

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

Céline Leroy, Eva Gril, Lynda Si Ouali, Sabrina Coste, Bastien Gérard,

Pascale Maillard, Helenice Mercier, Clement Stahl

▶ To cite this version:

Céline Leroy, Eva Gril, Lynda Si Ouali, Sabrina Coste, Bastien Gérard, et al.. Water and nutrient uptake capacity of leaf-absorbing trichomes vs. roots in epiphytic tank bromeliads. Environmental and Experimental Botany, 2019, 163, pp.112-123. 10.1016/j.envexpbot.2019.04.012 . hal-02149053

HAL Id: hal-02149053 https://hal.umontpellier.fr/hal-02149053

Submitted on 22 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Version of Record: https://www.sciencedirect.com/science/article/pii/S0098847219303065 Manuscript_df26f990dc47b6ba4c8701f5c3413a8d

- 1 Water and nutrient uptake capacity of leaf-absorbing trichomes vs. roots in epiphytic tank
- 2 bromeliads
- 3
- 4 Céline Leroy^{1,2*}, Eva Gril^{1,2}, Lynda Si Ouali³, Sabrina Coste⁴, Bastien Gérard³, Pascale Maillard³,
- 5 Helenice Mercier⁵, Clément Stahl⁶
- 6
- 7 ¹AMAP, IRD, CIRAD, CNRS, INRA, Université Montpellier, Montpellier, France
- 8 ²UMR EcoFoG, CNRS, CIRAD, INRA, AgroParisTech, Université des Antilles, Université de Guyane,
- 9 97310 Kourou, France
- 10 ³INRA, AgroParisTech, Université de Lorraine, UMR Silva, F-54000 Nancy, France
- ⁴UG, UMR EcoFoG, CNRS, CIRAD, INRA, AgroParisTech, Université des Antilles, Université de Guyane,
- 12 97310 Kourou, France
- 13 ⁵Department of Botany, Institute of Biosciences, University of São Paulo, CEP 05508-090, São Paulo,
- 14 SP, Brazil
- ¹⁵ ⁶INRA, UMR EcoFoG, CNRS, CIRAD, AgroParisTech, Université des Antilles, Université de Guyane,
- 16 97310 Kourou, France
- 17
- 18 *corresponding author Céline Leroy, celine.leroy@ird.fr
- 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 content, hydrenchyma thickness) and nutrient uptake (e.g., ¹⁵N-labelling). While the roots of A. 30 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, ¹⁵N labelling, Plant performance, Tank bromeliad,
 37 Water status
- 38

39 Highlights

- 40 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
- 44

46 **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.

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 systematic updates). Bromeliads account for a large proportion of vascular epiphyte species 56 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, C₃ or CAM Soil-Root (Pitcairnioideae and Bromelioideae); Type II, CAM Tank-65 Root (Bromelioideae); Type III, CAM Tank-Absorbing Trichome (Bromelioideae); Type IV, C3 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

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.

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 Tillandsioiseae 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 ± 23.1 mL, a number of leaves of 9.7 ± 0.2 , a total 129 height (distance from the bottom of the body to the top of the crown) of 27.1 ± 0.8 cm, a canopy 130 width (maximum distance between the tips of the leaves, two measurements taken at an angle of 131 90°) 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 ± 3.73 days.





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_{ad} = adaxial cuticle and epidermis, H_{ad} = 137 adaxial hydrenchyma, M = Mesophyll, H_{ab} = abaxial hydrenchyma, CE_{ab} = 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_{hairs} = root hairs, V = velamen, C_{outer} = outer cortex, 140 VC = vascular cylinder, C_{inner} = inner cortex, * = indicates the presence of LATs. Scale bars for A and B 141 = 10 cm and scale bars for all anatomical sections = 200 μ m. 142

143 Lutheria splendens (Brongn.) Lem. (Fig. 1B) is a Type IV tank-forming bromeliad in the subfamily 144 Tillandsioideae with C₃ 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 \pm 5.9 mL, a number of leaves of 11.2 \pm 0.4, a total height of 20.4 \pm 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.

