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Abstract

The water and nutrient uptake mechanisms used by vascular epiphytes have been the subject of a few studies. While leaf absorbing trichomes (LATs) are the main organ involved in resource uptake by bromeliads, little attention has been paid to the absorbing role of epiphytic bromeliad roots. This study investigates the water and nutrient uptake capacity of LATs *vs*. roots in two epiphytic tank bromeliads *Aechmea aquilega* and *Lutheria splendens*. The tank and/or the roots of bromeliads were watered, or not watered at all, in different treatments. We show that LATs and roots have different functions in resource uptake in the two species, which we mainly attributed to dissimilarities in carbon acquisition and growth traits (e.g., photosynthesis, relative growth rate, non-structural carbohydrates, malate), to water relation traits (e.g., water and osmotic potential, relative water content, hydrenchyma thickness) and nutrient uptake (e.g., ^{15}N-labelling). While the roots of *A. aquilega* did contribute to water and nutrient uptake, the roots of *L. splendens* were less important than the role played by the LATs in resource uptake. We also provide evidence for a synergistic effect of combined watering of tank and root in the Bromelioideae species. These results call for a more complex interpretation of LATs vs. roots in resource uptake in bromeliads.

Keywords: Carbon metabolism, Nutrient uptake, ^{15}N labelling, Plant performance, Tank bromeliad, Water status

Highlights

- *Lutheria splendens* and *Aechmea aquilega* are epiphytic tank bromeliad
- Leaf absorbing trichomes and roots have different functions in resource uptake in the two species
- The root system of *L. splendens* only plays a negligible role in resources uptake
- The root system of *A. aquilega* does contribute to water and nutrient uptake

1. Introduction

Vascular epiphytes, which grow on other plants without parasitism, have no contact with terrestrial soil resources, and consequently need to take up nutrients from rainfall, throughfall and stemflow water and/or from decomposing organic matter in the canopy (Gotsch et al., 2015). Epiphytes have evolved numerous remarkable adaptations (e.g., litter-trapping leaf arrangements, water-storing phytotelmata, leaf-absorbing trichomes, velamen radicum) to facilitate nutrient uptake (Benzing, 1990; Lüttge, 2008; Pridgeon, 1987). Bromeliads, one of the largest and most widespread families of vascular plants in the Neotropics, display many of these adaptations.

The Bromeliaceae family comprises 3,140 species distributed in three subfamilies: Bromelioideae, Tillandsioideae and Pitcairnioideae (Crayn et al., 2004 but see Givnish et al., 2011 for recent systematic updates). Bromeliads account for a large proportion of vascular epiphyte species distributed throughout the tropical and subtropical regions of the Americas. The ecological success of this wide geographic distribution may be explained by the development of key innovations (Givnish et al., 2014; Males, 2016): (i) epiphytism, (ii) leaf-absorbing trichomes (hereafter LATs), which facilitate water and nutrient uptake, (iii) tank growth form, in which a rosette of leaves forms a reservoir to trap rainwater, leaf litter and aquatic organisms, and (iv) Crassulacean acid metabolism (CAM) photosynthesis, which enables bromeliads to survive under dry environmental conditions. Characteristic combinations of these innovations have been used to define five functional types (Benzing, 2000): *Type I*, C_3 or CAM Soil-Root (Pitcairnioideae and Bromelioideae); *Type II*, CAM Tank-Root (Bromelioideae); *Type III*, CAM Tank-Absorbing Trichome (Bromelioideae); *Type IV*, C3 Tank-Absorbing Trichome (Tillandsioideae) and *Type V*, CAM Atmosphere-Absorbing Trichome (Tillandsioideae).

Bromeliads show varying degrees of dependency on LATs *vs.* roots for nutrient uptake depending on their functional type. The terrestrial species (*Types I* and *II*) have a well-developed root system for anchorage and resource uptake, whereas epiphytes (from *Types III* to *V*) are capable of absorbing water and nutrients through their LATs, thereby reducing the root function to pure mechanical support (Benzing, 2000; Martin, 1994; Winkler and Zotz, 2009). Some of the most "extreme" *Type V* epiphytes are rootless (e.g., *Tillandsia usneoides*) and depend solely on their LATs for water and mineral nutrition (Benzing and Ott, 1981). LATs enable very effective uptake of both inorganic and organic forms of nitrogen as well as various micronutrients (Inselsbacher et al., 2007; Winkler and Zotz, 2010, 2009). While a large panel of studies has focused on the structure and the importance of water and nutrient uptake by LATs (e.g., Benzing, 1976; North et al., 2013; Nyman et al., 1987), little attention has been paid to the structure and absorbing role of bromeliad root systems (but see Carvalho et al., 2017; Vanhoutte et al., 2016). To our knowledge, very few studies have investigated the role of LATs *vs.* roots in resource uptake, and their results are inconsistent. While some studies

failed to detect any (Nadkarni and Primack, 1989; Winkler and Zotz, 2009) or very little root nutrient uptake (Nievola and Mercier, 1996), others underlined efficient root nutrient uptake (Silva et al., 2018; Carvalho et al., 2017; Vanhoutte et al., 2017, 2016). More studies are thus needed to better grasp the role of roots in water and nutrient uptake in comparison to that of LATs.

These contradictory results could be due to the variety of experimental approaches used in each study (e.g., radioactive or isotopic labelling, gamma spectrometry, enzymatic activity). Additionally, in some studies, the role of roots was investigated while the tank continued to receive water (Carvalho et al., 2017; Vanhoutte et al., 2017). In such experimental conditions, the role of roots may be minimised as tank bromeliads can rely on the tank reservoir and water-storage tissues in the leaves (i.e., hydrenchyma) which may be responsible for external and internal water (and nutrient) storage, respectively (Freschi et al., 2010b; Males, 2016). A situation in which only the roots receive water and minerals, and not the tank, is unlikely to happen under natural conditions but this experimental design makes it possible to properly separate the functioning of LATs *vs*. roots in resource uptake, and subsequently in plant performance. An integrative approach with measurements of functional traits should provide information on resource capture, use and allocation.

The aim of the present study was to investigate the resource uptake capacity of LATs *vs.* roots in two common epiphytic tank bromeliad species: *Aechmea aquilega* (Salib.) Griseb and *Lutheria splendens* (Brongn.) Lem. These two species were chosen because they differ in their ontogenic development: *L. splendens* is a heteroblastic species which change from juvenile atmospheric to adult tank forms whereas *A. aquilega* is homoblastic. Thus, at the juvenile stage *L. splendens* have narrow, lanceolate leaves, densely covered with LATs (pers. obs., see also Meisner et al., (2013) for others Tillandsioiseae species), whereas *A. aquilega* do not have any LATs at the juvenile stage indicating that the roots is of prime importance for nutrient absorption (Leroy et al. 2019). On the contrary, at the adult tank form both species have LATs that are non-homogeneously distributed throughout the leaf blade. There is a longitudinal gradient of LATs density where the basal portion of the leaf, in contact with water and nutrients in the tank, has higher LATs density than the apical portion (Takahashi et al., 2007). The ontogenic specificities of these two species led us to speculate that there may be differences in the degree of dependence on LATs *vs*. roots for resource uptake at the adult tank form. Specifically, we hypothesised that *A. aquilega* would acquire water and nutrients through its roots more efficiently than *L. splendens,* subsequently providing greater nutritional benefits to the plant. To test these hypotheses, we used a semi-controlled experimental approach consisting of watering potted tank form bromeliads in a greenhouse using four different treatments: (i) watering both the tank and the roots, (ii) watering only the tank, (iii) watering only the roots, and (iv) not watering the plants at all. The last treatment, corresponding to drought conditions, enabled us to

identify symptoms of drought stress, which were then compared to the species responses under the other treatments. We compared the way the two bromeliad species responded to the water treatments by using a unique set of functional traits related to growth, carbon metabolism, water status, and nutrient uptake.

2. Materials and methods

2.1. Plant materials and growth conditions

Aechmea aquilega (Salib.) Griseb (**Fig. 1A**) is a Type III tank-forming bromeliad belonging to the subfamily Bromelioideae with CAM photosynthesis (Crayn et al., 2004). This species occurs as an epiphytic, rupicolous or secondary terrestrial bromeliad in full sun or partial shade environments (Leroy et al., 2013). Adult tank form *A. aquilega* growing in a shaded greenhouse at the *Campus agronomique* in Kourou French Guiana were used for the experiment. The plants (n=24) were characterised by a tank water volume of 116.2 ± 23.1 mL, a number of leaves of 9.7 ± 0.2, a total height (distance from the bottom of the body to the top of the crown) of 27.1 ± 0.8 cm, a canopy width (maximum distance between the tips of the leaves, two measurements taken at an angle of 90°) of 24.2 ± 1.4 cm and a length of 26.7 ± 0.9 cm, with a 4.5 ± 0.1 cm width for the longest leaf. The leaf appearance, estimated on a 6-month period, was in average every 26.03 ± 3.73 days.

