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1 **Water and nutrient uptake capacity of leaf-absorbing trichomes vs. roots in epiphytic tank**  
2 **bromeliads**

3

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19

20 **Abstract**

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

35

36 **Keywords:** Carbon metabolism, Nutrient uptake, <sup>15</sup>N labelling, Plant performance, Tank bromeliad,  
37 Water status

38

39 **Highlights**

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

44

45

## 46 1. Introduction

47 Vascular epiphytes, which grow on other plants without parasitism, have no contact with terrestrial  
48 soil resources, and consequently need to take up nutrients from rainfall, throughfall and stemflow  
49 water and/or from decomposing organic matter in the canopy (Gotsch et al., 2015). Epiphytes have  
50 evolved numerous remarkable adaptations (e.g., litter-trapping leaf arrangements, water-storing  
51 phytotelmata, leaf-absorbing trichomes, velamen radicum) to facilitate nutrient uptake (Benzing,  
52 1990; Lüttge, 2008; Pridgeon, 1987). Bromeliads, one of the largest and most widespread families of  
53 vascular plants in the Neotropics, display many of these adaptations.

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

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

81 failed to detect any (Nadkarni and Primack, 1989; Winkler and Zotz, 2009) or very little root nutrient  
82 uptake (Nievola and Mercier, 1996), others underlined efficient root nutrient uptake (Silva et al.,  
83 2018; Carvalho et al., 2017; Vanhoutte et al., 2017, 2016). More studies are thus needed to better  
84 grasp the role of roots in water and nutrient uptake in comparison to that of LATs.

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

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

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

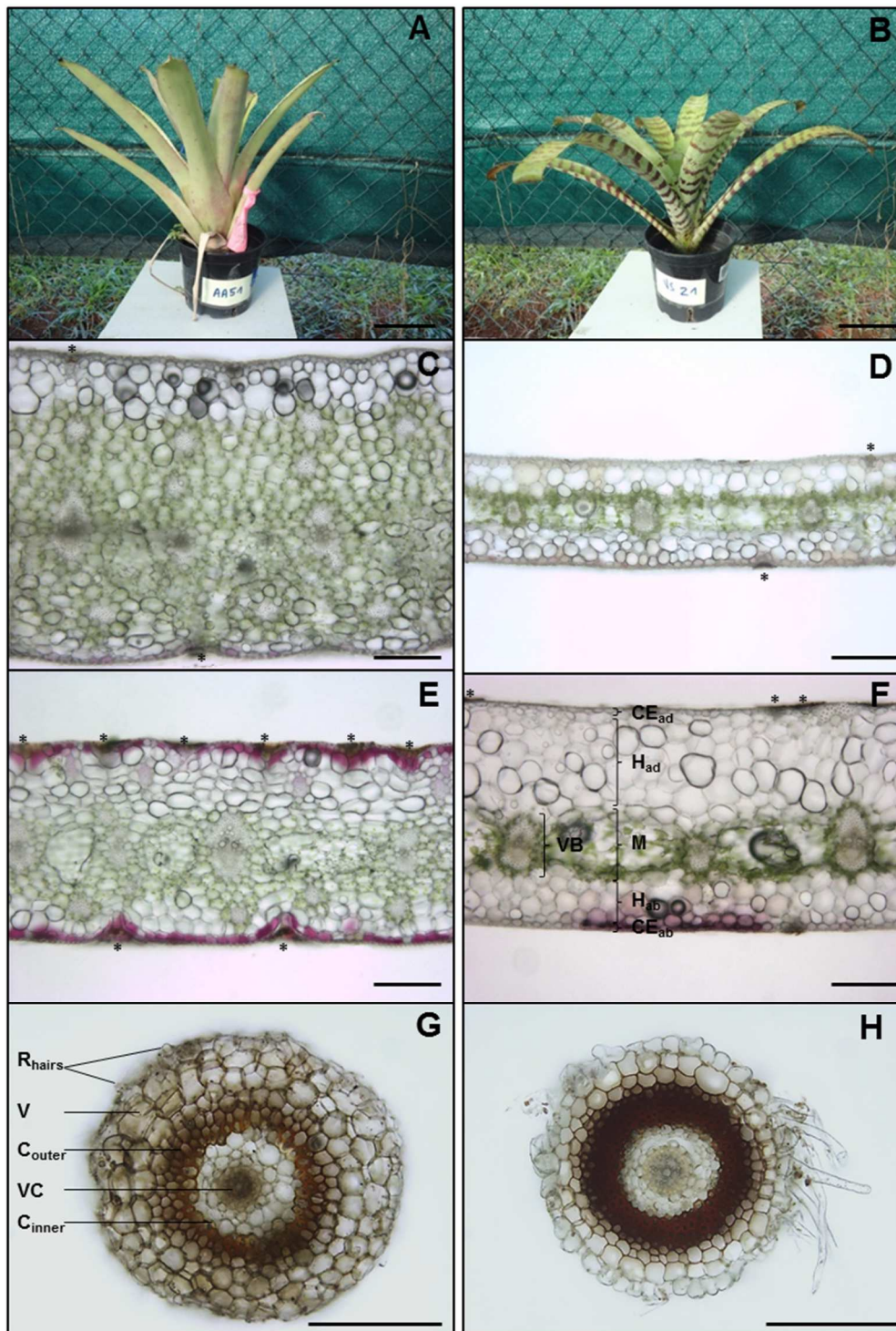
120

## 121 **2. Materials and methods**

### 122 **2.1. Plant materials and growth conditions**

123 *Aechmea aquilega* (Salib.) Griseb (**Fig. 1A**) is a Type III tank-forming bromeliad belonging to the  
124 subfamily Bromelioideae with CAM photosynthesis (Crayn et al., 2004). This species occurs as an  
125 epiphytic, rupicolous or secondary terrestrial bromeliad in full sun or partial shade environments  
126 (Leroy et al., 2013). **Adult tank** form *A. aquilega* growing in a shaded greenhouse at the *Campus*  
127 *agronomique* in Kourou French Guiana were used for the experiment. The plants ( $n=24$ ) were  
128 characterised by a tank water volume of  $116.2 \pm 23.1$  mL, a number of leaves of  $9.7 \pm 0.2$ , a total  
129 height (distance from the bottom of the body to the top of the crown) of  $27.1 \pm 0.8$  cm, a canopy  
130 width (maximum distance between the tips of the leaves, two measurements taken at an angle of  
131  $90^\circ$ ) of  $24.2 \pm 1.4$  cm and a length of  $26.7 \pm 0.9$  cm, with a  $4.5 \pm 0.1$  cm width for the longest leaf. **The**  
132 **leaf appearance, estimated on a 6-month period, was in average every  $26.03 \pm 3.73$  days.**





