled tank-bromeliads that were already full of water. We selected 19–32 plants for each of the eight tank-bromeliad species and five *S. longifolius* individuals (Table 1).

For each bromeliad, tank height and diameter (two successive 90 ° measurements) were recorded. We used a non-destructive sampling technique to extract the water and aquatic biota contained in the plants because the bromeliads could not be removed from their host trees either for legal reasons (the Nouragues station is located in a protected area) or because they were entirely embedded in the AGs. The percentages of total incident radiation above the bromeliads were calculated using hemispherical photographs. Photographs were taken near dusk to avoid direct sunlight, and from three to four positions per AG. We used a height-adjustable tripod and a digital camera (Nikon Coolpix 4500) equipped with a Nikon Fisheye converter lens (FC-E8 0·21X) that provides a 180 ° canopy view. We analysed the images using Gap Light Analyzer (GLA) 2·0 image processing software to calculate the percentage of total incident radiation (Frazer *et al.*, 1999). The main characteristics of the eight bromeliads studied are provided in Table 1.

Water samples were collected with a 10-mL micropipette with the end trimmed to widen the orifice (Jocqué *et al.*, 2010) and the volume extracted (WV, mL) from each bromeliad was measured using a graduated cylinder. The amount of fine particulate organic matter (FPOM; 1000–0·45 mm in size) was expressed as preserved volume (mm^3 after decantation in graduated test-tubes; see also Paradise, 2004). The samples were preserved in the field in 4 % formalin (final concentration). Aquatic invertebrates were sorted in the laboratory, identified to genus, species or morphospecies by a professional taxonomist (Dr A. G. B. Thomas, University of Toulouse 3, France), and enumerated (see species lists in Jabiol *et al.*, 2009; Céréghino *et al.*, 2011). Invertebrate morphospecies were partitioned into predators (i.e. carnivorous species which attack and consume live prey organisms) and prey (here, species which sift fine particulates from the water column and/or gather FPOM from the accumulated debris), and these categories were used to calculate predator/prey richness ratios (number of predatory taxa/number of prey taxa; hereafter, 'PPR'). The PPRs are one of the general patterns used to quantify trophic structure with a view to understanding the functioning of food webs (Warren and Gaston, 1992).

For each water sample collected, subsamples (1–5 mL) taken to count algae were stained with primulin, collected onto 0·8-μm pore-size black Nuclepore filters, mixed with oil and mounted between a glass slide and a cover slip and then stored at −20 °C. The slides were examined at ×1100 magnification using a Leica DC 300F epifluorescence microscope. Algae were identified by shape under UV light (340–380 nm) excitation and by detecting the red autofluorescence of chlorophyll a under blue light (450–490nm) excitation. A minimum of 200 cells per slide was counted and the densities expressed in cells mL^{-1}.

Nitrogen concentration and nitrogen stable isotope analyses

Leaf $\delta^{15}N$ is a useful indicator of the sources of N (Dawson *et al.*, 2002) and the nitrogen isotope ratios of consumers are typically 3–5 ‰ enriched in ^{15}N relative to their diets (DeNiro and Epstein, 1981). We thus hypothesized that a higher leaf $\delta^{15}N$ would show that the source of a great part of the nitrogen present came from the faeces of invertebrates released into the water and/or ants.

The median portion of one mature leaf, the fourth or fifth leaf from the centre of the rosette, was collected from five individuals for each bromeliad species. All of the samples collected were carefully cleaned and then vacuum-dried and ground into a homogeneous powder using a mixer mill. Plant samples (~1 g) were analysed for their $\delta^{15}N$ and N concentrations. Stable isotope analyses were conducted at the Stable Isotopes in Nature Laboratory (University of New Brunswick, Canada) using a Finnigan Delta^{Plus} gas isotope-ratio mass spectrometer interfaced with a Carlo Erba NC2500 elemental analyser. The natural abundance of ^{15}N was calculated as follows:

$$\delta^{15}N(‰) = [(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{standard} - 1)] \times 1000$$

where $(^{15}N/^{14}N)_{sample}$ is the N isotope ratio of the samples, and $(^{15}N/^{14}N)_{standard}$ is the N isotope ratio of the standard material (DeNiro and Epstein, 1978). The standard for stable N isotopes is atmospheric molecular.