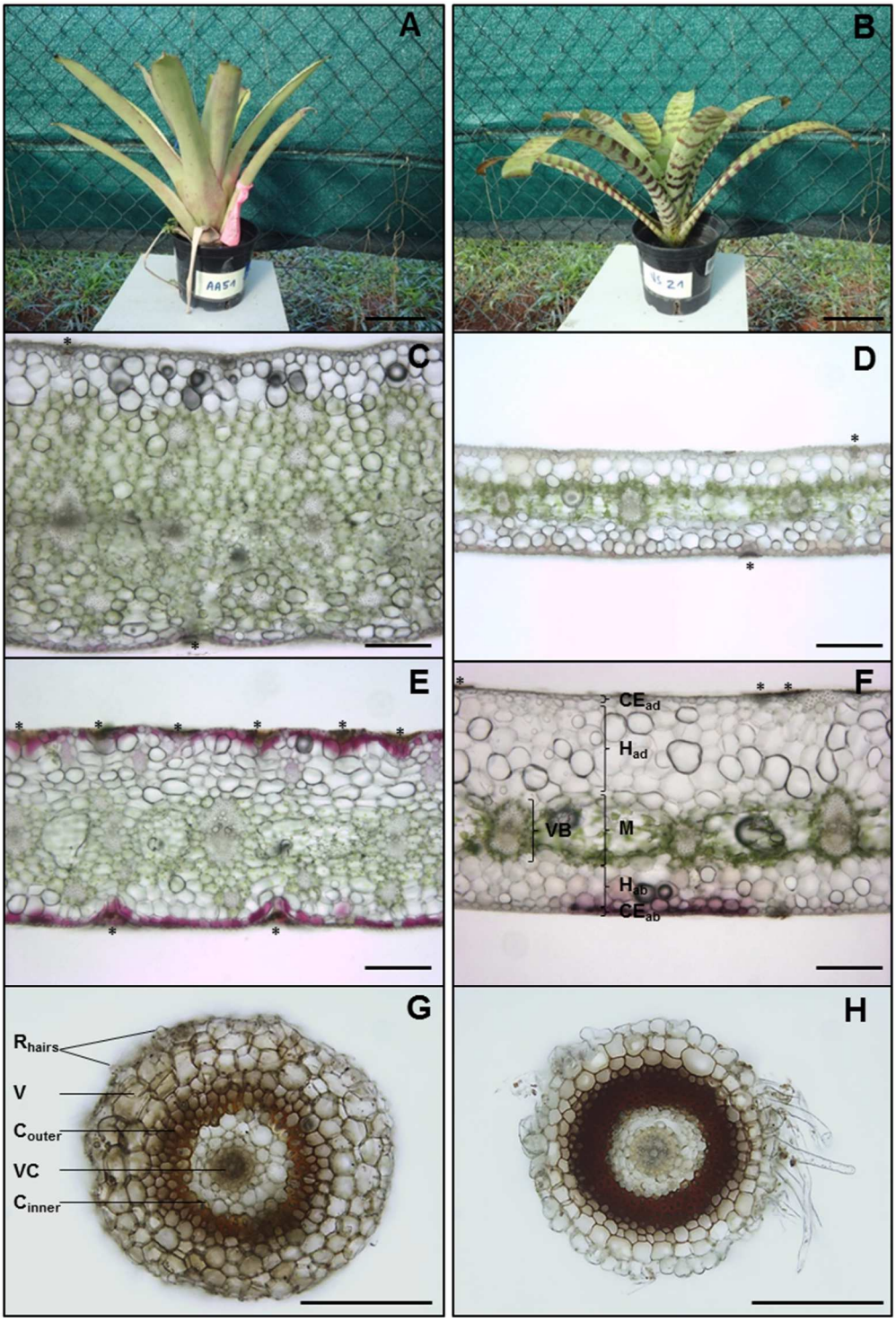

Fig. 1. Experimental (A) *Aechmea aquilega* and (B) *Lutheria splendens* in 1 litre horticultural plastic pot. Light micrographs of hand-cut transverse section of (C, D) the aerial and (E, F) the basal part of the lamina of (C, E) *A. aquilega* and (D, F) *L. splendens*. CE_{ad} = adaxial cuticle and epidermis, H_{ad} = adaxial hydrenchyma, M = Mesophyll, H_{ab} = abaxial hydrenchyma, CE_{ab} = abaxial cuticle and epidermis, VB = Vascular bundle. Light micrographs of hand-cut transverse section near the apex of the root of (G) *A. aquilega* and (H) *L. splendens*. R_{hairs} = root hairs, V = velamen, C_{outer} = outer cortex, VC = vascular cylinder, C_{inner} = inner cortex, * = indicates the presence of LATs. Scale bars for A and B = 10 cm and scale bars for all anatomical sections = 200 µm.

143 *Lutheria splendens* (Brongn.) Lem. (**Fig. 1B**) is a Type IV tank-forming bromeliad in the subfamily
144 Tillandsioideae with C_3 metabolism. This species occurs as an epiphyte and as a secondary terrestrial
145 plant in the understorey of pristine forests (Leroy et al., 2013). We collected 24 tank-form *L.*
146 *splendens* of similar size order than *A. aquilega* with a well-developed tank in a lowland rainforest
147 plot located near the Petit-Saut Dam, Sinnamary (05°03'43"N, 53°02'46"W), 55 km from the *Campus*
148 *agronomique* in Kourou. For acclimation in the shaded greenhouse, *L. splendens* were collected six
149 months prior to the start of the experiment. These plants (n=24) were characterised by a tank water
150 volume of 52.9 ± 5.9 mL, a number of leaves of 11.2 ± 0.4, a total height of 20.4 ± 0.8 cm, a canopy
151 width of 39.8 ± 1.9 cm and a length of 27.1 ± 1.1 cm, with a width of 3.9 ± 0.1 cm for the longest leaf.
152 The leaf appearance, estimated on a 6-month period, was in average every 32.88 ± 3.13 days.
153 Both species exhibited water storage tissue (hydrenchyma) on the adaxial and abaxial side of the
154 leaf formed by large non-chlorophyllous cells (**Fig. 1C-F**). The mesophyll, made up of the aerenchyma,
155 chlorenchyma and vascular bundles, was located in the central part of the lamina. The roots of *A.*
156 *aquilega* and *L. splendens* showed the typical anatomy of a monocot root (**Fig. 1G, H**) with a velamen
157 radicum, root hairs, a sclerified outer cortex, an inner cortex, and a vascular system.
158 The bromeliads were potted in 1 L horticultural plastic pots (105 mm height and 135 mm
159 diameter) containing a mixture of sand and forest soil (v:v 50:50). The pots were placed on two 2 x
160 1.2 m trays, making it possible to separate the species according to their natural light environments.
161 Cloths with two shade ratings created a medium light environment for *A. aquilega* and a low light
162 environment for *L. splendens*. Environmental HOBO sensors were used to characterise air relative
163 humidity, air temperature and light intensity (model UA-002-64, HOBO Pendant Tem Light – 64k and
164 model U23-001, HOBO Pro V2 Temp/RH Data logger, Amanvillers, France) at plant level. For *A.*
165 *aquilega*, the mean air relative humidity was 84.3 ± 0.1%, the mean air temperature was 28.3 ± 0.1°C
166 and the light intensity was *ca*. 30% of full external irradiance during the experiment. For *L. splendens*,
167 the mean relative humidity was 83.9 ± 0.06%, the mean temperature was 28.1 ± 0.1°C and the light
168 intensity was *ca*. 10% of full external irradiance. Mid-day photosynthetically active radiation (PAR)
169 was measured with a Li-Cor 6400XT portable photosynthesis system (Li-Cor, Inc., Lincoln, Nebraska,
170 USA) on two non-consecutive sunny days. Mid-day PAR was 496.5 ± 35.4 for *A. aquilega* and 202.5 ±
171 38.9 µmol m^{-2} s^{-1} for *L. splendens*, while the outside PAR was 1808.5 ± 103.1 µmol m^{-2} s^{-1}.
172
173 ***2.2 Water supply treatments***
174 All the plants were irrigated with fresh rainwater every second day for six months prior to the
175 experiment. Twenty-four bromeliads of similar shape and size of each of the two species were
176 organised homogeneously in four different watering treatments with a total of 6 replicates per
177 treatment. Every second day, the bromeliads were watered at soil capacity and full tank capacity

with fresh rainwater as follows: both the tank and the roots were watered (TR treatment), only the tank was watered (T treatment), only the roots were water (R treatment) or both the tank and the roots were not watered at all (D treatment). In the T treatment, we made a visual check that no water reached the roots. In the R and D treatments, water in the tank was gently removed with a pipette at the start of the experiment. The experiment was carried out on a 2-month period (see **Supplemental Table S1**) to have enough time for the plants to grow (i.e., appearance of *ca.* two leaves) and not too long time so that the plants from the D treatment would not die.

2.3 Growth and carbon metabolism

Leaf survival and growth— At beginning of the experiment (t1) and after 2 months (t2), we measured the total number of leaves and the length of one growing leaf in order to calculate the number of new leaves (NbN_{leaf}), the number of dead leaves (NbD_{leaf}) and the relative growth rate (RGR). The RGR ($ln(cm).day^{-1}$) was calculated based on Gonçalves et al. (2016) as: ((lnLength_t2 – lnLength_t1)/(t2 – t1)), with lnLength_t1 and lnLength_t2 as the means of natural logarithm transformed of the youngest leaf length at the beginning (t1) and at the end (t2) of the experiment period, respectively.

Gas exchange— For each species, net photosynthesis assimilation (A, µmol CO_2 m^{-2} s^{-1}) and stomatal conductance to H_2O (g_s, mol H_2O m^{-2} s^{-1}) were measured on three out of six individuals (on the first young expanded leaf, see **Supplemental Figure S2**) in each of the four watering treatments after 2 months. All measurements were made continuously throughout a day and a night from 9 AM to 8 AM the following day using three Li-Cor 6400XT portable photosynthesis systems. The light PAR level was set to 500 µmol m^{-2} s^{-1} for *A. aquilega* determined from direct PAR measurements in the greenhouse environment and to 200 µmol m^{-2} s^{-1} for *L. splendens* from preliminary light- curves (see **Supplemental Figure S3**) from 9 AM to 4 PM. Next, we switched to natural PAR conditions from 4 PM to 8 AM the following day by using the "track PAR out" mode. Leaf temperature, CO_2 concentration and air flow in the chamber were set at 27 °C, 400 ppm and 250 µmol s^{-1}, respectively. To compare treatments, we calculated maximum net photosynthesis assimilation (A_{max}, µmol CO_2 m^{-2} s^{-1}) and maximum stomatal conductance for water vapour (g_{smax}, mol H_2O m^{-2} s^{-1}) by averaging the five highest gas exchange (CO_2 and H_2O) values.

Metabolite sampling protocol— The tip of the second young expanded leaf (N=6 leaves per treatment) was harvested at 6 PM and the tip of the third young expanded leaf (see **Supplemental Figure S2**) the following morning at 6 AM corresponding to minimum and maximum malate concentrations (for the CAM species) and the reverse for storage carbohydrates (both CAM and C_3), respectively (Ceusters et al., 2008). Roots (N=3 per treatment) were harvested at 6 AM. Samples were immediately frozen in liquid nitrogen, then stored in a freezer until they were freeze-dried (Alpha 1-2 LD; Christ, Osterode am Harz, Germany). Each sample was ground to a fine powder in an

MM301 Mixer Mill, then stored in airtight vials in the dark until malate and non-structural carbohydrates (NSC) analyses.

Malate extraction— Malate was only extracted from the CAM *A. aquilega.* Extraction was performed in triplicate, and 100 mg of dry mass (DM) was placed in each micro-tube. The extraction method was based on Freschi et al., (2010a) with modifications. We used 500 µL of a methanol:chloroform:water (12:5:1- v/v/v) solution, added with salicylic acid (20 µg. mL^{-1}) as internal standard. The sample was mixed and incubated for 30 min at 60 °C. Then, 500 µL of distilled water were added and the extract was centrifuged at 18,000 × g for 10 min, the upper clear phase was used. The malic acid content (Malate, mg g^{-1} DW) was measured using High Performance Liquid Chromatography 1200 series system coupled with a Diode-Array Detector (Agilent Technologies, Santa Clara, CA, USA) (see Amorós et al., 2003).

Non-structural carbohydrate extraction— Soluble sugars were extracted from 10 to 15 mg powder mixed in 0.5 mL 80% ethanol (v/v) and incubated for 20 min at 80 °C. Extraction was repeated twice and all three supernatants were collected and dried (Refrigerated CentriVap Vacuum Concentrators, Labconco). The resulting soluble sugar extract was solubilised in 1.5 ml ultrapure water. Total soluble sugar concentrations were determined by spectrophotometry at 620 nm (spectrophotometer UV-visible DU 640 B, Beckman Coulter, USA) using anthrone reagent (Van Handel, 1965) and glucose as standard. The pellet containing starch was extracted in 1.5 ml of 0.2 M KOH solution and incubated for 20 min at 80 °C then hydrolysed in glucose molecules with amyloglucosidase (Sigma, EC 3.2.1.3). Starch concentrations were determined by spectrophotometry at 530 nm as described in (Chow and Landhausser, 2004) using a glucose oxidase and peroxidase/orthodianisidine reagent (Sigma, EC 1.11.1.7; EC 1.1.3.4; EC 243-737-5). Soluble sugars and starch concentrations, expressed as mg equivalent glucose g^{-1} DM, were thus determined for leaves (LSS and L_{starch}) and roots (RSS and R_{starch}).