Both species exhibited water storage tissue (hydrenchyma) on the adaxial and abaxial side of the leaf formed by large non-chlorophyllous cells (**Fig. 1C-F**). The mesophyll, made up of the aerenchyma, chlorenchyma and vascular bundles, was located in the central part of the lamina. The roots of *A*. *aquilega* and *L. splendens* showed the typical anatomy of a monocot root (**Fig. 1G, H**) with a velamen 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 $84.3 \pm 0.1\%$, 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 $83.9 \pm 0.06\%$, 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 μ mol m⁻² s⁻¹ for *L. splendens*, while the outside PAR was 1808.5 ± 103.1 μ mol m⁻² s⁻¹.

172

173 **2.2** Water supply treatments

All the plants were irrigated with fresh rainwater every second day for six months prior to the experiment. Twenty-four bromeliads of similar shape and size of each of the two species were organised homogeneously in four different watering treatments with a total of 6 replicates per 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.

185

186 $\,$ 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.

193 Gas exchange— For each species, net photosynthesis assimilation (A, µmol CO₂ m⁻² s⁻¹) and 194 stomatal conductance to H_2O (g_s mol H_2O m⁻² 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 µmol m⁻² s⁻¹ for A. aquilega determined from direct PAR measurements in the 199 greenhouse environment and to 200 µmol m⁻² 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, CO₂ concentration 202 and air flow in the chamber were set at 27 °C, 400 ppm and 250 µmol s⁻¹, respectively. To compare 203 treatments, we calculated maximum net photosynthesis assimilation (Amax, µmol CO2 m⁻² s⁻¹) and 204 maximum stomatal conductance for water vapour (g_{smax}, mol H₂O m⁻² s⁻¹) by averaging the five highest 205 gas exchange (CO_2 and H_2O) 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 C₃), 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 µL of a 218 methanol:chloroform:water (12:5:1- v/v/v) solution, added with salicylic acid (20 μ g. mL⁻¹) as internal 219 standard. The sample was mixed and incubated for 30 min at 60 °C. Then, 500 µL 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, 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 °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 °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/orthodianisidine 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 g^{-1} DM, were thus determined for leaves (LSS and L_{starch}) and roots (RSS and R_{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 °C 243 in the dark for 72 hours to determine turgid mass (TM), and finally dried at 60 °C for 72 hours to 244 determine dry mass (DM). The relative water content (RWC, %) was calculated as (FM-DM)/(TM-245 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

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, µm, 4 measurements), adaxial and abaxial epidermal wall and cuticle 254 thickness (CET, μ m, 6 measurements), adaxial and abaxial hydrenchyma thickness (HT, μ m, 6 255 measurements), and mesophyll thickness (MT, µm, 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 (\mathbb{D}_{MD}) was measured on the second young 259 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 260 261 (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 2_{MD} (MPa) corresponds to the mean of three samples (6.4 mm diameter 265 leaf discs).

266 Osmotic potential— Leaf osmotic potential (20 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 (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 (mmol kg⁻¹) was recorded from the osmometer when 273 the difference between consecutive measurements fell below 5 mmol kg⁻¹. This value was converted 274 to \mathbb{Z}_{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**

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 ¹⁵N. At the end of the 2-month experiment, we selected three plants per treatment (TR, T and R) for each species. The ¹⁵N-enriched solution consisted of 7 L of rainwater with 2 g of NH₄¹⁵NO₃ (10 atom % ¹⁵N, Isotec Inc., OH, USA) and 2 g of ¹⁵NH₄NO₃ (10 atom % ¹⁵N, 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 ¹⁵N-enriched solution was distributed in 285 all the leaf axil. In the TR and R treatments, the roots received 40 ml of ¹⁵N-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_{nat} and $\delta^{15}N_{nat}$) and one week after the $^{15}N_{-1}$ 288 enrichment period (i.e., from labelled plants to record the enrichment level, N_{lab} and $\delta^{15}N_{lab}$). 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 ¹⁵ N isotopic abundance (δ^{15} N, ‰). Stable isotope 291 analyses were conducted at the Cornell University Stable Isotope laboratory (Ithaca, NY, USA) using a 292 Thermo-Finnigan DELTA^{plus} 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 ^{15}N delta (δ ‰) versus an 295 international standard (N₂ in the air) as follows:

296 δ^{15} N‰ = (R_{sample}/R_{standard} - 1) ×1000 where R is the ratio ¹⁵ N/ ¹⁴ 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_{leaf} 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*.