Data analysis

To analyse the relationship between (1) nutrient stress (i.e. leaf N concentration) and (2) the nitrogen sources (i.e. leaf $\delta^{15}N$) and the explanatory variables, we used generalized linear modelling (GLM) on a sub-sample of five bromeliads per species. Since four variables were quantitative (i.e. WV, FPOM, PPR and algae abundance) and two were qualitative (i.e. ant-association and photosynthetic pathway), we performed an ANCOVA to analyse the overall effects of these explanatory variables on nitrogen concentrations and stable nitrogen isotopes. All three qualitative variables were transformed into explanatory dummy variables using the GLM function in R software V. 2·15·2 (R Development Core Team, 2011). All quantitative variables were rank-transformed to fit a normal distribution (Aulchenko *et al.*, 2007) and only simple effects were assessed (no interaction terms). We performed a stepwise backward removal procedure to select models based on the Akaike Information Criterion (AIC) of the full models (i.e. models considering all explanatory variables) for each dependent variable. Only models with the lowest AICs were presented. Departures from homoscedasticity and the normality of the residual errors were evaluated with a Shapiro–Wilk normality test and a Bartlett test of homogeneity of variances. For both models (leaf N concentration and leaf δ^{15}N), the *P*-values were non-significant, indicating normality and the homogeneity of the variance. Statistical analyses were evaluated under a 95 % confidence level and were conducted using R software.

Preliminary tests showed that most of the variables were not normally distributed (Shapiro Wilk's test) even after transformation. Thus, the non-parametric Kruskal–Wallis test was used followed by a Bonferroni corrected Mann–Whitney pairwise comparison to test differences in the biotic and abiotic parameters between bromeliad species. These statistical analyses were conducted using PAST v.2·17 software (Hammer *et al.*, 2001).

RESULTS

Model selection and influence of the biotic and abiotic variables on bromeliad nutrition

Models with the lowest AICs are presented in Table 2. Stable nitrogen isotopes and N concentrations were both positively correlated with the presence of ants ($P = 0{\cdot}0008$ and $P < 0{\cdot}0001$, respectively). Leaf δ^{15}N values were positively correlated with FPOM ($P = 0{\cdot}0002$), whereas no significant negative correlation was found with PPR ($P = 0{\cdot}113$). Nitrogen concentration was positively and significantly correlated with WV ($P = 0{\cdot}0237$) and bromeliad photosynthetic pathway ($P = 0{\cdot}0005$). Leaf N concentration was slightly higher for C_3-Tillandsioideae than for CAM-Bromelioideae (0·77 ± 0·03 and 0·69 ± 0·04 %, respectively). Finally, leaf N was negatively and significantly correlated with algal density ($P = 0{\cdot}0073$).

Biotic and abiotic characteristics of the bromeliad species

Water volume and FPOM differed significantly (Kruskal–Wallis tests: $H = 133{\cdot}2$, $P < 0{\cdot}001$ and $H = 123{\cdot}7$, $P < 0{\cdot}001$, respectively) between bromeliad species (Table 3). The highest WV was found in *A. aquilega* tanks and was significantly different from the WV of all other bromeliad species (Mann–Whitney pairwise comparisons, $P < 0{\cdot}05$). The tanks of *A. bromeliifolia* and *C. femoratus*-associated *A. mertensii* had the second highest WV (Table 3). *Aechmea aquilega* and *C. femoratus*-associated *A. mertensii* tanks collected the most FPOM compared with the other bromeliad species, whereas *C. berteroniana* had the lowest values (Mann–Whitney pairwise comparisons, $P < 0{\cdot}05$). Nevertheless, besides the small amount of FPOM, *C. berteroniana* tanks contained an average of 21·2 ± 17·3 mg dry mass of insect remains (mainly ants). There were no insect remains or they were negligible in the other bromeliad species.

PPRs differed significantly between bromeliads (Kruskal–Wallis tests: $H = 94{\cdot}08$, $P < 0{\cdot}001$). These ratios were higher for *C. femoratus*-associated *A. mertensii*, but not significantly different than for *A. aquilega*, *P. goeldii*-associated *A. mertensii* and *C. berterniana* (Mann–Whitney pairwise comparisons, $P > 0{\cdot}05$). The four other bromeliad species were characterized by a very low proportion of predators (or even no predators for *G. lingulata*).