2.4 Water status

Relative water content — The third young fully expanded leaf was sampled from both the aerial and basal part of the leaf to assess leaf relative water content (RWC). Eight and six 10-mm-diameter discs were collected with a cork borer from the aerial and basal part of the leaf, respectively. The discs were immediately weighed using an electronic balance (AB 204-S Mettler Toledo, Switzerland) to determine their fresh mass (FM), then stored in distilled water in sealed plastic bags and kept at 4 °C in the dark for 72 hours to determine turgid mass (TM), and finally dried at 60 °C for 72 hours to determine dry mass (DM). The relative water content (RWC, %) was calculated as (FM-DM)/(TM-DM)*100.

Leaf and tissue thicknesses — The second young expanded leaf was sampled for analysis of anatomical structure. Transverse sections of the middle portion of the aerial and basal part of the

fresh leaf were cut by hand using a sharp razor blade (**Fig. 1B-C, F-G**). Sections were immediately immersed in oil to stop water from moving out of the cells and to prevent cell shrinkage (Vanhoutte et al., 2016). Five pictures were taken of each of the two portions of the leaf using an inverted microscope (Olympus BX51-TF, Tokyo, Japan). Images were acquired with a digital camera (Lumenera LW1135C-IO, Ottawa, Canada) and processed using ImageJ 1.51 software. On each picture, we measured leaf thickness (LT, µm, 4 measurements), adaxial and abaxial epidermal wall and cuticle thickness (CET, µm, 6 measurements), adaxial and abaxial hydrenchyma thickness (HT, µm, 6 measurements), and mesophyll thickness (MT, µm, 6 measurements). Fresh hand-cut transversal sections of the roots of three additional control plants were made of each species in order to characterise their anatomical structure (**Figure 1D, H**).

Water potential— Mid-day leaf water potential (Ψ_{MD}) was measured on the second young expanded leaf with thermocouple psychrometers (76-1VC leaf cutter thermocouple psychrometer, Merrill Specialty Equipment, Logan, UT, USA) connected to a PsyPro water potential data-logger (Psypro; Wescor Inc., Logan, UT, USA). To ensure constant temperature, Psychrometers were placed in a water bath (25 °C) after sampling and left to equilibrate overnight. Water potential was then calculated from the initial slope of the psychrometric response curve, previously calibrated with NaCl solutions. Each individual Ψ_{MD} (MPa) corresponds to the mean of three samples (6.4 mm diameter leaf discs).

Osmotic potential— Leaf osmotic potential (Ψ_{osm}) was measured with a vapour pressure osmometer (VAPRO 5520, Wescor, Logan, UT, USA). One disc was collected in the middle of the aerial part of the third young expanded leaf with a 7-mm-diameter cork borer. The disc was wrapped in foil and frozen by immersion in liquid nitrogen (N_2) for at least 4 min, then immediately punctured 15 to 20 times with a sharp needle and sealed in the osmometer chamber. The disc was exposed to air for less than 40 s during all the steps between harvesting the disc and sealing it in the osmometer. The equilibrium solute concentration value c_0 (mmol kg^{-1}) was recorded from the osmometer when the difference between consecutive measurements fell below 5 mmol kg^{-1}. This value was converted to Ψ_{osm} (MPa) using the Van't Hoff equation relating solute concentration to vapour pressure (Bartlett et al., 2012; Maréchaux et al., 2015).

2.5 Nutrient uptake

^{15}N-labelling design— We further investigated the functional role of LATs *vs.* roots in water and nutrient uptake by supplying *A. aquilega* and *L. splendens* with a nitrogen solution artificially enriched with ^{15}N. At the end of the 2-month experiment, we selected three plants per treatment (TR, T and R) for each species. The ^{15}N-enriched solution consisted of 7 L of rainwater with 2 g of $NH_4{}^{15}NO_3$ (10 atom % ^{15}N, Isotec Inc., OH, USA) and 2 g of $^{15}NH_4NO_3$ (10 atom % ^{15}N, Isotec Inc., OH,

USA). The solution was provided every second day for 15 days according to each watering treatment. On each watering day, in the TR and T treatments, 40 ml of ^{15}N-enriched solution was distributed in all the leaf axil. In the TR and R treatments, the roots received 40 ml of ^{15}N-enriched solution.

Isotopic and elementary analyses— Pieces of young mature leaves were collected before (i.e., from unlabelled plants to record the natural abundance, N_{nat} and $\delta\,^{15}N_{nat}$) and one week after the ^{15}N-enrichment period (i.e., from labelled plants to record the enrichment level, N_{lab} and $\delta\,^{15}N_{lab}$). All the samples were freeze-dried before isotopic analyses. About 1 g of dried leaf sample was used to measure the concentration of N (N, %) and ^{15} N isotopic abundance ($\delta^{15}N$, ‰). Stable isotope analyses were conducted at the Cornell University Stable Isotope laboratory (Ithaca, NY, USA) using a Thermo-Finnigan DELTA^{plus} Advantage gas isotope-ratio mass spectrometer plumbed to a Carlo Erba NC2500 elemental analyser through a Conflo II open split interface for elemental and isotopic composition of samples. The isotopic signal for N was expressed as ^{15}N delta (δ ‰) versus an international standard (N_2 in the air) as follows:

$\delta^{15}N‰ = (R_{sample}/R_{standard} - 1) \times 1000$ where R is the ratio ^{15} N/ ^{14} N in the sample or in the standard.

2.6 Statistical analyses

Experiments were conducted using a full factorial randomised design for each species. Two-way ANOVAs were used to test for the effect of species, treatment and their interactions in all functional traits measured. Data were log or rank transformed (GenABEL package), when necessary, to satisfy the assumptions of the ANOVA. When significant, the ANOVAs were followed by a Tukey's honestly significant difference (HSD) test for a posteriori testing of multiple means. To determine which of the treatments affect the daily course of net photosynthesis assimilation we used a linear mixed model procedure with time and treatment as fixed factors. All statistical analyses were evaluated using a 95% confidence interval and were conducted using R version, 2.14.1. (R Development Core Team, 2015). The results are presented as means ± 1 standard error.

3. Results

Species and treatment had significant effects on most of the measured traits (**Table 1**). The interaction between species and treatment had significant effects on some of the traits, indicating that the two species responded differently to the treatments.

3.1 Growth, photosynthesis and carbohydrate content

In *L. splendens,* NbN_{leaf} and the RGR were significantly lower in the R and D treatments than in the TR treatment, while in *A. aquilega,* significant differences were only found between the D treatment

and the three other treatments (**Table 1, Fig. 2**). Similarly, the NbD_{leaf} was significantly higher in the D treatment than in the other treatments in *A. aquilega*. The results were less striking in *L. splendens*.

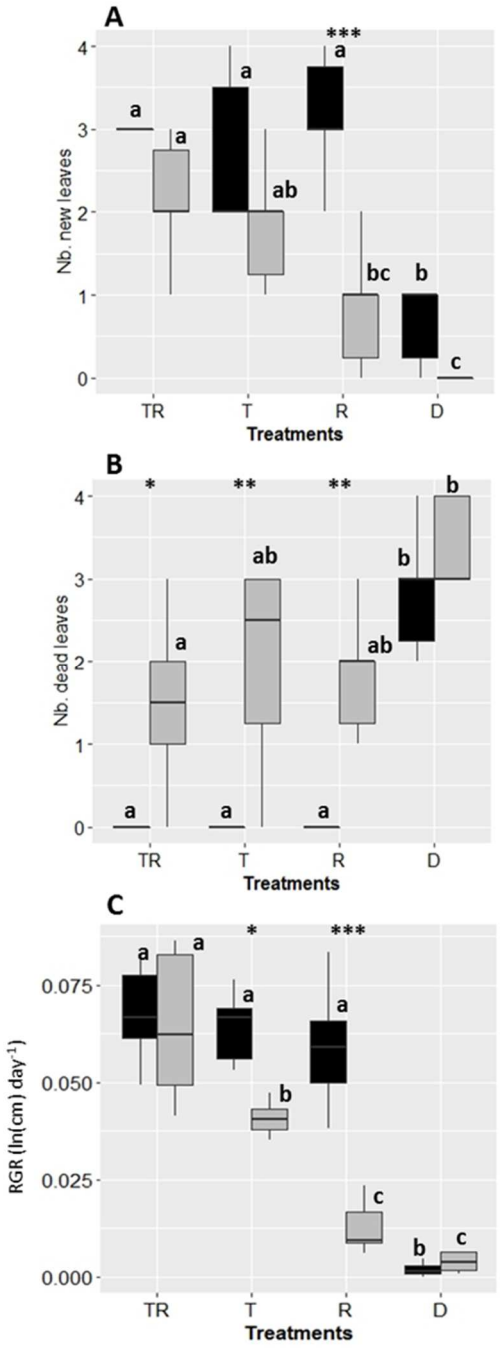

Fig. 2. Effect of water supply on plant growth. Effects of treatments (TR, T, R and D) on (A) the number of new leaves (NbN_{leaf}, N=6 for each treatment), (B) number of dead leaves (NbD_{leaf}, N=6 for each treatment) and (C) RGR in *Aechmea aquilega* (black) and *Lutheria splendens* (grey). Error bars above and below the boxes indicate the 90th and 10th percentiles, and the ends of the boxes indicate the 25th and 75th percentiles and solid circles indicate outliers. Different letters indicate significant differences for each species between treatments (Tukey's test, P<0.05). Asterisks indicate statistically significant differences for each treatment between species (Tukey's test, P<0.05; *, <0.05; **, <0.005 and *** < 0.0005).

The daily course of the net photosynthesis assimilation did not differ in the TR and T treatments in *A. aquilega* but was significantly reduced in the R treatment and nil in the D treatment (**Table 1, Fig. 3A**). When only the roots were watered (R treatment), A_{max} (1.12 ± 0.32 µmol m^{-2} s^{-1}) and g_{smax} (0.011 ± 0.002 mol m^{-2} s^{-1}) were reduced but not significantly different compared to the TR (1.56 ± 0.25 µmol m^{-2} s^{-1} and 0.013 ± 0.003 mol m^{-2} s^{-1}, respectively) and T treatments (1.81 ± 0.34 µmol m^{-2} s^{-1} and 0.015 ± 0.003 mol m^{-2} s^{-1}, respectively). The malate content in the R treatment (2.40 ± 0.40 mg g^{-1}) was at the same level as in the TR and T treatments (2.25 ± 0.22 mg g^{-1} and 2.28 ± 0.20 mg g^{-1}, respectively; **Table 1, Fig. 3A, and Supplemental Figure S4**). A_{max} (0.13 ± 0.07 µmol m^{-2} s^{-1}) and g_{smax} (0.0006 ± 0.0002 mol m^{-2} s^{-1}) were almost nil and the malate content (0.33 ± 0.07 mg g^{-1}) was significantly reduced under water shortage (D treatment). In *L. splendens*, the daily course of the net photosynthesis assimilation was significantly reduced in the T treatment compared to the TR treatment and was nil in the R and D treatments (**Table 1, Fig. 3B**). Significant reductions in A_{max} and g_{smax} were observed in the D *versus* the TR treatments (**Table 1, Fig. 3B, and Supplemental Figure S4**). When only the root system of *L. splendens* was watered (R treatment), A_{max} (0.04 ± 0.02 µmol m^{-2} s^{-1}) and g_{smax} (0.0001 ± 0.001 mol m^{-2} s^{-1}) values did not significantly differ from those measured in plants in the D treatment (0.10 ± 0.13 mol m^{-2} s^{-1} and 0.0008 ± 0.0007 mg g^{-1}, respectively).