133

134 **Fig. 1.** Experimental (A) *Aechmea aquilega* and (B) *Lutheria splendens* in 1 litre horticultural plastic  
 135 pot. Light micrographs of hand-cut transverse section of (C, D) the aerial and (E, F) the basal part of  
 136 the lamina of (C, E) *A. aquilega* and (D, F) *L. splendens*. CE<sub>ad</sub> = adaxial cuticle and epidermis, H<sub>ad</sub> =  
 137 adaxial hydrenchyma, M = Mesophyll, H<sub>ab</sub> = abaxial hydrenchyma, CE<sub>ab</sub> = abaxial cuticle and  
 138 epidermis, VB = Vascular bundle. Light micrographs of hand-cut transverse section near the apex of  
 139 the root of (G) *A. aquilega* and (H) *L. splendens*. R<sub>hairs</sub> = root hairs, V = velamen, C<sub>outer</sub> = outer cortex,  
 140 VC = vascular cylinder, C<sub>inner</sub> = inner cortex, \* = indicates the presence of LATs. Scale bars for A and B  
 141 = 10 cm and scale bars for all anatomical sections = 200 μm.  
 142

143 *Lutheria splendens* (Brongn.) Lem. (**Fig. 1B**) is a Type IV tank-forming bromeliad in the subfamily  
144 Tillandsioideae with C<sub>3</sub> 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<sup>-2</sup> s<sup>-1</sup> for *L. splendens*, while the outside PAR was 1808.5 ± 103.1 μmol m<sup>-2</sup> s<sup>-1</sup>.

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



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

185

### 186 2.3 Growth and carbon metabolism

187 *Leaf survival and growth*— At beginning of the experiment (t1) and after 2 months (t2), we measured  
188 the total number of leaves and the length of one growing leaf in order to calculate the number of  
189 new leaves ( $N_{\text{Nleaf}}$ ), the number of dead leaves ( $N_{\text{Dleaf}}$ ) and the relative growth rate (RGR). The RGR  
190 ( $\ln(\text{cm}) \cdot \text{day}^{-1}$ ) was calculated based on Gonçalves et al. (2016) as:  $((\ln \text{Length}_{\text{t2}} - \ln \text{Length}_{\text{t1}}) / (\text{t2} -$   
191  $\text{t1}))$ , with  $\ln \text{Length}_{\text{t1}}$  and  $\ln \text{Length}_{\text{t2}}$  as the means of natural logarithm transformed of the  
192 youngest leaf length at the beginning (t1) and at the end (t2) of the experiment period, respectively.

193 *Gas exchange*— For each species, net photosynthesis assimilation ( $A$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and  
194 stomatal conductance to  $\text{H}_2\text{O}$  ( $g_s$ ,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) were measured on three out of six individuals (on  
195 the first young expanded leaf, see Supplemental Figure S2) in each of the four watering treatments  
196 after 2 months. All measurements were made continuously throughout a day and a night from 9 AM  
197 to 8 AM the following day using three Li-Cor 6400XT portable photosynthesis systems. The light PAR  
198 level was set to  $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$  for *A. aquilega* determined from direct PAR measurements in the  
199 greenhouse environment and to  $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$  for *L. splendens* from preliminary light- curves (see  
200 Supplemental Figure S3) from 9 AM to 4 PM. Next, we switched to natural PAR conditions from 4 PM  
201 to 8 AM the following day by using the “track PAR out” mode. Leaf temperature,  $\text{CO}_2$  concentration  
202 and air flow in the chamber were set at  $27^\circ\text{C}$ , 400 ppm and  $250 \mu\text{mol s}^{-1}$ , respectively. To compare  
203 treatments, we calculated maximum net photosynthesis assimilation ( $A_{\text{max}}$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and  
204 maximum stomatal conductance for water vapour ( $g_{\text{smax}}$ ,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) by averaging the five highest  
205 gas exchange ( $\text{CO}_2$  and  $\text{H}_2\text{O}$ ) values.

206 *Metabolite sampling protocol*— The tip of the second young expanded leaf (N=6 leaves per  
207 treatment) was harvested at 6 PM and the tip of the third young expanded leaf (see Supplemental  
208 Figure S2) the following morning at 6 AM corresponding to minimum and maximum malate  
209 concentrations (for the CAM species) and the reverse for storage carbohydrates (both CAM and  $\text{C}_3$ ),  
210 respectively (Ceusters et al., 2008). Roots (N=3 per treatment) were harvested at 6 AM. Samples  
211 were immediately frozen in liquid nitrogen, then stored in a freezer until they were freeze-dried  
212 (Alpha 1-2 LD; Christ, Osterode am Harz, Germany). Each sample was ground to a fine powder in an

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

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

224 *Non-structural carbohydrate extraction*— Soluble sugars were extracted from 10 to 15 mg powder  
225 mixed in 0.5 mL 80% ethanol (v/v) and incubated for 20 min at  $80^\circ\text{C}$ . Extraction was repeated twice  
226 and all three supernatants were collected and dried (Refrigerated CentriVap Vacuum Concentrators,  
227 Labconco). The resulting soluble sugar extract was solubilised in 1.5 ml ultrapure water. Total soluble  
228 sugar concentrations were determined by spectrophotometry at 620 nm (spectrophotometer UV-  
229 visible DU 640 B, Beckman Coulter, USA) using anthrone reagent (Van Handel, 1965) and glucose as  
230 standard. The pellet containing starch was extracted in 1.5 ml of 0.2 M KOH solution and incubated  
231 for 20 min at  $80^\circ\text{C}$  then hydrolysed in glucose molecules with amyloglucosidase (Sigma, EC 3.2.1.3).  
232 Starch concentrations were determined by spectrophotometry at 530 nm as described in (Chow and  
233 Landhausser, 2004) using a glucose oxidase and peroxidase/orthodiansidine reagent (Sigma, EC  
234 1.11.1.7; EC 1.1.3.4; EC 243-737-5). Soluble sugars and starch concentrations, expressed as mg  
235 equivalent glucose  $\text{g}^{-1}$  DM, were thus determined for leaves (LSS and  $L_{\text{starch}}$ ) and roots (RSS and  $R_{\text{starch}}$ ).  
236