Algae were found in all eight tank-bromeliad species and their densities were significantly different between bromeliad taxa (Kruskal–Wallis test: $H = 93{\cdot}97$, $P < 0{\cdot}001$). The highest values of algal densities were found in *A. aquilega* compared with the other bromeliad species (Mann–Whitney pairwise comparisons, $P < 0{\cdot}05$). *Catopsis berteroniana* and *C. femoratus*-associated *A. mertensii* were characterized by high algal densities, whereas very low values were recorded for all of the other bromeliad species. Moreover, we found that algal density was related to incident radiation (Spearman correlation, $r = 0{\cdot}48$, $P < 0{\cdot}001$).

Leaf δ^{15}N and leaf N were significantly different between bromeliad species (Kruskal–Wallis tests: $H = 51{\cdot}96$, $P < 0{\cdot}001$ and $H = 26{\cdot}01$, $P = 0{\cdot}001$, respectively). The total leaf nitrogen concentration was below 1 % for all of the bromeliad species and the leaf δ^{15}N ranged from −1·5 to +2 ‰ (Table 3).

Camponotus femoratus-associated *A. mertensii* and *C. berteroniana* were characterized by the highest and the lowest leaf δ^{15}N (1·73 ± 0·18 vs. −1·05 ± 0·36 ‰, respectively) compared with all of the bromeliad species (Mann–Whitney pairwise comparisons, $P < 0{\cdot}05$). Leaf δ^{15}N values for the tankless species *S. longifolius* (−0·41 ± 0·33 ‰) were intermediate and significantly different from those recorded for *C. femoratus*-associated *A. mertensii* ($P = 0{\cdot}012$). Leaf nitrogen concentration was the lowest for *A. aquilega* whereas *C. femoratus*-associated *A. mertensii* and *V. pleiosticha* were characterized by the highest amounts of nitrogen (Mann–Whitney pairwise comparisons, $P < 0{\cdot}05$). Leaf N values for *S. longifolius* were also intermediate (0·78 ± 0·10 %) and significantly different from the values for *A. aquilega* ($P = 0{\cdot}036$).

DISCUSSION

This study sheds new light on the roles played by biotic and abiotic variables in nitrogen acquisition (nutrient stress and nitrogen sources) by tank-bromeliads. Among epiphytes, bromeliads are characterized by one of the lowest leaf N concentrations with values below 1 % dry mass (Zotz and Hietz, 2001; Cardelus and Mack, 2010; this study), whereas values range from 1 to 5 % in other families (Hietz *et al.*, 1999; Lorenzo *et al.*, 2010). The low N concentration for bromeliad species could be due to lower N requirements because they use N more efficiently than other plant species (Cardelus and Mack, 2010). Our model shows that the photosynthetic pathway is significantly correlated

TABLE 2. *Models assessing the influence of biotic and abiotic variables on the natural abundance of leaf* δ^{15}*N (‰) and leaf nitrogen concentration (N, %) for the nine bromeliad morphospecies* ($n = 5$ *for all species)*

Fixed effects	Estimate ± s.e.	*t*-value	d.f.	*P*	AIC (full model)
Leaf δ^{15}N					114·59 (121·24)
Intercept	−1·268 ± 0·37	−3·419	41	0·0014	
Slope					
FPOM	0·563 ± 0·14	4·151		0·0002	
PPR	−0·239 ± 0·15	−1·621		0·113	
Ants	0·962 ± 0·26	3·635		0·0008	
Leaf N					113·78 (118·7)
Intercept	−5·067 ± 1·14	−4·429	40	<0·0001	
Slope					
WV	0·473 ± 0·24	1·928		0·0614	
Algae	−0·51 ± 0·18	−2·826		0·0073	
Ants	2·371 ± 0·53	4·513		<0·0001	
Metabolism	2·024 ± 0·54	3·766		0·0005	

Algae, algal abundance; Ant, presence of associated ants; FPOM, fine particulate organic matter (mL); metabolism, C_3 vs. CAM photosynthetic pathway; PPR, number of predatory taxa/number of prey taxa; WV, water volume (mL).