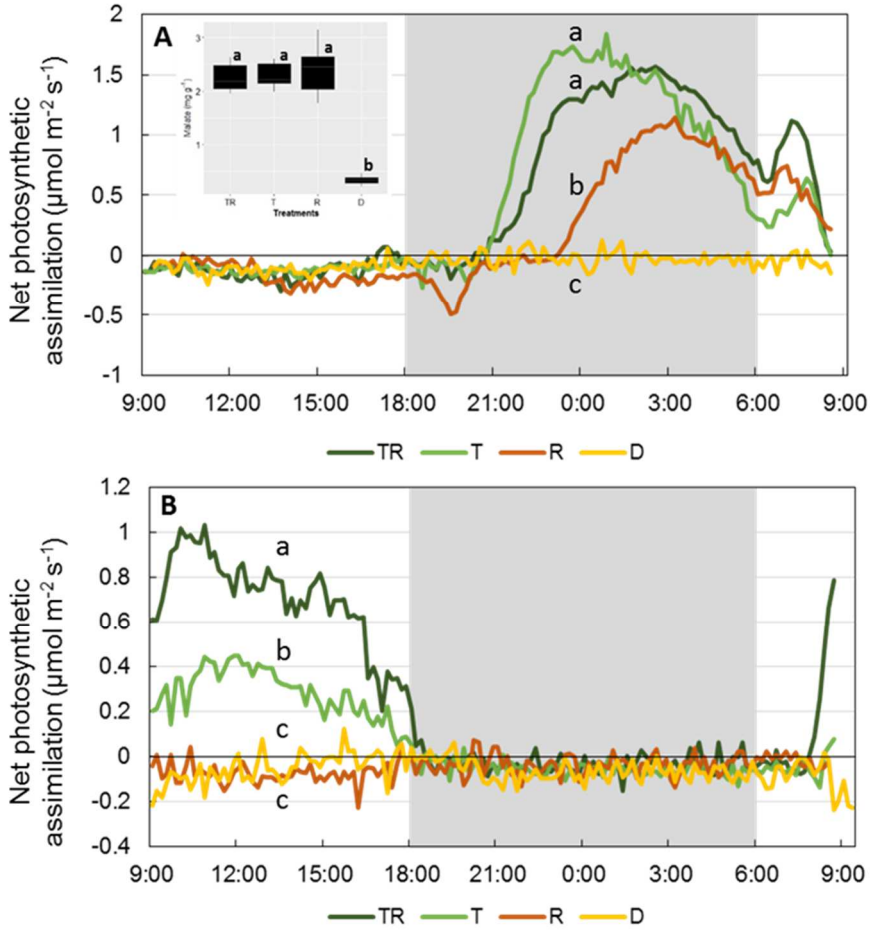

Fig. 3. Diel course of net photosynthesis assimilation (µmol m^{-2} s^{-1}) of (A) *Aechmea aquilega* (CAM photosynthetic pathway, N=3 for each treatment) and (B) *Lutheria splendens* (C3 photosynthetic pathway, N=3 for each treatment) according to the four watering treatments (TR, T, R and D). The grey area indicates the night period. Different letters indicate significant differences (P<0.05) between treatments (TR, T, R and D). In (A) the inset graph shows the malate content (mg g^{-1} DW) of *A. aquilega* according to the treatments (N=6 for each treatment).

No significant differences in leaf soluble sugars (LSS) at dusk were found between species or among treatments (**Table 1, Fig. 4A, B**). Mean values of LSS in *A. aquilega* ranged from 81 to 57 mg g^{-1} at dusk and from 69 to 45 mg g^{-1} at dawn, while mean values in *L. splendens* ranged from 87 to 58 mg g^{-1} at dusk and 69 to 53 mg g^{-1} at dawn. In contrast, leaf starch differed significantly as a function of the species, the treatments, and their interactions (**Table 1**). L_{starch} was significantly higher in *A. aquilega* than in *L. splendens,* which did not contain any starch in the leaves (**Fig. 4C, D**). Higher mean values of L_{starch} were found in *A. aquilega* at dusk in the TR and T treatments (60 and 48 mg g^{-1}, respectively) and lower values in the D treatment (15 mg g^{-1}), with intermediate contents of 33 mg g^{-1} in the R treatment. At dawn, L_{starch} was significantly reduced with mean values below 10 mg g^{-1}, meaning that starch was remobilised during the night. In the root system, soluble sugars (RSS) did not differ with the treatment but did differ between the two species (**Table 1**). *Aechmea aquilega* showed significantly higher mean values (20 mg g^{-1}) of RSS than *L. splendens* in which values were below 5 mg g^{-1} (**Fig. 4E**). Remarkably, the roots of both species did not contain any starch, indicating that the roots are not a starch storage organ.

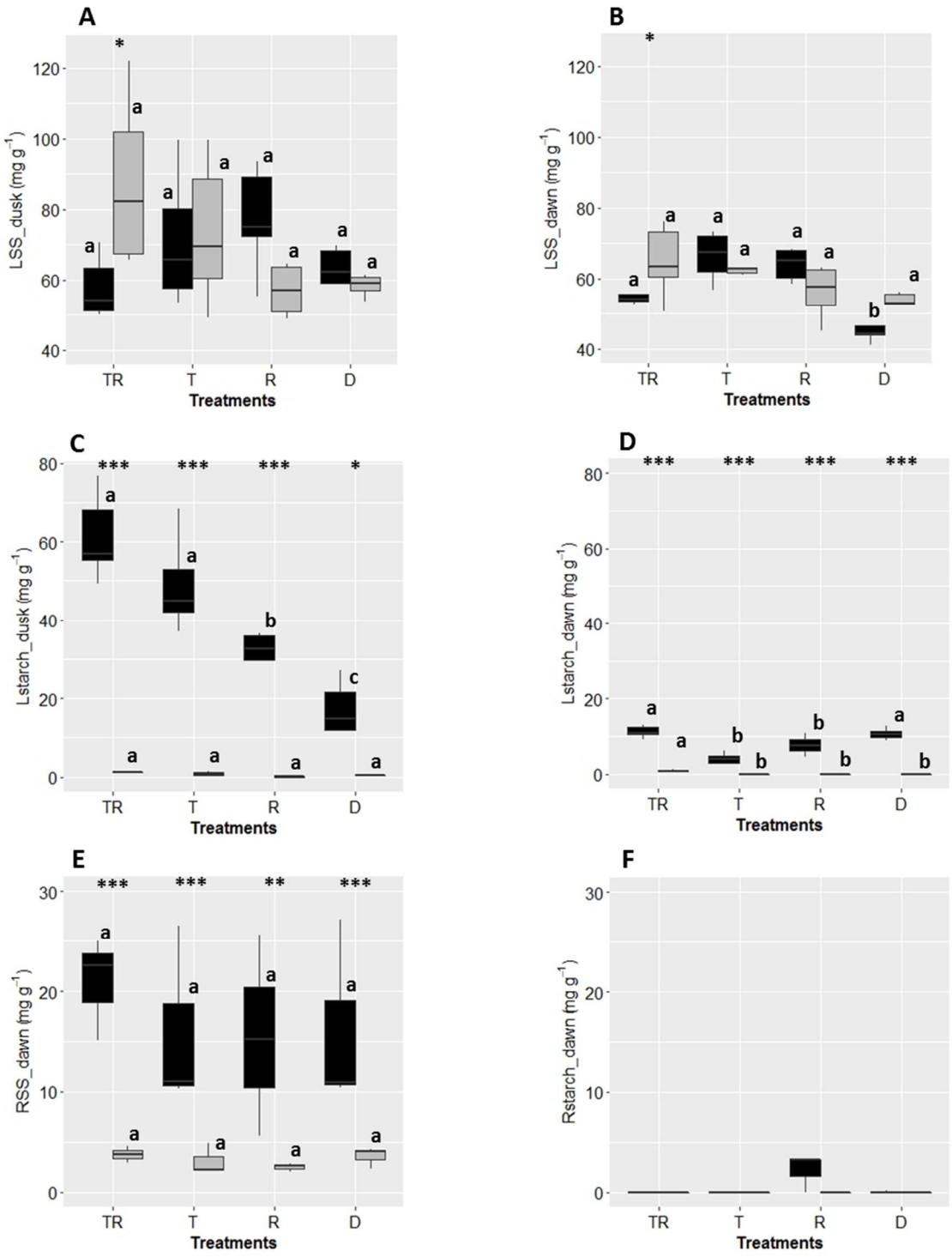

Fig. 4. Effects of treatments on the leaf soluble sugars (LSS, mg g^{-1}, N=6 for each treatment) at (**A**) dusk and (**B**) dawn, and leaf starch (L_{starch}, mg g^{-1}, N=6 for each treatment) at (**C**) dusk and (**D**) dawn, and (E) root soluble sugars (RSS, mg g^{-1}, N=3 for each treatment) at dawn and (F) root starch (R_{starch}, mg g^{-1}, N=3 for each treatment) in *Aechmea aquilega* (black) and *Lutheria splendens* (grey). Error bars above and below the boxes indicate the 90th and 10th percentiles, and the ends of the boxes indicate the 25th and 75th percentiles and solid circles indicate outliers. Different letters for each lamina tissues indicate significant differences (Tukey's test, P<0.05) between treatments (TR, T, R and D). Asterisks indicate statistically significant differences for each treatment between species (Tukey's test, P<0.05; *, <0.05; **, <0.005 and *** < 0.0005).

3.2 Water status

Mid-day water potential (Ψ_{md}) and osmotic potential (Π_{osm}) were very high in both species in well-watered condition but were significantly reduced after 2 months of drought, with respectively -0.72 ± 0.09 MPa and -1.07 ± 0.07 MPa, in *A. aquilega* and with respectively -1.71 ± 0.13 MPa and -1.65 ± 0.14 MPa, in *L. splendens* (**Table 1, Fig. 5A, B**). When only the plant roots were watered (R treatment), Ψ_{md} and Π_{osm} of *A. aquilega* did not differ significantly from the values recorded in the TR and T treatments, whereas, in *L. splendens, these* values were intermediate between the TR or the T treatment, and the D treatment.

Similarly, the RWC and the leaf thickness (LT) of the aerial and basal parts of the leaves of *A. aquilega* were significantly reduced by 45-55% and 30-50%, respectively in the D treatment compared with in the other treatments (**Table 1, Fig. 5C-F**). In *L. splendens*, RWC and LT were significantly reduced, by 35% and 10-25% respectively, when only the plant roots were watered (R treatment) compared to well-watered plants (TR), indicating that this species suffers from water stress. When *L. splendens* individuals were not watered, RWC and LT were reduced by 50-65% and 30-45%, respectively, compared to well-watered plants.

The decrease in leaf thickness was mainly due to a decrease in hydrenchyma thickness (HT) (**Table 1, Supplemental Figure S5**). For *L. splendens*, the abaxial HT of the aerial part and the adaxial HT of the basal part of the leaves were significantly reduced in the R treatment compared to the TR and T treatments. Hydrenchyma of *A. aquilega* leaves were reduced in both the adaxial and abaxial parts but only for the aerial part of the leaves. Differences were more pronounced in both species in the D treatment, with a decrease in the mesophyll thickness (MT) as well.