## 237 **2.4 Water status**

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

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

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

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

266 *Osmotic potential*— Leaf osmotic potential ( $\Psi_{\text{osm}}$ ) was measured with a vapour pressure  
267 osmometer (VAPRO 5520, Wescor, Logan, UT, USA). One disc was collected in the middle of the  
268 aerial part of the third young expanded leaf with a 7-mm-diameter cork borer. The disc was wrapped  
269 in foil and frozen by immersion in liquid nitrogen ( $\text{N}_2$ ) for at least 4 min, then immediately punctured  
270 15 to 20 times with a sharp needle and sealed in the osmometer chamber. The disc was exposed to  
271 air for less than 40 s during all the steps between harvesting the disc and sealing it in the osmometer.  
272 The equilibrium solute concentration value  $c_0$  ( $\text{mmol kg}^{-1}$ ) was recorded from the osmometer when  
273 the difference between consecutive measurements fell below  $5 \text{ mmol kg}^{-1}$ . This value was converted  
274 to  $\Psi_{\text{osm}}$  (MPa) using the Van't Hoff equation relating solute concentration to vapour pressure (Bartlett  
275 et al., 2012; Maréchaux et al., 2015).

276

## 277 **2.5 Nutrient uptake**

278  *$^{15}\text{N}$ -labelling design*— We further investigated the functional role of LATs vs. roots in water and  
279 nutrient uptake by supplying *A. aquilega* and *L. splendens* with a nitrogen solution artificially  
280 enriched with  $^{15}\text{N}$ . At the end of the 2-month experiment, we selected three plants per treatment  
281 (TR, T and R) for each species. The  $^{15}\text{N}$ -enriched solution consisted of 7 L of rainwater with 2 g of  
282  $\text{NH}_4^{15}\text{NO}_3$  (10 atom %  $^{15}\text{N}$ , Isotec Inc., OH, USA) and 2 g of  $^{15}\text{NH}_4\text{NO}_3$  (10 atom %  $^{15}\text{N}$ , Isotec Inc., OH,

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

286 *Isotopic and elementary analyses*— Pieces of young mature leaves were collected before (i.e.,  
287 from unlabelled plants to record the natural abundance, N<sub>nat</sub> and δ<sup>15</sup>N<sub>nat</sub>) and one week after the <sup>15</sup>N-  
288 enrichment period (i.e., from labelled plants to record the enrichment level, N<sub>lab</sub> and δ<sup>15</sup>N<sub>lab</sub>). All the  
289 samples were freeze-dried before isotopic analyses. About 1 g of dried leaf sample was used to  
290 measure the concentration of N (N, %) and <sup>15</sup>N isotopic abundance (δ<sup>15</sup>N, ‰). Stable isotope  
291 analyses were conducted at the Cornell University Stable Isotope laboratory (Ithaca, NY, USA) using a  
292 Thermo-Finnigan DELTA<sup>plus</sup> Advantage gas isotope-ratio mass spectrometer plumbed to a Carlo Erba  
293 NC2500 elemental analyser through a ConFlo II open split interface for elemental and isotopic  
294 composition of samples. The isotopic signal for N was expressed as <sup>15</sup>N delta (δ ‰) versus an  
295 international standard (N<sub>2</sub> in the air) as follows:

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

297

## 298 **2.6 Statistical analyses**

299 Experiments were conducted using a full factorial randomised design for each species. Two-way  
300 ANOVAs were used to test for the effect of species, treatment and their interactions in all functional  
301 traits measured. Data were log or rank transformed (GenABEL package), when necessary, to satisfy  
302 the assumptions of the ANOVA. When significant, the ANOVAs were followed by a Tukey's honestly  
303 significant difference (HSD) test for a posteriori testing of multiple means. **To determine which of the**  
304 **treatments affect the daily course of net photosynthesis assimilation we used a linear mixed model**  
305 **procedure with time and treatment as fixed factors.** All statistical analyses were evaluated using a  
306 95% confidence interval and were conducted using R version, 2.14.1. (R Development Core Team,  
307 2015). The results are presented as means ± 1 standard error.

308

## 309 **3. Results**

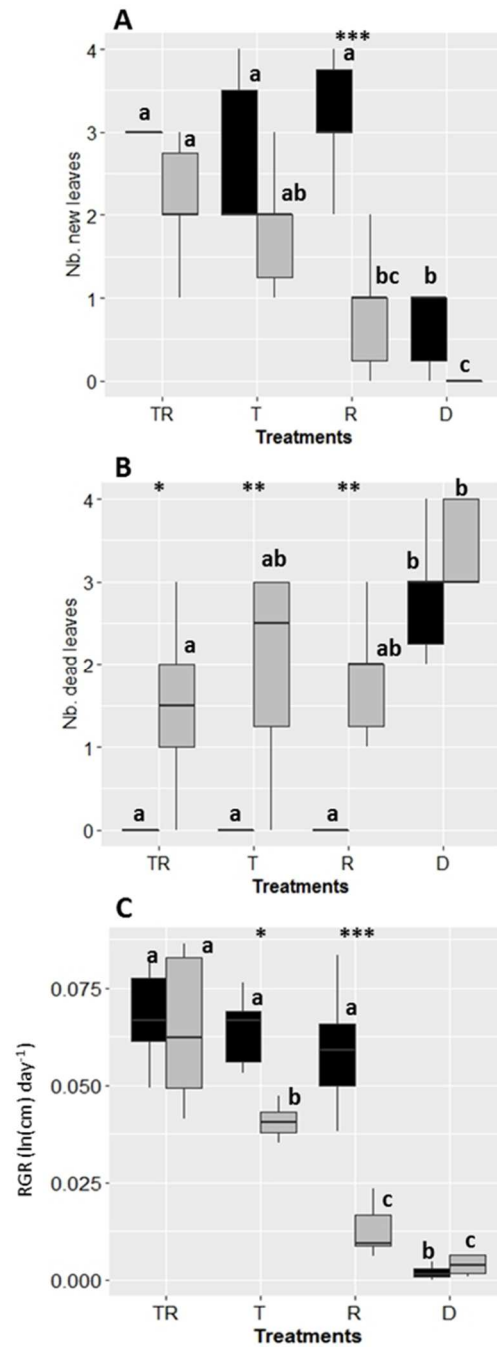
310 Species and treatment had significant effects on most of the measured traits (**Table 1**). The  
311 interaction between species and treatment had significant effects on some of the traits, indicating  
312 that the two species responded differently to the treatments.