to nitrogen concentration, suggesting that CAM or C_3 pathways might be linked to bromeliad nutrition. It has been suggested that the additional reducing power and energy produced at night from citrate synthesis in atmospheric CAM bromeliads could be diverted for the nocturnal reduction and assimilation of nitrate (Freschi *et al.*, 2010). By contrast, in our study, the leaf N concentration was slightly higher for C_3 compared with CAM bromeliads, but concerned tank-bromeliad and not atmospheric ones. Yet, the hypothetical links connecting CAM photosynthesis and nitrogen metabolism have remained largely unexplored and certainly deserve further study as their connections are still elusive (Freschi *et al.*, 2010).

Variations in δ^{15}N in bromeliad species are mainly due to plant size (Hietz and Wanek, 2003) and growth habits (Nievola *et al.*, 2001). Atmospheric bromeliads that depend only on nutrients deposited by the atmosphere are characterized by strong ^{15}N depletion (i.e. δ^{15}N values from −14 to −6 ‰), whereas tank-bromeliads that also depend on rainwater but store it together with decaying organic matter are less ^{15}N depleted (i.e. δ^{15}N values from −2 to +3 ‰) (Hietz and Wanek, 2003; Wanek and Zotz, 2011). In our study, δ^{15}N ranged from −1·5 to +2 ‰. These disparities might be due to the quantity of accumulated FPOM, which might positively influence the leaf δ^{15}N (this study). These disparities might also be due to the quality of canopy-derived nitrogen. For example, Roggy *et al.* (1999) found great differences in the δ^{15}N signatures of canopy leaves. Moreover, ^{15}N depletion might be also attributed to N_2 fixation by the cyanobacteria contained in the tank (Bermudes and Benzing, 1991). Leaf δ^{15}N values also reflect the part of nitrogen derived from invertebrate faeces released into the water (Huxley, 1980; Bazile *et al.*, 2012). An increase in leaf δ^{15}N might be due to higher numbers of aquatic invertebrates (Leroy *et al.*, 2009) and/or trophic levels within food webs (Ngai and Srivastava, 2006). Surprisingly, we found that the PPR had a negative effect on bromeliad nutrition (see also Romero and Srivastava, 2010). But because these variables were not significantly correlated (see Table 2) and because other studies have shown contrasting results or considered the predator/prey biomass ratio rather than the richness ratio (Ruetz *et al.*, 2002; Ngai and Srivastava, 2006), no general conclusion can be drawn. Clearly, further investigations are needed to accurately determine the contribution of invertebrate detritivores and predators to tank-bromeliad nutrition.

Nitrogen acquisition showed a strong positive correlation with the presence of ants. When associated with the ant *C. femoratus*, the tank-bromeliad *A. mertensii* hosted more aquatic invertebrate morphospecies and had higher leaf N and δ^{15}N values than when associated with *P. goeldii* (although the two associations coexist on a local scale). Thus, the species of the mutualistic ant partner matters because it has consequences for bromeliad nutrition. The ant *C. femoratus* can indirectly provide nutritional benefits to tank-bromeliads by determining the location of the seedling under tree canopies (where litter inputs are greater), thereby influencing the phytotelm food web (Leroy *et al.*, 2009; Céréghino *et al.*, 2010, 2011). It is also likely that AG-ants provide the bromeliads with nitrogen directly through the roots because, when these ants were provided with ^{15}N-enriched food, the bromeliad tissues were subsequently found to be enriched in ^{15}N (Leroy *et al.*, 2012). *Streptocalyx longifolius*, which also roots in *C. femoratus* AGs, does not benefit from reservoir-assisted nutrition. However, its leaf δ^{15}N values are similar to some of the other tank-bromeliads studied (i.e. *G. lingulata*, *V. pleiosticha*) and are even higher than in other epiphytic, tankless bromeliads (Hietz and Wanek, 2003). It is thus likely that *C. femoratus*-associated *S. longifolius* bromeliads benefit from ant-derived nutrients (e.g. faeces, insect remains) through their roots.