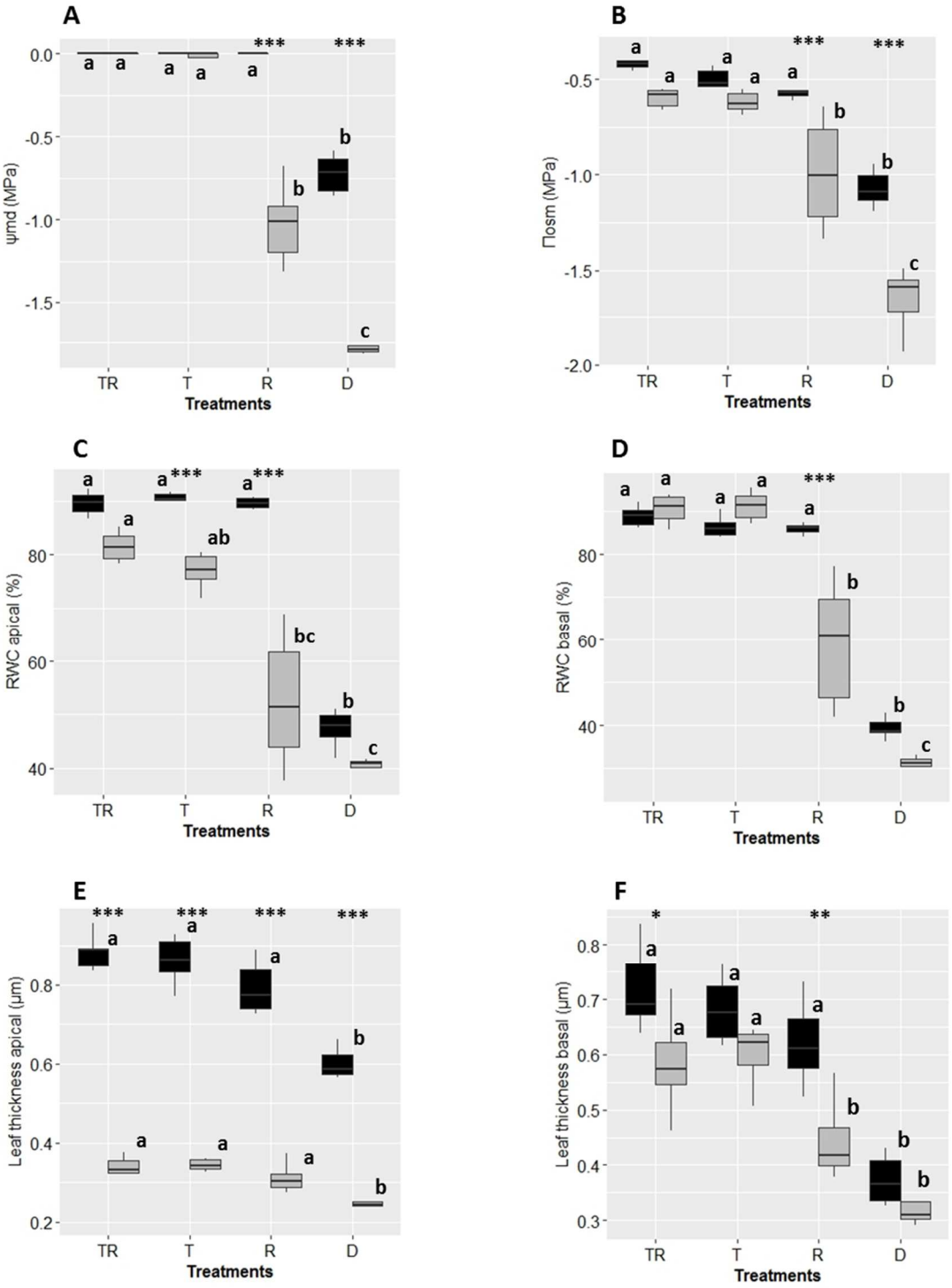

Fig. 5. Effects of treatments on (**A**) midday water potential (Ψ_{md}, MPa, N=6 for each treatment) and (**B**) osmotic potential (Π_{osm}, MPa, N=6 for each treatment), on the relative water content (RWC, %, N=6 for each treatment) at (**C**) the apical and (**D**) basal part of the leaf, and finally on the leaf thickness (LT, µm, N=6 for each treatment) at (**E**) the apical and (**F**) basal part of the leaf in *Aechmea aquilega* (black) and *Lutheria splendens* (grey). Error bars above and below the boxes indicate the 90th and 10th percentiles, and the ends of the boxes indicate the 25th and 75th percentiles and solid circles indicate outliers. Different letters for each lamina tissues indicate significant differences (Tukey's test, P<0.05) between treatments (TR, T, R and D). Asterisks indicate statistically significant

differences for each treatment between species (Tukey's test, P<0.05; *, <0.05; **, <0.005 and *** < 0.0005).

3.3 Nutrient uptake

The leaf N content (N_{nat}, %) and the leaf $\delta^{15}N$ ($\delta^{15}N_{nat}$, ‰) of unlabelled plants did not differ significantly between treatments except in *A. aquilega* in the D treatment with higher values than in the other treatments (**Table 1, Supplemental Figure S6**). Supplying the *A. aquilega* root system with the ^{15}N-enriched solution resulted in a significant increase in leaf $\delta^{15}N$ ($\delta^{15}N_{lab}$ =4012 ± 1195 ‰) and leaf N (N_{lab} = 0.71 ± 0.1 %) compared to natural abundance in the same treatment (10.28 ± 2.5‰ and 0.48 ± 0.01%, Tukey's test, p= 0.01 and p= <0.0001, respectively) (**Table 1, Fig. 6**). On the contrary, in *L. splendens*, when the root system was watered with ^{15}N-labelled solution, leaf $\delta^{15}N$ increased only marginally compared to the natural abundance (48 ± 15 ‰ and 7.5 ± 1.4 ‰, Tukey's test, p= 0.009, respectively) and did not enable significant N uptake as the leaf N remained constant at 0.78% (**Table 1, Fig 6, and Supplemental Figure S6**). Finally, absorption of N and ^{15}N were significantly higher when the *A. aquilega* tank was watered compared to when the roots were watered, and were significantly higher when both the tank and the roots were watered (**Fig. 6**).

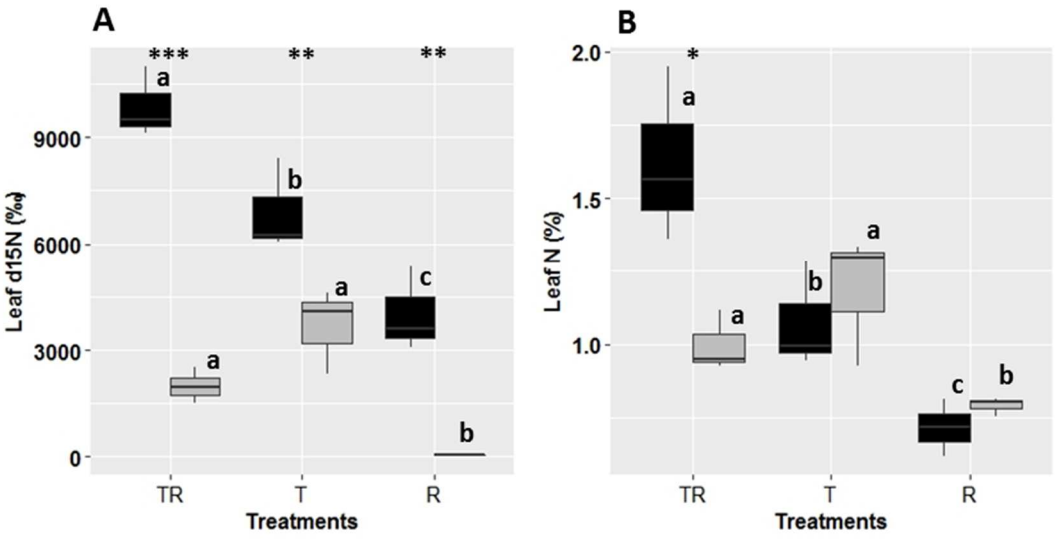

Fig. 6. Effects of ^{15}N labelling on (**A**) leaf $\delta^{15}N$ (‰, N=6 for each treatment) and (**B**) leaf N (%, N=6 for each treatment) according to the watering treatment for *Aechmea aquilega* (black) and *Lutheria splendens* (grey). Error bars above and below the boxes indicate the 90th and 10th percentiles, and the ends of the boxes indicate the 25th and 75th percentiles and solid circles indicate outliers. Different letters for each lamina tissues indicate significant differences (Tukey's test, P<0.05) between treatments (TR, T, R and D). Asterisks indicate statistically significant differences for each treatment between species (Tukey's test, P<0.05; *, <0.05; **, <0.005 and *** < 0.0005).

4. Discussion

Our study revealed that the two bromeliads species differ substantially in the role played by LATs *vs.* roots in resource uptake. In *A. aquilega,* both LATs and roots absorbed water and nutrients whereas in *L. splendens,* roots were less important than the role played by LATs. These results were supported by a unique set of functional traits related to species response to water depletion.

4.1 Physiological response of tank form bromeliads to water depletion

Our study showed that 2 months of drought stress significantly reduced bromeliad metabolism, since growth, carbon acquisition and storage (RGR, A_{max}, g_{smax}, malate and starch content), water storage (RWC, LT, HT), and water and osmotic potential (Ψ_{md}, Π_{osm}) were all reduced compared to well-watered plants. Symptoms of drought stress have also been documented in various bromeliad species (e.g., Bader et al., 2009; Ceusters et al., 2009; Nowak and Martin, 1997; Stiles and Martin, 1996; Vanhoutte et al., 2016) and in other epiphytic or terrestrial families (e.g., Chiang et al., 2013; Herrera et al., 2000; Schmidt and Kaiser, 1987; Zhang et al., 2016).

Drought stress significantly reduced nocturnal acidification in *A. aquilega* because stomatal closure prevents nocturnal fixation of external CO_2 through a notable reduction of malate content in leaves. Additionally, because starch is considered as the only source of hexose for acid synthesis (Popp et al., 2003), a reduction in starch content with drought was correlated with a reduction in malate content. While starch might act as a storage compound in *A. aquilega*, its low level (about 0.2%) in *L. splendens* led us to hypothesise that other biochemical forms of C storage exist. In fact, in most of higher plants, in addition to starch, which is a common storage compound (Martínez-Vilalta et al., 2016; Plavcová et al., 2016), other species dependent biochemical forms of storage may accumulate, such as fructans in grassland species (Zwicke et al., 2015) or neutral lipids (triacylglycerols) in fat trees (Fischer and Höll, 1991; Hoch, 2015; Hoch et al., 2003; Moraes et al., 2016). In contrast, drought stress did not significantly modify leaf and root soluble sugars in the two species studied here. Maintenance of the level of soluble carbohydrate contents while photosynthetic activity was low, could be explained by (i) mobilisation of starch and/or other storage compounds and their interconversion into soluble sugars and by (ii) reduced growth. Obviously, our knowledge of the composition of storage compounds in bromeliads is still poor, as is their importance in mechanisms involved in desiccation tolerance in these species (Vieira et al., 2017). Because the types of carbohydrates involved (e.g. glucose, fructose, sucrose, starch, fructans, etc.) differ across bromeliad species (Christopher and Holtum, 1998), to better understand the regulatory mechanisms of carbon metabolism involved in response to drought stress, further quantification of carbohydrate diversity is required in the two species.