313

### 314 **3.1 Growth, photosynthesis and carbohydrate content**

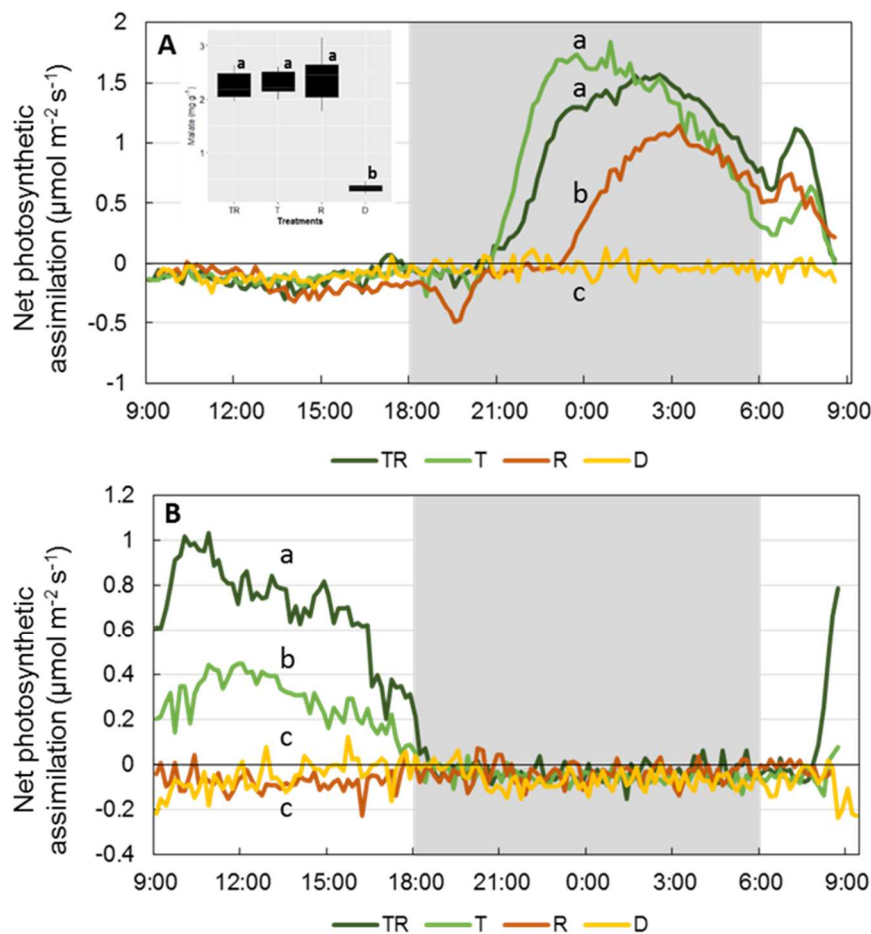
315 In *L. splendens*, NbN<sub>leaf</sub> and the RGR were significantly lower in the R and D treatments than in the TR  
316 treatment, while in *A. aquilega*, significant differences were only found between the D treatment

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



319  
 320 **Fig. 2.** Effect of water supply on plant growth. Effects of treatments (TR, T, R and D) on (A) the  
 321 number of new leaves ( $NbN_{leaf}$ ,  $N=6$  for each treatment), (B) number of dead leaves ( $NbD_{leaf}$ ,  $N=6$  for  
 322 each treatment) and (C) RGR in *Aechmea aquilega* (black) and *Lutheria splendens* (grey). Error bars  
 323 above and below the boxes indicate the 90th and 10th percentiles, and the ends of the boxes  
 324 indicate the 25th and 75th percentiles and solid circles indicate outliers. Different letters indicate  
 325 significant differences for each species between treatments (Tukey's test,  $P<0.05$ ). Asterisks indicate  
 326 statistically significant differences for each treatment between species (Tukey's test,  $P<0.05$ ; \*,  
 327  $<0.05$ ; \*\*,  $<0.005$  and \*\*\*  $<0.0005$ ).  
 328

329 The daily course of the net photosynthesis assimilation did not differ in the TR and T treatments in  
 330 *A. aquilega* but was significantly reduced in the R treatment and nil in the D treatment (Table 1, Fig.  
 331 3A). When only the roots were watered (R treatment),  $A_{max}$  ( $1.12 \pm 0.32 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and  $g_{smax}$  ( $0.011$   
 332  $\pm 0.002 \text{ mol m}^{-2} \text{s}^{-1}$ ) were reduced but not significantly different compared to the TR ( $1.56 \pm 0.25$   
 333  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and  $0.013 \pm 0.003 \text{ mol m}^{-2} \text{s}^{-1}$ , respectively) and T treatments ( $1.81 \pm 0.34 \mu\text{mol m}^{-2} \text{s}^{-1}$   
 334 and  $0.015 \pm 0.003 \text{ mol m}^{-2} \text{s}^{-1}$ , respectively). The malate content in the R treatment ( $2.40 \pm 0.40 \text{ mg g}^{-1}$ )  
 335 was at the same level as in the TR and T treatments ( $2.25 \pm 0.22 \text{ mg g}^{-1}$  and  $2.28 \pm 0.20 \text{ mg g}^{-1}$ ,  
 336 respectively; Table 1, Fig. 3A, and Supplemental Figure S4).  $A_{max}$  ( $0.13 \pm 0.07 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and  $g_{smax}$   
 337 ( $0.0006 \pm 0.0002 \text{ mol m}^{-2} \text{s}^{-1}$ ) were almost nil and the malate content ( $0.33 \pm 0.07 \text{ mg g}^{-1}$ ) was  
 338 significantly reduced under water shortage (D treatment). In *L. splendens*, the daily course of the net  
 339 photosynthesis assimilation was significantly reduced in the T treatment compared to the TR  
 340 treatment and was nil in the R and D treatments (Table 1, Fig. 3B). Significant reductions in  $A_{max}$  and  
 341  $g_{smax}$  were observed in the D versus the TR treatments (Table 1, Fig. 3B, and Supplemental Figure  
 342 S4). When only the root system of *L. splendens* was watered (R treatment),  $A_{max}$  ( $0.04 \pm 0.02 \mu\text{mol m}^{-2}$   
 343  $\text{s}^{-1}$ ) and  $g_{smax}$  ( $0.0001 \pm 0.001 \text{ mol m}^{-2} \text{s}^{-1}$ ) values did not significantly differ from those measured in  
 344 plants in the D treatment ( $0.10 \pm 0.13 \text{ mol m}^{-2} \text{s}^{-1}$  and  $0.0008 \pm 0.0007 \text{ mg g}^{-1}$ , respectively).