Surprisingly, *C. berteroniana* individuals, which depend on prey-derived N inputs (i.e. insects trapped by the waxy leaves; Gaume *et al.*, 2004), were more ^{15}N depleted than the other tank-bromeliads studied. Carnivorous plants, which derive N from ^{15}N-rich insect tissues, are usually characterized by higher δ^{15}N values compared with non-carnivorous plants (Schulze *et al.*, 1997; Moran *et al.*, 2001; Bazile *et al.*, 2012). Nevertheless, carnivorous plants might shift from nutrient uptake through prey capture to nutrient uptake through their roots when soil N availability increases, resulting in variability in the amount of leaf δ^{15}N (Millett *et al.*, 2003, 2012; Thorén *et al.*, 2003). However, concerning the epiphytic *C. berteroniana*, root-derived N might be very low. Furthermore, compared with other carnivorous

TABLE 3. *Main characteristics of the eight tank-bromeliad morphospecies sampled*

	CAM-Bromelioideae					C_3-Tillandsioideae			
Metabolism ID	AA	AB	SL_CF	AM_CF	AM_PG	CB	GL	VP	VS
Ants	No	No	Yes	Yes	Yes	No	No	No	No
WV***	949·2 ± 104·3^{a}	137·8 ± 21·1^{b}	–	129·0 ± 16·2^{b}	41·1 ± 5·8^{c}	40·6 ± 3·7^{c}	17·4 ± 2·5^{d}	73·2 ± 9·1^{e}	48·5 ± 5·0^{c}
FPOM***	10·8 ± 1·3^{a}	1·1 ± 0·2^{b}	–	10·9 ± 2·2^{a}	1·5 ± 0·3^{b}	0·7 ± 0·08^{b}	1·0 ± 0·1^{b}	1·7 ± 0·2^{b}	4·2 ± 0·4^{c}
PPR***	0·34 ± 0·02^{a}	0·14 ± 0·04^{b}	–	0·43 ± 0·04^{a}	0·37 ± 0·06^{a}	0·42 ± 0·07^{a}	0·19 ± 0·10^{b}	0	0·06 ± 0·03^{b}
Algae***	454·4 ± 226·3^{a}	0·27 ± 0·10^{c}	–	123·8 ± 65·0^{b}	2·1 ± 0·7^{d}	60·2 ± 33·7^{b}	0·06 ± 0·01^{c}	0·3 ± 0·1^{c}	1·3 ± 0·7^{cd}
δ^{15}N***	–0·2 ± 0·3^{abc}	0·5 ± 0·3^{ab}	–0·4 ± 0·3^{abc}	1·7 ± 0·2^{e}	0·5 ± 0·2^{ac}	–1·1 ± 0·3^{cd}	–0·5 ± 0·3^{bd}	–0·2 ± 0·1^{abcd}	0·6 ± 0·1^{cd}
N***	0·46 ± 0·03^{a}	0·64 ± 0·02^{bf}	0·8 ± 0·1^{bcef}	0·89 ± 0·04^{d}	0·68 ± 0·02^{be}	0·64 ± 0·03^{be}	0·83 ± 0·05^{cde}	0·90 ± 0·03^{cd}	0·71 ± 0·05^{bcdef}

Shown are mean (± s.e.) water volume (WV, mL), fine particulate organic matter (FPOM, mL), predator/prey richness ratio (PPR), algal density (algae, 10^3 mL^{-1}), natural abundance of stable nitrogen isotope (δ^{15}N, ‰) and nitrogen concentration (N, %). AA, *Aechmea aquilega*; AB, *Aechmea bromeliifolia*; SL_CF, *Streptocalyx longifolius* associated with *Camponotus femoratus*; AM_CF, *Aechmea mertensii* associated with *Camponotus femoratus*; AM_PG, *Aechmea mertensii* associated with *Pachycondyla goeldii*; CB, *Catopsis berteroniana*; GL, *Guzmania lingulata*; VP, *Vriesea pleiosticha*; VS, *Vriesea splendens*.