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





Fig. 3. Diel course of net photosynthesis assimilation (μ mol 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).

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 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 58 355 mg g⁻¹ at dusk and 69 to 53 mg g⁻¹ at dawn. In contrast, leaf starch differed significantly as a function 356 357 of the species, the treatments, and their interactions (Table 1). L_{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_{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⁻¹ 360 361 in the R treatment. At dawn, L_{starch} was significantly reduced with mean values below 10 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 mg g⁻¹) of RSS than *L. splendens* in which values were 365 below 5 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.





377 **3.2 Water status**

Mid-day water potential (Ψ_{md}) and osmotic potential (Π_{osm}) were very high in both species in wellwatered 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.







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

409

410 **3.3 Nutrient uptake**

411 The leaf N content (N_{nat}, %) and the leaf δ^{15} N (δ^{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 ¹⁵N-enriched solution resulted in a significant increase in leaf $\delta^{15}N$ ($\delta^{15}N_{lab}$ =4012 ± 1195 ‰) and 415 leaf N (N_{lab} = 0.71 ± 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 *L. splendens*, when the root system was watered with ¹⁵N-labelled solution, leaf δ^{15} N increased only 417 418 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 419 420 1, Fig 6, and Supplemental Figure S6). Finally, absorption of N and ¹⁵N 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 ¹⁵N labelling on (**A**) leaf δ¹⁵N (‰, 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 90th and 10th percentiles, and the 428 ends of the boxes indicate the 25th and 75th 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).

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 $\,$

- 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 wellwatered 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).
- 448 Drought stress significantly reduced nocturnal acidification in A. aquilega because stomatal 449 closure prevents nocturnal fixation of external CO_2 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 Ψ_{md} , Π_{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. aquilaga. 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, ψ_{md} , Π_{osm}) or similar to drought 492 stressed plants (i.e., NbN_{leaf}, RGR, LT, A_{sat}, g_{ssat}).

493 The ¹⁵N-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. aquilega, when only the roots received the ¹⁵N-labelled solution, our results are evidence for a higher 500 501 leaf δ^{15} N compared to *L. splendens*, and subsequently, an increase of the leaf N if compared to plants 502 before the ¹⁵N 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 ¹⁵N-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 merits further investigation. 535

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
- 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 δ^{15} N and leaf N.
- 584

585 Acknowledgements

586 We would like to thank Jocelyn Cazal and Jean-Yves Goret for their help in the field, in the 587 greenhouse installation and for technical assistances, Aline Bertinatto Cruz for malate HPLC analyses 588 and SILVATECH, ISC from UMR 1434 SILVA, 1136 IAM, 1138 BEF and 4370 EA LERMAB research 589 center INRA Nancy-Lorraine for it contribution to NSC analyses. SILVATECH facility is supported by 590 the French National Researcher Agency through the Laboratory of Excellence ARBRE (ANR-11-LABX-591 0002-01). We are grateful to Lore Verryckt from the Imbalance-P project (European Research Council 592 Synergy grant ERC-2013-SyG-610028 IMBALANCE-P) for letting us use the three Li-Cor 6400XT 593 portable photosynthesis systems and for her technical help. We would like to thank Daphne 594 Goodfellow for proofreading the manuscript and three anonymous referees for their valuable 595 comments. This work received financial support from an "Investissement d'Avenir" grants managed 596 by the Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-25-01).

597

598 References

- 599 Amorós, A., Zapata, P., Pretel, M.T., Botella, M.A., Serrano, M., 2003. Physico-chemical and
- 600 physiological changes during fruit development and ripening of five loquat (*Eriobotrya Japonica*
- 601 Lindl.) cultivars. Food Sci. Technol. Int. 9, 43–51.
- 602 Bader, M.Y., Menke, G., Zotz, G., 2009. Pronounced drought tolerance characterizes the early life
- 603 stages of the epiphytic bromeliad *Tillandsia flexuosa*. Funct. Ecol. 23, 472–479.
- 604 Bartlett, M.K., Scoffoni, C., Ardy, R., Zhang, Y., Sun, S., Cao, K., Sack, L., 2012. Rapid determination of
- 605 comparative drought tolerance traits: using an osmometer to predict turgor loss point. Methods
- 606 Ecol. Evol. 3, 880–888.