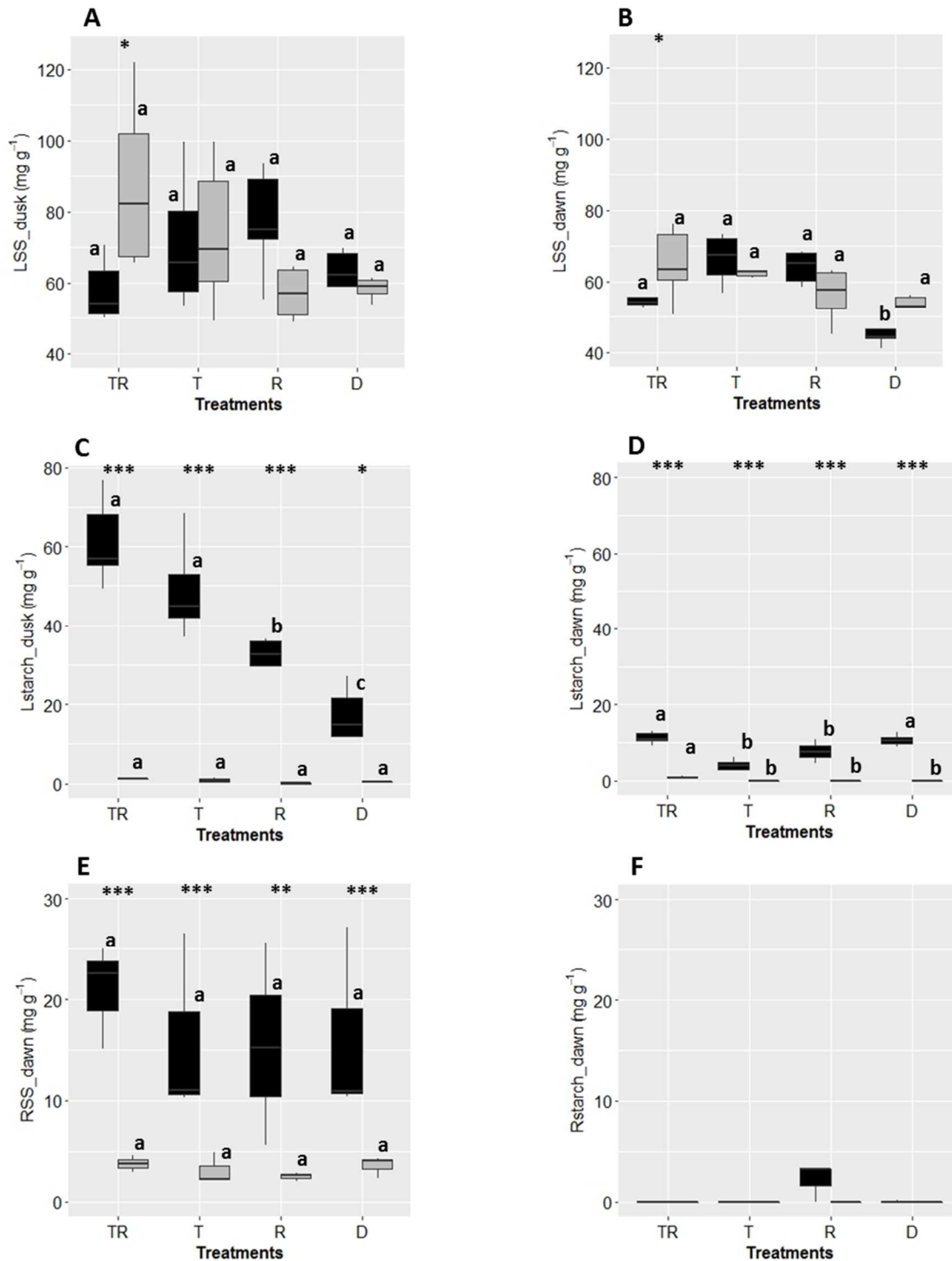


345



346 **Fig. 3.** Diel course of net photosynthesis assimilation ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) of (A) *Aechmea aquilega* (CAM  
347 photosynthetic pathway, N=3 for each treatment) and (B) *Lutheria splendens* (C3 photosynthetic  
348 pathway, N=3 for each treatment) according to the four watering treatments (TR, T, R and D). The  
349 grey area indicates the night period. Different letters indicate significant differences ( $P < 0.05$ )  
350 between treatments (TR, T, R and D). In (A) the inset graph shows the malate content ( $\text{mg g}^{-1} \text{DW}$ ) of  
351 *A. aquilega* according to the treatments (N=6 for each treatment).  
352