Water stress can cause failure of soluble sugar transport in the phloem, thus limiting carbohydrate use (McDowell, 2011). The marked reduction in Ψ_{md}, Π_{osm}, RWC and leaf thickness with drought indicated that the two bromeliads suffered from water stress, which likely prevented the transport of soluble sugar to enable constant amounts of sugars to be maintained. Additionally, the decrease in leaf thickness was mainly due to dehydration and shrinkage of the hydrenchyma in both species and also of the mesophyll in *A. aquilaga*. Hydrenchyma is considered as a water reservoir to be used maintain a favourable water status in the mesophyll (Freschi et al., 2010b). In *Tillandsia ionantha* (Nowak and Martin, 1997) and *Guzmania monostachia* (Freschi et al., 2010b), cell shrinkage was detected in the hydrenchyma whereas the mesophyll mainly maintained their original size even after 2 months of drought stress. However, in our study, we also observed mesophyll tissue dehydration and cell shrinkage in both species, suggesting a strong impact on hydraulic conductance properties and photosynthetic activity.

4.2 Evidence for a contrasted role for roots in water and nutrient uptake in the two species

Because epiphytism favours LATs and *vice versa* (Givnish et al., 2014), the role of LATs in resource uptake in all Tillandsioideae species and tank-forming Bromelioideae no longer needs demonstrating (see Benzing, 1976; North et al., 2013; Papini et al., 2010). Our results, as well, showed that LATs played an essential role in water and nutrient uptake in both species. Although it has been widely accepted that the roots of epiphytic bromeliads are often reduced to holdfasts (Benzing, 2000), we clearly showed that in *A. aquilega,* roots also play a role in resource uptake. When only their root system was watered, *A. aquilega* individuals showed traits similar to well-watered plants (except for net photosynthesis assimilation, leaf starch at dusk and dawn, and the adaxial hydrenchyma thickness of the apical portion of the lamina) whereas *L. splendens* trait values were intermediate between well-watered and drought stressed plants (i.e., RWC, ψ_{md}, Π_{osm}) or similar to drought stressed plants (i.e., NbN_{leaf}, RGR, LT, A_{sat}, g_{ssat}).

The ^{15}N-labelling further indicated that the roots of *A. aquilega* and *L. splendens* play a contrasted role in resource uptake. The roots of *L. splendens* enabled only minor resource uptake which was not sufficient to avoid water stress, as most of the traits were considerably reduced. *Lutheria splendens* consequently appeared to absorb water and nutrients mainly via the LATs, as also found for *Guzmania lingulata* (Nadkarni and Primack, 1989). On the contrary, the root system of the horticultural Tillandsioideae *Guzmania* 'Rana', *Guzmania lingulata* and *Vriesea* 'Harmony' contributed to water and nutrient uptake (Silva et al., 2018; Vanhoutte et al., 2016). Concerning *A. aquilega*, when only the roots received the ^{15}N-labelled solution, our results are evidence for a higher leaf δ^{15}N compared to *L. splendens*, and subsequently, an increase of the leaf N if compared to plants before the ^{15}N enrichment. Thus, roots of *A. aquilega* contribute to the plant's nutrition. Also,

503 because water status traits (i.e., RWC, ψmd, Πosm) were not reduced compared to those in well-
504 watered plants, we further provide evidence that resource uptake solely by the roots of *A. aquilega*
505 enables sufficient carbon exchange and conservation (although the net photosynthetic assimilation
506 and leaf starch were reduced) to maintain plant growth (e.g, RGR, new leaves) compared to well-
507 watered plants. Thus, based on traits measured after 2-month experiment, resource uptake by the
508 roots of *A. aquilega* seems to be as efficient as uptake by the LATs. The roots of *Nidularium minutum*
509 and *A. fasciata* were found to be more efficient in providing water and nutrient uptake than the
510 LATs, thereby enhancing plant performance (Carvalho et al., 2017; Kämpf, 1994).
511 The results of our ^{15}N-labelling experiment are evidence for a synergistic effect of combined
512 watering of tank and roots in *A. aquilega*. These results showed that resource uptake was higher (i.e.,
513 higher leaf N and δ^{15}N) when both the tank and the roots of the plants were watered compared to
514 only the LATs (or the roots). Sieber (1955) reported higher growth in *A. fasciata* and *Nidularium*
515 *innocentii* when both the LATs and roots were supplied with nutrients instead of only supplying the
516 LATs. Over 2-month experiment, we did observe only higher net photosynthesis assimilation and leaf
517 starch content for the TR compared to T treatment, certainly because bromeliads, like vascular
518 epiphytes in general, are slow-growing species (e.g., Laube and Zotz, 2003; Schmidt and Zotz, 2002).
519 It thus cannot be excluded that, over a longer period of time, secondary rooted *A. aquilega*
520 individuals might perform better than epiphytic ones.
521
522 ***4.3 Similar root anatomy but distinct root metabolism***
523 Based on the existence of root hairs, velamen radicum and vascular cylinder in the distal part of the
524 roots of the two bromeliads (**Fig. 1G-H**), resource uptake capacity is likely to be similar in the two
525 species. Equivalent root anatomy was found in *Nidularium minutum*, a tank bromeliad, but with a
526 terrestrial habit, for which the roots contributed to nutrient uptake most likely assisted by the
527 presence of velamen (Carvalho et al., 2017). Although the general assumption has been that
528 bromeliads lack a velamen radicum, a few studies demonstrated the existence of this structure in
529 both terrestrial and epiphytic species (e.g., Pita and Menezes, 2002; Proença and das Graças Sajo,
530 2008; Silva and Scatena, 2011). Although it is known that the velamen of orchids roots facilitates
531 water and nutrient uptake (Zotz and Winkler, 2013), to our knowledge, no studies have investigated
532 its functional aspects in bromeliads. The contrasting responses of *A. aquilega* and *L. splendens*
533 individuals when only their roots were watered suggests that the absorption and/or transportation
534 capacity of resources may differ among species. This question is still unexplored in bromeliads and
535 merits further investigation.
536 Our results support major differences in NSC content in the two species, which may explain the
537 contrasting role of roots in water and nutrient uptake. While the roots of the two species are

undoubtedly not starch storage organs, starch was found in the leaves and soluble sugars were found in both the leaves and the roots of *A. aquilega*, whereas by contrast, no starch was found in the leaves and only negligible amounts of soluble sugars were found in the roots of *L. splendens*. Soluble sugars are known to perform a variety of functions which support functions involving rapid consumption (e.g., growth, respiration, defense) and play a role in non-consumption functions such as intermediary metabolites, osmolytes, substrates for transport and ion uptake (Farrar and Jones, 2000; Martínez-Vilalta et al., 2016). Because soluble sugars are fundamental metabolites involved in the regulation of root metabolism (Delhon et al., 1996; Rufty et al., 1989), our results indirectly suggest that the roots of *A. aquilega* were metabolically active whereas those of *L. splendens* were not. For now, studies of NSC contents in bromeliads are still incomplete and further investigations are required for a better overview of their partitioning, particularly the segmentation between roots and the rosette leaf part, and according to root and leaf ontogenies.

The contribution of roots to nutrient uptake has been shown to diverge even in the same bromeliad species. For example, in *Aechmea fasciata*, Kämpf (1994) found the root system to be more efficient than the LATs in resource uptake whereas, in greenhouse conditions, Winkler and Zotz (2009) concluded that the same species was unable to take up phosphorous via its roots. These confusing results suggest a possible change in biochemical properties of nutrient-membrane transporters in bromeliad roots. Such membrane transporters have already been described for a number of different N-sources in bromeliad leaves (Inselsbacher et al., 2007; Meisner et al., 2013). Today, the biochemical properties of nutrient-membrane transporters in bromeliad roots remain to be elucidated to better understand the functional role of the roots in water and nutrient uptake.

5. Conclusion

The two tank-forming bromeliads investigated in this study exhibited substantial differences in their carbon, water, and nutrient-related traits when only their root system was watered, *A. aquilega* having trait values indicative of well-watered plants and *L. splendens* having trait values indicative of drought-stressed plants. Consequently, the LATs and roots of the two species play contrasted roles in resource uptake that confirm our hypothesis. Specifically, the roots of *A. aquilega* contributed significantly to water and nutrient uptake, whereas the roots of *L. splendens* were less important than the role played by LATs in resource uptake (not sufficient to maintain baseline metabolism). We further provide evidence for a synergistic effect of combined watering of tank and roots in *A. aquilega*. Finally, the results of our study call for a more complex interpretation of LATs *vs.* roots metabolism. Roots of epiphytic bromeliads do not only play a role in anchoring the plant, as reported in numerous studies, rather there appears to be a continuum, from roots able to take up resources to roots unable to uptake resources, depending on the species. Future works should explore the role of

roots in resource uptake according to lineages (at the sub-family level), functional types, and ontogenic development to better understand the plasticity of epiphytic bromeliad species.

Appendix Supplementary data

Table S1. Time schedule of the 2-month experiment.

Figure S2. Schematic representation of longitudinal section of a tank-bromeliad showing where each trait was measured.

Figure S3. Photosynthetic light-response curve of *Lutheria splendens*.

Figure S4. Effect of water supply on gas exchange.

Figure S5. Effect of water supply on the thickness of the different leaf tissues.

Figure S6. Effect of water supply on natural abundance of leaf $\delta^{15}N$ and leaf N.

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References

Amorós, A., Zapata, P., Pretel, M.T., Botella, M.A., Serrano, M., 2003. Physico-chemical and physiological changes during fruit development and ripening of five loquat (*Eriobotrya Japonica* Lindl.) cultivars. Food Sci. Technol. Int. 9, 43–51.

Bader, M.Y., Menke, G., Zotz, G., 2009. Pronounced drought tolerance characterizes the early life stages of the epiphytic bromeliad *Tillandsia flexuosa*. Funct. Ecol. 23, 472–479.

Bartlett, M.K., Scoffoni, C., Ardy, R., Zhang, Y., Sun, S., Cao, K., Sack, L., 2012. Rapid determination of comparative drought tolerance traits: using an osmometer to predict turgor loss point. Methods Ecol. Evol. 3, 880–888.