607 Benzing, D.H., 2000. Bromeliaceae: profile of an adaptive radiation. Cambridge University Press,

608 Cambridge, UK, Cambridge.

- Benzing, D.H., 1990. Vascular epiphytes: general biology and related biota. Cambridge University
 Press, Cambridge.
- Benzing, D.H., 1976. Bromeliad trichomes: structure, function, and ecological significance. Selbyana 1,
 330–348.
- Benzing, D.H., Ott, D.W., 1981. Vegetative reduction in epiphytic Bromeliaceae and Orchidaceae: its
 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 CO₂. 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 C₃ 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 NO₃ uptake in soybean plants
- IV. Dependence on current photosynthesis and sugar availability to the roots. J. Exp. Bot. 47, 893–900.
- 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.

- 640 Freschi, L., Rodriguez, M.A., Tiné, M.A.S., Mercier, H., 2010a. Correlation between citric acid and
- 641 nitrate metabolisms during CAM cycle in the atmospheric bromeliad *Tillandsia pohliana*. J. Plant
 642 Physiol. 167, 1577–1583.
- 643 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
- 645 tank bromeliad *Guzmania monostachia* perform distinct functions in response to water shortage.
- 646 J. Plant Physiol. 167, 526–533.
- 647 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.,
- 649 Berry, P.E., Sytsma, K.J., 2011. Phylogeny, adaptive radiation, and historical biogeography in
- 650 Bromeliaceae: Insights from an eight-locus plastid phylogeny. Am. J. Bot. 98, 872–895.
- 651 Givnish, T.J., Barfuss, M.H., Ee, B.V., Riina, R., Schulte, K., Horres, R., Gonsiska, P.A., Jabaily, R.S., S, R.,
- 652 Crayn, D.M., Smith, J.A.C., Winter, K., Brown, G.K., Evans, T.M., Holst, B.K., Luther, H., Till, W., Zizka,
- 653 G., Berry, P.E., Sytsma, K.J., 2014. Adaptive radiation, correlated and contingent evolution, and net 654 species diversification in Bromeliaceae. Mol. Phylogenet. Evol. 71, 55–78.
- 655 Gonçalves, A.Z., Mercier, H., Oliveira, R.S., Romero, G.Q., 2016. Trade-off between soluble protein 656 production and nutritional storage in Bromeliaceae. Ann. Bot. 118, 1199–1208.
- 657 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.
- 662 Hoch, G., 2015. Carbon reserves as indicators for carbon limitation in trees, in: Lüttge, U., Beyschlag,
- 663 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.
- 666 Inselsbacher, E., Cambui, C.A., Stange, C.F., Mercier, H., Wanek, W., 2007. Microbial activities and
- 667 foliar uptake of nitrogen in the epiphytic bromeliad *Vriesea gigantea*. New Phytol. 175, 311–320.
- 668 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.
- 671 Leroy, C., Maes, A. Q., Louisanna, E., Séjalon-Delmas, N., in press. How significant are endophytic
- 672 fungi in seeds and seedlings? Effects on germination, survival and performances of two epiphytic
- 673 plant species. Fungal Ecol. 39, 296-306.