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



367

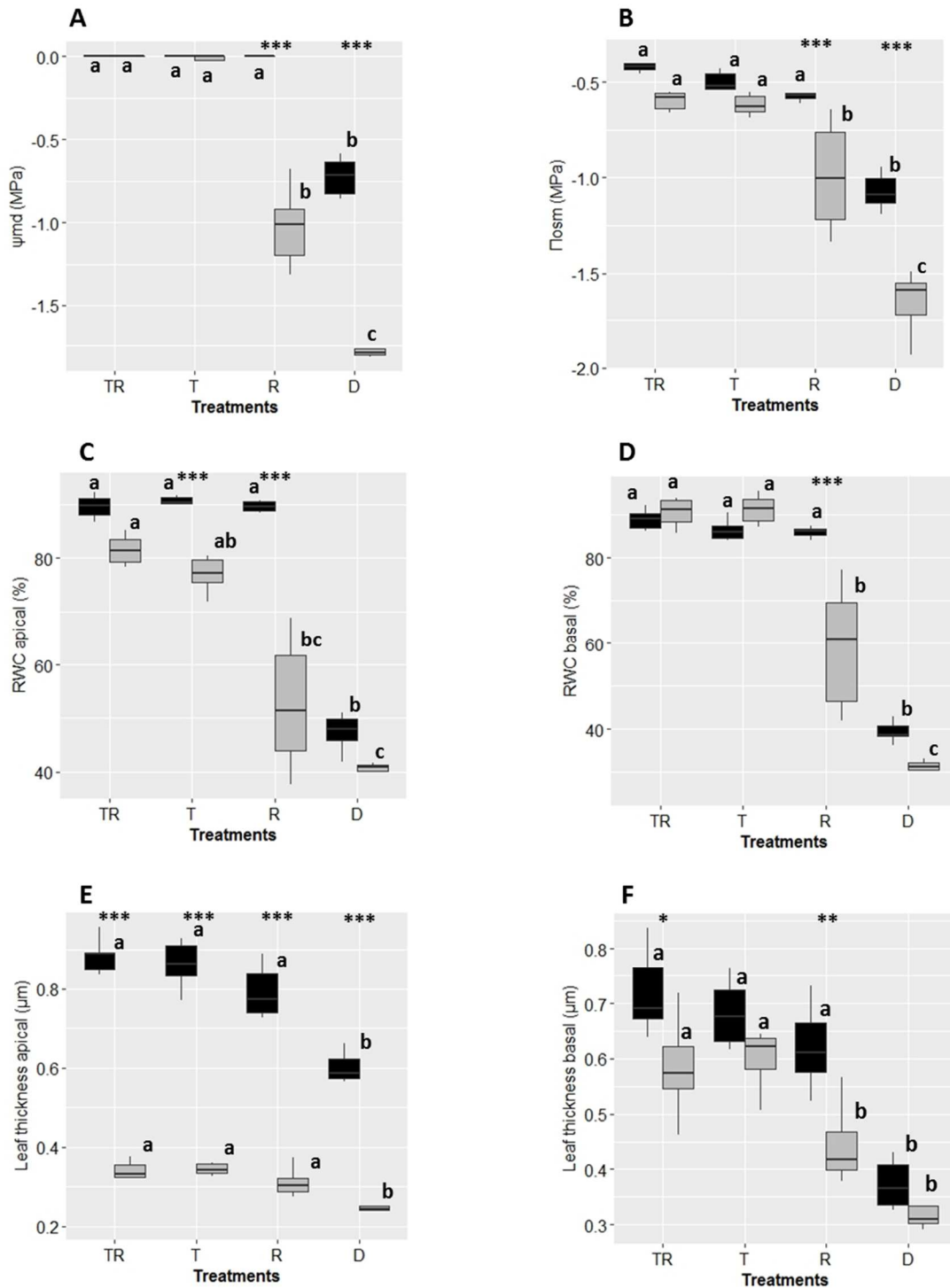
368 **Fig. 4.** Effects of treatments on the leaf soluble sugars (LSS,  $\text{mg g}^{-1}$ , N=6 for each treatment) at (A)  
 369 dusk and (B) dawn, and leaf starch ( $L_{\text{starch}}$ ,  $\text{mg g}^{-1}$ , N=6 for each treatment) at (C) dusk and (D) dawn,  
 370 and (E) root soluble sugars (RSS,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

### 377 3.2 Water status

378 Mid-day water potential ( $\Psi_{\text{md}}$ ) and osmotic potential ( $\Pi_{\text{osm}}$ ) were very high in both species in well-  
379 watered condition but were significantly reduced after 2 months of drought, with respectively  $-0.72 \pm$   
380  $0.09$  MPa and  $-1.07 \pm 0.07$  MPa, in *A. aquilega* and with respectively  $-1.71 \pm 0.13$  MPa and  $-1.65 \pm$   
381  $0.14$  MPa, in *L. splendens* (Table 1, Fig. 5A, B). When only the plant roots were watered (R  
382 treatment),  $\Psi_{\text{md}}$  and  $\Pi_{\text{osm}}$  of *A. aquilega* did not differ significantly from the values recorded in the TR  
383 and T treatments, whereas, in *L. splendens*, these values were intermediate between the TR or the T  
384 treatment, and the D treatment.

385 Similarly, the RWC and the leaf thickness (LT) of the aerial and basal parts of the leaves of *A.*  
386 *aquilega* were significantly reduced by 45-55% and 30-50%, respectively in the D treatment  
387 compared with in the other treatments (Table 1, Fig. 5C-F). In *L. splendens*, RWC and LT were  
388 significantly reduced, by 35% and 10-25% respectively, when only the plant roots were watered (R  
389 treatment) compared to well-watered plants (TR), indicating that this species suffers from water  
390 stress. When *L. splendens* individuals were not watered, RWC and LT were reduced by 50-65% and  
391 30-45%, respectively, compared to well-watered plants.

392 The decrease in leaf thickness was mainly due to a decrease in hydrenchyma thickness (HT) (Table  
393 1, Supplemental Figure S5). For *L. splendens*, the abaxial HT of the aerial part and the adaxial HT of  
394 the basal part of the leaves were significantly reduced in the R treatment compared to the TR and T  
395 treatments. Hydrenchyma of *A. aquilega* leaves were reduced in both the adaxial and abaxial parts  
396 but only for the aerial part of the leaves. Differences were more pronounced in both species in the D  
397 treatment, with a decrease in the mesophyll thickness (MT) as well.