607 Benzing, D.H., 2000. Bromeliaceae: profile of an adaptive radiation. Cambridge University Press,
608 Cambridge, UK, Cambridge.
609 Benzing, D.H., 1990. Vascular epiphytes: general biology and related biota. Cambridge University
610 Press, Cambridge.
611 Benzing, D.H., 1976. Bromeliad trichomes: structure, function, and ecological significance. Selbyana 1,
612 330–348.
613 Benzing, D.H., Ott, D.W., 1981. Vegetative reduction in epiphytic Bromeliaceae and Orchidaceae: its
614 origin and significance. Biotropica 13, 131–140.
615 Carvalho, J.L., Hayashi, A.H., Kanashiro, S., Tavares, A.R., 2017. Anatomy and function of the root
616 system of bromeliad *Nidularium minutum*. Aust. J. Bot. 65, 550–555.
617 Ceusters, J., Borland, A.M., Londers, E., Verdoodt, V., Godts, C., De Proft, M.P., 2009. Differential
618 usage of storage carbohydrates in the CAM bromeliad *Aechmea* 'Maya' during acclimation to
619 drought and recovery from dehydration. Physiol. Plant. 135, 174–184.
620 Ceusters, J., Borland, A.M., Londers, E., Verdoodt, V., Godts, C., De Proft, M.P., 2008. Diel shifts in
621 carboxylation pathway and metabolite dynamics in the CAM Bromeliad *Aechmea* 'Maya' in
622 response to elevated CO_2. Ann. Bot. 102, 389–397.
623 Chiang, J.-M., Lin, T.-C., Luo, Y.-C., Chang, C.-T., Cheng, J.-Y., Martin, C.E., 2013. Relationships among
624 rainfall, leaf hydrenchyma, and Crassulacean acid metabolism in *Pyrrosia lanceolata* (L.) Fraw.
625 (Polypodiaceae) in central Taiwan. Flora - Morphol. Distrib. Funct. Ecol. Plants 208, 343–350.
626 Chow, P.S., Landhausser, S.M., 2004. A method for routine measurements of total sugar and starch
627 content in woody plant tissues. Tree Physiol. 24, 1129–1136.
628 Christopher, J.T., Holtum, J.A.M., 1998. Carbohydrate partitioning in the leaves of Bromeliaceae
629 performing C_3 photosynthesis or Crassulacean acid metabolism. Funct. Plant Biol. 25, 371–376.
630 https://doi.org/10.1071/pp98005
631 Crayn, D.M., Winter, K., Smith, J.A.C., 2004. Multiple origins of crassulacean acid metabolism and the
632 epiphytic habit in the Neotropical family Bromeliaceae. Proceeding Natl. Acad. Sci. 101, 3703–
633 3708.
634 Delhon, P., Gojon, A., Tillard, P., Passama, L., 1996. Diurnal regulation of $N0_3$ uptake in soybean plants
635 IV. Dependence on current photosynthesis and sugar availability to the roots. J. Exp. Bot. 47, 893–
636 900.
637 Farrar, J.F., Jones, D.L., 2000. The control of carbon acquisition by roots. New Phytol. 147, 43–53.
638 Fischer, C., Höll, W., 1991. Food reserves of Scots pine (*Pinus sylvestris* L.). Trees - Struct. Funct. 5,
639 187–195.

Freschi, L., Rodriguez, M.A., Tiné, M.A.S., Mercier, H., 2010a. Correlation between citric acid and nitrate metabolisms during CAM cycle in the atmospheric bromeliad *Tillandsia pohliana*. J. Plant Physiol. 167, 1577–1583.

Freschi, L., Takahashi, C.A., Cambui, C.A., Semprebom, T.R., Cruz, A.B., Mioto, P.T., de Melo Versieux, L., Calvente, A., Latansio-Aidar, S.R., Aidar, M.P.M., Mercier, H., 2010b. Specific leaf areas of the tank bromeliad *Guzmania monostachia* perform distinct functions in response to water shortage. J. Plant Physiol. 167, 526–533.

Givnish, T.J., Barfuss, M.H., Ee, B.V., Riina, R., Schulte, K., Horres, R., Gonsiska, P.A., Jabaily, R.S., Crayn, D.M., Smith, J.A., Winter, K., Brown, G.K., Evans, T.M., Holst, B.K., Luther, H., Till, W., Zizka, G., Berry, P.E., Sytsma, K.J., 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny. Am. J. Bot. 98, 872–895.

Givnish, T.J., Barfuss, M.H., Ee, B.V., Riina, R., Schulte, K., Horres, R., Gonsiska, P.A., Jabaily, R.S., S, R., Crayn, D.M., Smith, J.A.C., Winter, K., Brown, G.K., Evans, T.M., Holst, B.K., Luther, H., Till, W., Zizka, G., Berry, P.E., Sytsma, K.J., 2014. Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae. Mol. Phylogenet. Evol. 71, 55–78.

Gonçalves, A.Z., Mercier, H., Oliveira, R.S., Romero, G.Q., 2016. Trade-off between soluble protein production and nutritional storage in Bromeliaceae. Ann. Bot. 118, 1199–1208.

Gotsch, S.G., Nadkarni, N., Darby, A., Glunk, A., Dix, M., Davidson, K., Dawson, T.E., 2015. Life in the treetops: ecophysiological strategies of canopy epiphytes in a tropical montane cloud forest. Ecol. Monogr. 85, 393–412.

Herrera, A., Fernandez, M., Taisma, M.A., 2000. Effects of drought on CAM and water relations in Plants of *Peperomia carnevalii*. Ann. Bot. 86, 511–517.

Hoch, G., 2015. Carbon reserves as indicators for carbon limitation in trees, in: Lüttge, U., Beyschlag, W. (Eds.), Progress in Botany: Vol. 76. Springer International Publishing, pp. 321–346.

Hoch, G., Richter, A., Körner, C., 2003. Non-structural carbon compounds in temperate forest trees. Plant Cell Environ. 26, 1067–1081.

Inselsbacher, E., Cambui, C.A., Stange, C.F., Mercier, H., Wanek, W., 2007. Microbial activities and foliar uptake of nitrogen in the epiphytic bromeliad *Vriesea gigantea*. New Phytol. 175, 311–320.

Kämpf, A.N., 1994. Adubação foliar em *Aechmea fasciata* (Lindley) Baker. Bromélia 16–20.

Laube, S., Zotz, G., 2003. Which abiotic factors limit vegetative growth in a vascular epiphyte? Funct. Ecol. 17, 598–604.

Leroy, C., Maes, A. Q., Louisanna, E., Séjalon-Delmas, N., in press. How significant are endophytic fungi in seeds and seedlings? Effects on germination, survival and performances of two epiphytic plant species. Fungal Ecol. 39, 296-306.

Leroy, C., Carrias, J.-F., Corbara, B., Pélozuelo, L., Dézerald, O., Brouard, O., Dejean, A., Céréghino, R., 2013. Mutualistic ants contribute to tank-bromeliad nutrition. Ann. Bot. 112, 919–926.

Lüttge, U., 2008. Physiological ecology of tropical plants, Second ed. Springer Verlag, Berlin.

Males, J., 2016. Think tank: water relations of Bromeliaceae in their evolutionary context. Bot. J. Linn. Soc. 181, 415–440.

Maréchaux, I., Bartlett, M.K., Sack, L., Baraloto, C., Engel, J., Joetzjer, E., Chave, J., 2015. Drought tolerance as predicted by leaf water potential at turgor loss point varies strongly across species within an Amazonian forest. Funct. Ecol. 29, 1268–1277.

Martin, C.E., 1994. Physiological ecology of the Bromeliaceae. Bot. Rev. 60, 1–82.

Martínez-Vilalta, J., Sala, A., Asensio, D., Galiano, L., Hoch, G., Palacio, S., Piper, F.I., Lloret, F., 2016. Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. Ecol. Monogr. 86, 495–516.

McDowell, N.G., 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. PlantT Physiol. 155, 1051–1059.

Meisner, K., Winkler, U., Zotz, G., 2013. Heteroblasty in bromeliads – anatomical, morphological and physiological changes in ontogeny are not related to the change from atmospheric to tank form. Funct. Plant Biol. 40, 251–262.

Moraes, M.G., Carvalho, M.A.M., Franco, A.C., Pollock, C.J., Figueiredo-Ribeiro, R. de C.L., 2016. Fire and drought: soluble carbohydrate storage and survival mechanisms in herbaceous plants from the Cerrado. BioScience 66, 107–117.

Nadkarni, N.M., Primack, R.B., 1989. The use of gamma spectrometry to measure within plant nutrient allocation of a tank bromeliad, *Guzmania lingulata*. Selbyana 11, 22–25.

Nievola, C.C., Mercier, H., 1996. The importance of leaf and root systems in nitrate assimilation in *Vriesea fosteriana*. Bromélia 3, 14–18.

North, G.B., Lynch, F.H., Maharaj, F.D., Phillips, C.A., Woodside, W.T., 2013. Leaf hydraulic conductance for a tank bromeliad: axial and radial pathways for moving and conserving water. Front. Plant Sci. 4, 78–78.

Nowak, E.J., Martin, C.E., 1997. Physiological and anatomical responses to water deficits in the CAM epiphyte *Tillandsia ionantha* (Bromeliaceae). Int. J. Plant Sci. 158, 818–826.

Nyman, L.P., Davis, J.P., O'Dell, S.J., Arditti, J., Stephens, G.C., Benzing, D.H., 1987. Active uptake of amino acids by leaves of an epiphytic vascular plant, *Tillandsia paucifolia* (Bromeliaceae). Plant Physiol. 83, 681–684.

Papini, A., Tani, G., Di Falco, P., Brighigna, L., 2010. The ultrastructure of the development of *Tillandsia* (Bromeliaceae) trichome. Flora 205, 94–100.

Pita, P.B., Menezes, N.L., 2002. Anatomia da raiz de espécies de *Dyckia* Schult. f. e *Encholirium* Mart. ex Schult. & Schult. f. (Bromeliaceae, Pitcairnioideae) da Serra do Cipó (Minas Gerais, Brasil), com especial referência ao velame. Rev. Bras. Bot. 25, 25–34.

Plavcová, L., Hoch, G., Morris, H., Ghiasi, S., Jansen, S., 2016. The amount of parenchyma and living fibers affects storage of non tructural carbohydrates in young stems and roots of temperate trees. Am. J. Bot. 103, 603–612.

Popp, M., Janett, H.P., Medina, E., 2003. Metabolite gradients and carbohydrate translocation in rosette leaves of CAM and C_3 bromeliads. New Phytol. 157, 649–656.

Pridgeon, A.M., 1987. The velamen and exodermis of orchid roots, in: Arditti, J. (Ed.), Orchid Biology, Reviews and Perspectives, IV. Ithaca, NY: Cornell University Press, pp. 139–192.

Proença, S.L., das Graças Sajo, M., 2008. Rhizome and root anatomy of 14 species of Bromeliaceae. Rodriguésia 59, 113–128.

R Development Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.Rproject.org/

Rufty, T.W., MacKown, C.T., Volk, R.J., 1989. Effects of altered carbohydrate availability on whole-Plant. Plant Physiol. 89, 457–463.

Schmidt, G., Zotz, G., 2002. Inherently slow growth in two Caribbean epiphytic species: A demographic approach. J. Veg. Sci. 13, 527–534.

Schmidt, J.E., Kaiser, W.M., 1987. Response of the succulent leaves of *Peperomia magnoliaefolia* to dehydration: water relations and solute movement in chlorenchyma and hydrenchyma. Plant Physiol. 83, 190–194.

Sieber, J., 1955. Untersuchungen über die wasser- und Nährstoffaufnahme bei epiphytischen trichterbildenden bromeliaceen. Gartenbauwissenschaft 141–164.

Silva, K.G. da, Ferreira, M.L., Silva, E.A. da, Kanashiro, S., Camargo, P.B. de, Tavares, A.R., 2018. Nitrogen efficiency indexes for evaluating nitrogen uptake and use in ornamental bromeliad's root system and tank. Pesq. Agropec. Bras. 53, 703–709.

Silva, I.V. da, Scatena, V.L., 2011. Anatomia de raízes de nove espécies de Bromeliaceae (Poales) da região amazônica do estado de Mato Grosso, Brasil. Acta Bot. Bras. 25, 618–627.