Asterisks indicate significant differences after the Kruskall-Wallis test (***$P < 0{\cdot}001$). Values marked with the same letter are not significantly different (Bonferroni corrected Mann–Whitney pairwise comparisons, $P < 0{\cdot}05$).

plants, *C. berteroniana* is rather seen as a protocarnivore because it does not have specialized glands that produce digestive fluids but relies on bacteria and other micro-organisms to break down prey (Givnish *et al.*, 1984). A variety of detritivorous bacteria capable of degrading pectin, cellulosic materials as well as chitin (the main component in the exoskeletons of arthropods) have already been observed inside the tanks of *C. berteroniana* (Pittl *et al.*, 2010; Goffredi *et al.*, 2011). Based on these assumptions, *C. berteroniana* might have an alternative means of nutrient acquisition that deserves further study. Because, *C. berteroniana* is found in sunny areas, this species may harbour in its tanks cyanobacteria able to fix N_2 as has been observed in some other bromeliads (Bermudes and Benzing, 1991) and might explain the unexpectedly low δ^{15}N. Neither *C. berteroniana* nor *A. aquilega* (both grow in sun-exposed areas) maintained N concentrations comparable to bromeliads growing in the understorey sites, which suggests a high degree of nutrient stress for both species. This feature may be attributed to the lack of leaf-litter input (see Romero *et al.*, 2008) that constitutes the main source of nutrients for the aquatic food web. Indeed, *A. aquilega* tanks contain the highest FPOM volume. However, considering the volume of water, this indicates that the FPOM is highly diluted compared to the FPOM in other bromeliads. Although detritus is a main source of energy for tank-bromeliads (Benzing, 2000), recent research has shown the role of algae as a potential energy source (Brouard *et al.*, 2011, 2012; Marino *et al.*, 2011). Algae represent a higher-quality trophic resource than leaf-litter and could, thus, be more relevant to the faunal food web than their relative biomass would suggest (McNeely *et al.*, 2007). Nevertheless, our study has shown that algae might negatively affect tank-bromeliad nutrition. Indeed, the algal density in *A. aquilega* and *C. berteroniana* tanks is significantly higher than in the tanks of understorey bromeliads such as *A. bromeliifolia*, *G. lingulata*, *V. pleiosticha* and *V. splendens*. We thus hypothesize that algae probably compete with the bromeliad for dissolved inorganic nutrients such as ammonium (NH_4^+), which is the primary source of nitrogen for both the bromeliad (Inselsbacher *et al.*, 2007) and the algae (Sigee, 2005).

For *A. mertensii*, algal density is higher when the plant is associated with *C. femoratus* and exposed to incident light levels that are two times lower than when associated with *P. goeldii* (Table 1). In this case, the nutrients provided by the decomposition of organic matter, rather than light, could be a key factor controlling algal growth (Carrias *et al.*, 2012). Moreover, Euglenophyceae are abundant in shaded and partially shaded bromeliads (Brouard *et al.*, 2012). Many of these micro-organisms, which are categorized as 'algae', are able to absorb organic rather than inorganic nitrogen (Amblard, 1991) and therefore should compete less with the bromeliad for these nutrients. However, the interactions between bromeliads and algae remain poorly understood (Marino *et al.*, 2011). Further investigations are needed to accurately determine the implications of algae as competitors of tank-bromeliads for nutrients and to better understand the regulation (bottom-up vs. top-down effects) of algae in bromeliad systems.

Overall, we found that the presence of mutualistic ants was the most important factor contributing to between-species differences in bromeliad nitrogen acquisition. We provide evidence that ants have a positive influence on bromeliad (both tank and tankless) nitrogen acquisition, and that the extent of the benefit depends on the associated ant species. Conversely, a protocarnivorous bromeliad not associated with mutualistic ants was thought to obtain nitrogen from ant carcasses. However, the relatively low leaf δ^{15}N and N concentrations for this species compared with other bromeliads suggest that it is more advantageous for a bromeliad to use myrmecotrophy via its roots than to use carnivory via its tank. Our study also suggests that the contribution of phytotelm communities to bromeliad nutrition is more complex than previously thought. Finally, this study highlights a gap in our knowledge of the reciprocal interactions between bromeliads and the various trophic levels (from bacteria to large metazoan predators) that intervene in reservoir-assisted nutrition.

ACKNOWLEDGEMENTS

We are grateful to the staff of the laboratory HYDRECO in Petit Saut and to the staff of the Nouragues Research Station for technical and logistical support. We would also like to thank three anonymous reviewers for valuable comments on a previous version of the manuscript. Andrea Yockey-Dejean proofread the text and corrected the English. This study has benefited from an 'Investissement d'Avenir' grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-0025).

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