398

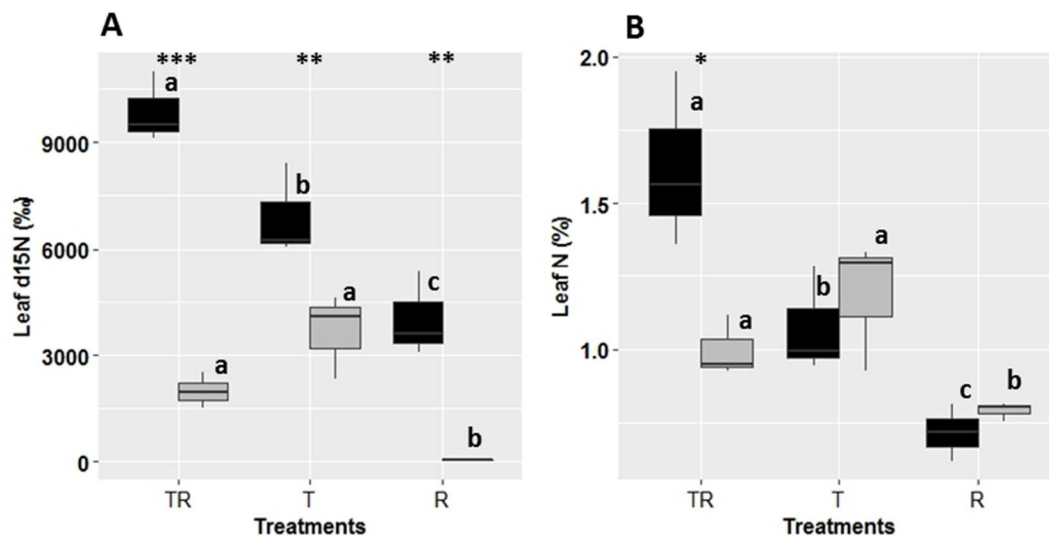
399 **Fig. 5.** Effects of treatments on (A) midday water potential ( $\Psi_{md}$ , MPa, N=6 for each treatment) and  
 400 (B) osmotic potential ( $\Pi_{osm}$ , MPa, N=6 for each treatment), on the relative water content (RWC, %, N=6 for each treatment) at (C) the apical and (D) basal part of the leaf, and finally on the leaf  
 401 thickness (LT,  $\mu\text{m}$ , N=6 for each treatment) at (E) the apical and (F) basal part of the leaf in *Aechmea*  
 402 *aquilega* (black) and *Lutheria splendens* (grey). Error bars above and below the boxes indicate the  
 403 90<sup>th</sup> and 10<sup>th</sup> percentiles, and the ends of the boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles and solid  
 404 circles indicate outliers. Different letters for each lamina tissues indicate significant differences  
 405 (Tukey's test, P<0.05) between treatments (TR, T, R and D). Asterisks indicate statistically significant  
 406

407 differences for each treatment between species (Tukey's test,  $P < 0.05$ ; \*,  $< 0.05$ ; \*\*,  $< 0.005$  and \*\*\*  $<$   
 408 0.0005).

409

### 410 3.3 Nutrient uptake

411 The leaf N content ( $N_{nat}$ , %) and the leaf  $\delta^{15}N$  ( $\delta^{15}N_{nat}$ , ‰) of unlabelled plants did not differ  
 412 significantly between treatments except in *A. aquilega* in the D treatment with higher values than in  
 413 the other treatments (Table 1, Supplemental Figure S6). Supplying the *A. aquilega* root system with  
 414 the  $^{15}N$ -enriched solution resulted in a significant increase in leaf  $\delta^{15}N$  ( $\delta^{15}N_{lab} = 4012 \pm 1195$  ‰) and  
 415 leaf N ( $N_{lab} = 0.71 \pm 0.1$  %) compared to natural abundance in the same treatment ( $10.28 \pm 2.5$ ‰ and  
 416  $0.48 \pm 0.01$ %, Tukey's test,  $p = 0.01$  and  $p = < 0.0001$ , respectively) (Table 1, Fig. 6). On the contrary, in  
 417 *L. splendens*, when the root system was watered with  $^{15}N$ -labelled solution, leaf  $\delta^{15}N$  increased only  
 418 marginally compared to the natural abundance ( $48 \pm 15$  ‰ and  $7.5 \pm 1.4$  ‰, Tukey's test,  $p = 0.009$ ,  
 419 respectively) and did not enable significant N uptake as the leaf N remained constant at 0.78% (Table  
 420 1, Fig 6, and Supplemental Figure S6). Finally, absorption of N and  $^{15}N$  were significantly higher when  
 421 the *A. aquilega* tank was watered compared to when the roots were watered, and were significantly  
 422 higher when both the tank and the roots were watered (Fig. 6).



423

424

425 **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  
 426 each treatment) according to the watering treatment for *Aechmea aquilega* (black) and *Lutheria*  
 427 *splendens* (grey). Error bars above and below the boxes indicate the 90<sup>th</sup> and 10<sup>th</sup> percentiles, and the  
 428 ends of the boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles and solid circles indicate outliers. Different  
 429 letters for each lamina tissues indicate significant differences (Tukey's test,  $P < 0.05$ ) between  
 430 treatments (TR, T, R and D). Asterisks indicate statistically significant differences for each treatment  
 431 between species (Tukey's test,  $P < 0.05$ ; \*,  $< 0.05$ ; \*\*,  $< 0.005$  and \*\*\*  $< 0.0005$ ).

432

433

#### 434 4. Discussion

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

439

#### 440 4.1 Physiological response of tank form bromeliads to water depletion

441 Our study showed that 2 months of drought stress significantly reduced bromeliad metabolism, since  
442 growth, carbon acquisition and storage (RGR,  $A_{max}$ ,  $g_{smax}$ , malate and starch content), water storage  
443 (RWC, LT, HT), and water and osmotic potential ( $\Psi_{md}$ ,  $\Pi_{osm}$ ) were all reduced compared to well-  
444 watered plants. Symptoms of drought stress have also been documented in various bromeliad  
445 species (e.g., Bader et al., 2009; Ceusters et al., 2009; Nowak and Martin, 1997; Stiles and Martin,  
446 1996; Vanhoutte et al., 2016) and in other epiphytic or terrestrial families (e.g., Chiang et al., 2013;  
447 Herrera et al., 2000; Schmidt and Kaiser, 1987; Zhang et al., 2016).

448 Drought stress significantly reduced nocturnal acidification in *A. aquilega* because stomatal  
449 closure prevents nocturnal fixation of external CO<sub>2</sub> through a notable reduction of malate content in  
450 leaves. Additionally, because starch is considered as the only source of hexose for acid synthesis  
451 (Popp et al., 2003), a reduction in starch content with drought was correlated with a reduction in  
452 malate content. While starch might act as a storage compound in *A. aquilega*, its low level (about  
453 0.2%) in *L. splendens* led us to hypothesise that other biochemical forms of C storage exist. In fact, in  
454 most of higher plants, in addition to starch, which is a common storage compound (Martínez-Vilalta  
455 et al., 2016; Plavcová et al., 2016), other species dependent biochemical forms of storage may  
456 accumulate, such as fructans in grassland species (Zwicke et al., 2015) or neutral lipids  
457 (triacylglycerols) in fat trees (Fischer and Höll, 1991; Hoch, 2015; Hoch et al., 2003; Moraes et al.,  
458 2016). In contrast, drought stress did not significantly modify leaf and root soluble sugars in the two  
459 species studied here. Maintenance of the level of soluble carbohydrate contents while  
460 photosynthetic activity was low, could be explained by (i) mobilisation of starch and/or other storage  
461 compounds and their interconversion into soluble sugars and by (ii) reduced growth. Obviously, our  
462 knowledge of the composition of storage compounds in bromeliads is still poor, as is their  
463 importance in mechanisms involved in desiccation tolerance in these species (Vieira et al., 2017).  
464 Because the types of carbohydrates involved (e.g. glucose, fructose, sucrose, starch, fructans, etc.)  
465 differ across bromeliad species (Christopher and Holtum, 1998), to better understand the regulatory  
466 mechanisms of carbon metabolism involved in response to drought stress, further quantification of  
467 carbohydrate diversity is required in the two species.