Stiles, K.C., Martin, C.E., 1996. Effects of drought stress on CO_2 exchange and water relations in the CAM epiphyte *Tillandsia utriculata* (Bromeliaceae). J. Plant Physiol. 149, 721–728.

Takahashi, C. A., Ceccantini, G. C. T., & Mercier, H., 2007. Differential capacity of nitrogen assimilation between apical and basal leaf portions of a tank epiphytic bromeliad. Braz. J. Plant Physiol. 19, 119–126.

Van Handel, E., 1965. Estimation of glycogen in small amounts of tissue. Anal. Biochem. 256–265.

743 Vanhoutte, B., Ceusters, J., De Proft, M.P., 2016. The ‘tubing’ phenomenon in commercial cultivation
744 of *Guzmania*: morphology, physiology and anatomy. Sci. Hortic. 205, 112–118.
745 Vanhoutte, B., Schenkels, L., Ceusters, J., Proft de, M.P., 2017. Water and nutrient uptake in *Vriesea*
746 cultivars: Trichomes vs. Roots. Environ. Exp. Bot. 136, 21–30.
747 Vieira, E.A., Silva, K.R., Oriani, A., Moro, C.F., Braga, M.R., 2017. Mechanisms of desiccation tolerance
748 in the bromeliad *Pitcairnia burchellii* Mez: biochemical adjustments and structural changes. Plant
749 Physiol. Biochem. 121, 21–30.
750 Winkler, U., Zotz, G., 2010. ‘And then there were three’: highly efficient uptake of potassium by foliar
751 trichomes of epiphytic bromeliads. Ann. Bot. 106, 421-427.
752 Winkler, U., Zotz, G., 2009. Highly efficient uptake of phosphorus in epiphytic bromeliads. Ann. Bot.
753 103, 477–484.
754 Zhang, W., Hu, H., Zhang, S.B., 2016. Divergent adaptive strategies by two co-occurring epiphytic
755 orchids to water stress: escape or avoidance? Front. Plant Sci. 7, 588–588.
756 Zotz, G., Winkler, U., 2013. Aerial roots of epiphytic orchids: the velamen radicum and its role in
757 water and nutrient uptake. Oecologia 171, 733–741.
758 Zwicke, M., Picon-Cochard, C., Morvan-Bertrand, A., Prud’homme, M.-P., Volaire, F., 2015. What
759 functional strategies drive drought survival and recovery of perennial species from upland
760 grassland? Ann. Bot. 116, 1001–1015.

Table 1. Results from the 2-way ANOVA for the effects of species (*Aechmea aquilega* and *Lutheria splendens*), treatments (TR, T, R and D) and their interaction. F-values and P-values are displayed. See text for abbreviations.

Traits	Species		Treatment		Species*Treatment	
	F	P	F	P	F	P
Growth, photosynthesis and Carbohydrates content						
NbN_{leaf}	20.09	**<0.0001**	18.50	**<0.0001**	4.06	**0.0132**
NbD_{leaf}	38.07	**<0.0001**	25.43	**<0.0001**	1.81	0.161
RGR (cm cm^{-1} day^{-1})	19.29	**<0.0001**	57.18	**<0.0001**	10.27	**<0.0001**
A_{sat} (µmol m^{-2} s^{-1})	38.87	**<0.0001**	19.40	**<0.0001**	3.61	**0.036**
g_{ssat} (mol m^{-2} s^{-1})	7.88	**0.0126**	17.83	**<0.0001**	1.36	0.289
Malate	-	-	8.79	**0.0006**	-	-
LSS_dusk (mg g^{-1})	0.07	0.78	0.28	0.83	6.79	**<0.0001**
LSS_dawn (mg g^{-1})	1.32	0.256	9.01	**<0.0001**	5.37	**0.003**
RSS_dawn (mg g^{-1})	36.35	**<0.0001**	0.77	0.527	0.27	0.84
L_{starch}_dusk (mg g^{-1})	29.22	**<0.0001**	17.36	**<0.0001**	5.01	**0.004**
L_{starch}_dawn (mg g^{-1})	303.48	**<0.0001**	23.81	**<0.0001**	4.46	**0.008**
R_{starch}_dawn (mg g^{-1})	4.71	**0.045**	2.25	0.12	2.25	0.512
Water status						
Aerial part of the leaf						
RWC (%)	65.82	**<0.0001**	31.52	**<0.0001**	3.38	**0.0275**
LT (µm)	188.42	**<0.0001**	20.73	**<0.0001**	0.64	0.591
CET_sup (µm)	11.39	**0.0016**	4.35	**0.009**	2.26	0.0962
HT_sup (µm)	25.04	**<0.0001**	45.13	**<0.0001**	17.51	**<0.0001**
MT (µm)	86.01	**<0.0001**	2.53	0.0706	1.06	0.376
HT_inf (µm)	0.75	0.389	39.99	**<0.0001**	3.66	**0.031**
CET_inf (µm)	0.04	0.8439	3.46	**0.0252**	0.76	0.521
Ψ_{md} (MPa)	0.49	0.484	25.03	**<0.0001**	1.75	0.173
Π_{osm} (MPa)	66.29	**<0.0001**	56.91	**<0.0001**	1.56	0.214
Basal part of the leaf						
RWC (%)	11.22	**0.0018**	221.11	**<0.0001**	18.30	**<0.0001**
LT (µm)	26.85	**<0.0001**	49.47	**<0.0001**	2.38	0.084
CET_sup (µm)	0.33	0.567	8.28	**0.0002**	1.92	0.142
HT_sup (µm)	0.02	0.886	24.81	**<0.0001**	2.09	0.116
MT (µm)	54.14	**<0.0001**	7.85	**0.0003**	2.08	0.118
HT_inf (µm)	2.65	0.112	11.35	**<0.0001**	0.82	0.489
CET_inf (µm)	20.99	**<0.0001**	4.03	**0.013**	0.512	0.676
Nutrient uptake						
Natural abundance						
N_{nat} (%)	72.24	**<0.0001**	6.17	**0.005**	2.06	0.153
$\delta^{15}N_{nat}$ (‰)	15.89	**0.001**	1.17	0.34	3.67	**0.003**
^{15}N-labelling						
N_{lab} (%)	118.31	**<0.0001**	27.32	**<0.0001**	9.69	**0.003**
$\delta^{15}N_{lab}$ (‰)	2.97	0.10	14.92	**<0.0001**	7.95	**0.006**

Bold characters indicate that the P-value is significant.

Figure captions

Fig. 1. Experimental (**A**) *Aechmea aquilega* and (**B**) *Lutheria splendens* in 1 litre horticultural plastic pot. Light micrographs of hand-cut transverse section of (**C, D**) the aerial and (**E, F**) the basal part of the lamina of (**C, E**) *A. aquilega* and (**D, F**) *L. splendens*. CE_{ad} = adaxial cuticle and epidermis, H_{ad} = adaxial hydrenchyma, M = Mesophyll, H_{ab} = abaxial hydrenchyma, CE_{ab} = abaxial cuticle and epidermis, VB = Vascular bundle. Light micrographs of hand-cut transverse section near the apex of the root of (**G**) *A. aquilega* and (**H**) *L. splendens*. R_{hairs} = root hairs, V = velamen, C_{outer} = outer cortex, VC = vascular cylinder, C_{inner} = inner cortex. * = indicates the presence of LATs. Scale bars for A and B = 10 cm and scale bars for all anatomical sections = 200 µm.

Fig. 2. Effect of water supply on plant growth. Effects of treatments (TR, T, R and D) on (A) the number of new leaves (NbN_{leaf}, N=6 for each treatment), (B) number of dead leaves (NbD_{leaf}, N=6 for each treatment) and (C) RGR (N=6 for each treatment) in *Aechmea aquilega* (black) and *Lutheria splendens* (grey). Error bars above and below the boxes indicate the 90th and 10th percentiles, and the ends of the boxes indicate the 25th and 75th percentiles and solid circles indicate outliers. Different letters indicate significant differences for each species between treatments (Tukey's test, P<0.05). Asterisks indicate statistically significant differences for each treatment between species (Tukey's test, P<0.05; *, <0.05; **, <0.005 and *** < 0.0005).

Fig. 3. Diel course of net photosynthesis assimilation (µmol m^{-2} s^{-1}) of (**A**) *Aechmea aquilega* (CAM photosynthetic pathway, N=3 for each treatment) and (**B**) *Lutheria splendens* (C_3 photosynthetic pathway, N=3 for each treatment) according to the four watering treatments (TR, T, R and D). The grey area indicates the night period. Different letters indicate significant differences (Tukey's test, P<0.05) between treatments (TR, T, R and D). In (**A**) the inset graph shows the malate content (mg g^{-1}) of *A. aquielaga* according to the treatments.

Fig. 4. Effects of treatments on the leaf soluble sugars (LSS, mg g^{-1}, N=6 for each treatment) at (**A**) dusk and (**B**) dawn, and leaf starch (L_{starch}, mg g^{-1}, N=6 for each treatment) at (**C**) dusk and (**D**) dawn, and (E) root soluble sugars (RSS, mg g^{-1}, N=3 for each treatment) at dawn and (F) root starch (R_{starch}, mg g^{-1}, N=3 for each treatment) in *Aechmea aquilega* (black) and *Lutheria splendens* (grey). Error bars above and below the boxes indicate the 90th and 10th percentiles, and the ends of the boxes indicate the 25th and 75th percentiles and solid circles indicate outliers. Different letters for each lamina tissues indicate significant differences (Tukey's test, P<0.05) between treatments (TR, T, R and D). Asterisks indicate statistically significant differences for each treatment between species (Tukey's test, P<0.05; *, <0.05; **, <0.005 and *** < 0.0005).

Fig. 5. Effects of treatments on (**A**) midday water potential (Ψ_{md}, MPa, N=6 for each treatment) and (**B**) osmotic potential (Π_{osm}, MPa, N=6 for each treatment), on the relative water content (RWC, %, N=6 for each treatment) at (**C**) the apical and (**D**) basal part of the leaf, and finally on the leaf thickness (LT, µm, N=6 for each treatment) at (**E**) the apical and (**F**) basal part of the leaf in *Aechmea aquilega* (black) and *Lutheria splendens* (grey). Error bars above and below the boxes indicate the 90^{th} and 10^{th} percentiles, and the ends of the boxes indicate the 25^{th} and 75^{th} percentiles and solid circles indicate outliers. Different letters for each lamina tissues indicate significant differences (Tukey's test, P<0.05) between treatments (TR, T, R and D). Asterisks indicate statistically significant differences for each treatment between species (Tukey's test, P<0.05; *, <0.05; **, <0.005 and *** < 0.0005).

Fig. 6. Effects of ^{15}N labelling on (**A**) leaf $\delta^{15}N$ (‰, N=6 for each treatment) and (**B**) leaf N (%, N=6 for each treatment) according to the watering treatment for *Aechmea aquilega* (black) and *Lutheria splendens* (grey). Error bars above and below the boxes indicate the 90^{th} and 10^{th} percentiles, and the ends of the boxes indicate the 25^{th} and 75^{th} percentiles and solid circles indicate outliers. Different letters for each lamina tissues indicate significant differences (Tukey's test, P<0.05) between treatments (TR, T, R and D). Asterisks indicate statistically significant differences for each treatment between species (Tukey's test, P<0.05; *, <0.05; **, <0.005 and *** < 0.0005).