- 674 Leroy, C., Carrias, J.-F., Corbara, B., Pélozuelo, L., Dézerald, O., Brouard, O., Dejean, A., Céréghino, R.,
- 675 2013. Mutualistic ants contribute to tank-bromeliad nutrition. Ann. Bot. 112, 919–926.
- 676 Lüttge, U., 2008. Physiological ecology of tropical plants, Second ed. Springer Verlag, Berlin.
- 677 Males, J., 2016. Think tank: water relations of Bromeliaceae in their evolutionary context. Bot. J. Linn.
- 678 Soc. 181, 415–440.
- 679 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.
- 682 Martin, C.E., 1994. Physiological ecology of the Bromeliaceae. Bot. Rev. 60, 1–82.
- 683 Martínez-Vilalta, J., Sala, A., Asensio, D., Galiano, L., Hoch, G., Palacio, S., Piper, F.I., Lloret, F., 2016.
- 684 Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. Ecol. Monogr.
 685 86, 495–516.
- McDowell, N.G., 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation
 mortality. PlantT Physiol. 155, 1051–1059.
- 688 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.
- 691 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.
- 694 Nadkarni, N.M., Primack, R.B., 1989. The use of gamma spectrometry to measure within plant
- 695 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.
- 700 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.
- 703 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.

- 708 Pita, P.B., Menezes, N.L., 2002. Anatomia da raiz de espécies de *Dyckia* Schult. f. e *Encholirium* Mart.
- 709 ex Schult. & Schult. f. (Bromeliaceae, Pitcairnioideae) da Serra do Cipó (Minas Gerais, Brasil), com
- 710 especial referência ao velame. Rev. Bras. Bot. 25, 25–34.
- 711 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.
- 713 Am. J. Bot. 103, 603–612.
- Popp, M., Janett, H.P., Medina, E., 2003. Metabolite gradients and carbohydrate translocation in
- 715 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.
- 718 Proença, S.L., das Graças Sajo, M., 2008. Rhizome and root anatomy of 14 species of Bromeliaceae.
- 719 Rodriguésia 59, 113–128.
- 720 R Development Core Team. 2015. R: a language and environment for statistical computing. R
- 721 Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.
- 722 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.
- 725 Schmidt, G., Zotz, G., 2002. Inherently slow growth in two Caribbean epiphytic species: A
- demographic approach. J. Veg. Sci. 13, 527–534.
- 727 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.

- 730 Sieber, J., 1955. Untersuchungen über die wasser- und Nährstoffaufnahme bei epiphytischen
- 731 trichterbildenden bromeliaceen. Gartenbauwissenschaft 141–164.
- 732 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.
- 735 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.
- 737 Stiles, K.C., Martin, C.E., 1996. Effects of drought stress on CO₂ exchange and water relations in the
- 738 CAM epiphyte *Tillandsia utriculata* (Bromeliaceae). J. Plant Physiol. 149, 721–728.
- 739 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. PlantPhysiol. 19, 119–126.
- Van Handel, E., 1965. Estimation of glycogen in small amounts of tissue. Anal. Biochem. 256–265.

- Vanhoutte, B., Ceusters, J., De Proft, M.P., 2016. The 'tubing' phenomenon in commercial cultivation
 of *Guzmania*: morphology, physiology and anatomy. Sci. Hortic. 205, 112–118.
- Vanhoutte, B., Schenkels, L., Ceusters, J., Proft de, M.P., 2017. Water and nutrient uptake in *Vriesea*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
- in the bromeliad *Pitcairnia burchellii* Mez: biochemical adjustments and structural changes. Plant
- 749 Physiol. Biochem. 121, 21–30.
- Winkler, U., Zotz, G., 2010. 'And then there were three': highly efficient uptake of potassium by foliar
 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.
- 103, 477–484.
- Zhang, W., Hu, H., Zhang, S.B., 2016. Divergent adaptive strategies by two co-occurring epiphytic
 orchids to water stress: escape or avoidance? Front. Plant Sci. 7, 588–588.
- Zotz, G., Winkler, U., 2013. Aerial roots of epiphytic orchids: the velamen radicum and its role in
 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.

Traits	Species		Treatment		Species*Treatment	
	F	Р	F	Р	F	Р
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
<u>Water status</u>						
<u>Aerial part of the leaf</u>						
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
П _{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
δ 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
δ ¹⁵ N _{lab} (‰)	2.97	0.10	14.92	<0.0001	7.95	0.006

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.

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).

Fig. 3. Diel course of net photosynthesis assimilation (μ mol 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 (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, μ 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 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; **, <0.005 and *** < 0.0005).

Fig. 6. Effects of ¹⁵N labelling on (**A**) leaf δ^{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).