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

480

#### 481 **4.2 Evidence for a contrasted role for roots in water and nutrient uptake in the two species**

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

493 The  $^{15}N$ -labelling further indicated that the roots of *A. aquilega* and *L. splendens* play a contrasted  
494 role in resource uptake. The roots of *L. splendens* enabled only minor resource uptake which was not  
495 sufficient to avoid water stress, as most of the traits were considerably reduced. *Lutheria splendens*  
496 consequently appeared to absorb water and nutrients mainly via the LATs, as also found for  
497 *Guzmania lingulata* (Nadkarni and Primack, 1989). On the contrary, the root system of the  
498 horticultural Tillandsioideae *Guzmania* 'Rana', *Guzmania lingulata* and *Vriesea* 'Harmony'  
499 contributed to water and nutrient uptake (Silva et al., 2018; Vanhoutte et al., 2016). Concerning *A.*  
500 *aquilega*, when only the roots received the  $^{15}N$ -labelled solution, our results are evidence for a higher  
501 leaf  $\delta^{15}N$  compared to *L. splendens*, and subsequently, an increase of the leaf N if compared to plants  
502 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,  $\psi_{md}$ ,  $\Pi_{osm}$ ) 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 <sup>15</sup>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  $\delta^{15}N$ ) 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

538 undoubtedly not starch storage organs, starch was found in the leaves and soluble sugars were found  
539 in both the leaves and the roots of *A. aquilega*, whereas by contrast, no starch was found in the  
540 leaves and only negligible amounts of soluble sugars were found in the roots of *L. splendens*. Soluble  
541 sugars are known to perform a variety of functions which support functions involving rapid  
542 consumption (e.g., growth, respiration, defense) and play a role in non-consumption functions such  
543 as intermediary metabolites, osmolytes, substrates for transport and ion uptake (Farrar and Jones,  
544 2000; Martínez-Vilalta et al., 2016). Because soluble sugars are fundamental metabolites involved in  
545 the regulation of root metabolism (Delhon et al., 1996; Rufty et al., 1989), our results indirectly  
546 suggest that the roots of *A. aquilega* were metabolically active whereas those of *L. splendens* were  
547 not. For now, studies of NSC contents in bromeliads are still incomplete and further investigations  
548 are required for a better overview of their partitioning, particularly the segmentation between roots  
549 and the rosette leaf part, and according to root and leaf ontogenies.

550 The contribution of roots to nutrient uptake has been shown to diverge even in the same  
551 bromeliad species. For example, in *Aechmea fasciata*, Kämpf (1994) found the root system to be  
552 more efficient than the LATs in resource uptake whereas, in greenhouse conditions, Winkler and Zotz  
553 (2009) concluded that the same species was unable to take up phosphorous via its roots. These  
554 confusing results suggest a possible change in biochemical properties of nutrient-membrane  
555 transporters in bromeliad roots. Such membrane transporters have already been described for a  
556 number of different N-sources in bromeliad leaves (Inselsbacher et al., 2007; Meisner et al., 2013).  
557 Today, the biochemical properties of nutrient-membrane transporters in bromeliad roots remain to  
558 be elucidated to better understand the functional role of the roots in water and nutrient uptake.

559

## 560 **5. Conclusion**

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

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

575

#### 576 **Appendix Supplementary data**

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

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

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

581 **Figure S4.** Effect of water supply on gas exchange.

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

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

584

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597

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

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<sub>ad</sub> = adaxial cuticle and epidermis, H<sub>ad</sub> = adaxial hydrenchyma, M = Mesophyll, H<sub>ab</sub> = abaxial hydrenchyma, CE<sub>ab</sub> = 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<sub>hairs</sub> = root hairs, V = velamen, C<sub>outer</sub> = outer cortex, VC = vascular cylinder, C<sub>inner</sub> = inner cortex. \* = indicates the presence of LATs. Scale bars for A and B = 10 cm and scale bars for all anatomical sections = 200  $\mu\text{m}$ .

**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<sub>leaf</sub>, N=6 for each treatment), (B) number of dead leaves (NbD<sub>leaf</sub>, 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 90<sup>th</sup> and 10<sup>th</sup> percentiles, and the ends of the boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> 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 ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) of (A) *Aechmea aquilega* (CAM photosynthetic pathway, N=3 for each treatment) and (B) *Lutheria splendens* (C<sub>3</sub> 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 ( $\text{mg g}^{-1}$ ) of *A. aquilega* according to the treatments.

**Fig. 4.** Effects of treatments on the leaf soluble sugars (LSS,  $\text{mg g}^{-1}$ , N=6 for each treatment) at (A) dusk and (B) dawn, and leaf starch (L<sub>starch</sub>,  $\text{mg g}^{-1}$ , N=6 for each treatment) at (C) dusk and (D) dawn, and (E) root soluble sugars (RSS,  $\text{mg g}^{-1}$ , N=3 for each treatment) at dawn and (F) root starch (R<sub>starch</sub>,  $\text{mg g}^{-1}$ , N=3 for each treatment) in *Aechmea aquilega* (black) and *Lutheria splendens* (grey). Error bars above and below the boxes indicate the 90<sup>th</sup> and 10<sup>th</sup> percentiles, and the ends of the boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> 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 ( $\Psi_{md}$ , MPa, N=6 for each treatment) and (B) osmotic potential ( $\Pi_{osm}$ , 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,  $\mu\text{m}$ , 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<sup>th</sup> and 10<sup>th</sup> percentiles, and the ends of the boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> 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 <sup>15</sup>N labelling on (A) leaf  $\delta^{15}\text{N}$  (‰, 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<sup>th</sup> and 10<sup>th</sup> percentiles, and the ends of the boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> 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 \*\*\*  $< 0.0005$ ).