Effect of substrate fertility on tank-bromeliad performances

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Research Article

Abstract

Purpose

Members of the plant family Bromeliaceae can uptake nutrients directly from their leaves via leaf absorbing trichomes and their roots have long been reduced to anchorage function,thus overlooked. Recently, evidence has accumulated for a significant role for the roots of some species of tank bromeliads in both water and nutrients absorption. However, to date, little attention has been paid to the importance of the substrate fertility for the structure of the roots and the growth and performance of tank bromeliads.

Methods

This study investigated the effect of substrate fertility on *Aechmea aquilega*. Seeds of this tank bromeliad were sowed in a greenhouse in French Guiana in three different substrates: a nutrient-poor, a nutrient-rich and a mixed substrate. The performances of 15-month-old A. aquilega were assessed by measuring leaf and root traits related to nutrient acquisition and resources capture.

Results

We show that plants growing in nutrient-poor substrate grew twice slower and were smaller than plants grown on the nutrient-rich substrate with fewer leaves and roots, lower total dry mass, and smaller leaves and root length. Overall, 70% of measured traits responded significantly to the experimental treatments indicating that the response of A. aquilega to nutrient availability is a combination of physiological processes, leaf and root structure, and chemistry.

Conclusion

This study is the first to show that the fertility of the substrate on which the bromeliad A. aquilega grows has a strong and lasting effect on the plant performance and may be a relevant factor for bromeliad ecology.

Introduction

One of the key challenges faced by sessile plants is ensuring their uptake of sufficient nutrients and water for their growth and survival. Soil is the main source of water and nutrients for most plants, but as nutrients are unevenly spatially distributed, the availability of nutrients for plants also varies (Cain et al. 1999) as do the plants response strategies (Bloom et al. 1985; Kraiser et al. 2011). Ecologists use the functional traits of leaves, stems and roots to obtain information on the resource (i.e., light, water, nutrients) acquisition strategies of species (Sterck et al. 2011). Cross-species comparisons have revealed

major axes of trait variations reflecting strategies (i.e. leaves and roots economic spectra) which range from rapid acquisition to conservation of resources (Roumet et al. 2016; Wright et al. 2004). The same range can also be found within a single plant species across environmental gradients (Buchanan et al. 2019; Delpiano et al. 2020; Fajardo and Siefert 2018; Hajek et al. 2013; Hayes et al. 2019; Isaac et al. 2017). Plants facing harsh conditions (i.e. low resources availability) are expected to have more conservative traits than plants growing in nutrient-rich environments (Lambers and Poorter 1992; Wright et al. 2004). Functional trait approaches have mainly been used in trees and herbaceous species (e.g., Fort et al., 2013; Freschet et al., 2013) while such studies of epiphytes are rare (de Paula Oliveira et al. 2021; Richards and Damschen 2021).

Epiphytes, plants that live non-parasitically on trees, have to cope with heterogeneous and intermittent nutrient and water availability (Zotz 2016a). These plants have consequently developed numerous morphological adaptations (e.g., leaf-absorbing trichomes, velamen radicum, water-storage tanks, pseudo-bulbs) to optimise water and nutrient uptake and conservation (Males 2016; Zotz 2016a). Epiphytic plant species can be obligate or facultative and can be found growing on different substrates including bark, rocks, canopy soil, or the ground (Wu et al. 2020; Zhang et al. 2021). Such different substrates can modify the morphological, anatomical, physiological and stoichiometric traits of conspecific individuals (Chen et al. 2019; Lu et al. 2015; Wu et al. 2020; Zhang et al. 2021). Hoeber & Zotz (2021) recently found that accidental epiphytic individuals performed better than terrestrial conspecifics due to the beneficial growth conditions in the forks of branches filled with organic-rich arboreal soil. The nutrient use strategies of facultative epiphytes have been shown to be flexible, thus enabling facultative epiphytes to exploit different substrate interchangeably (Wu et al. 2020; Zhang et al. 2021).

Plants belonging to the Bromeliaceae family display many remarkable morphological, anatomical and physiological adaptations to facilitate nutrient uptake and conservation (Givnish et al. 2014; Leroy et al. 2016; Males 2016). Bromeliads grow on different substrates (terrestrial, lithophyte or epiphyte) and differ in their ability to retain water and nutrients (i.e., tank-forming, or tankless) and in the photosynthetic pathway (i.e., C_3 or CAM). The leaves are the most important vegetative organ in bromeliads because they perform essential physiological functions including photosynthesis, nutrient assimilation and water conservation (Benzing 2000). In tank bromeliads, the basal part of the leaf is devoted to water and nutrient absorption through absorbing trichomes and to nutrient uptake thanks to transporters, plus nitrate reductase activity similar to that found in the roots of terrestrial plants (Gonçalves et al. 2020; Kleingesinds et al. 2018). The middle and apical portions of the leaf are devoted to photosynthesis and glutamine synthetase activity (Gonçalves et al. 2020).

The functional importance of leaves in bromeliad nutrition led scientists to overlook the roots, which were thought to only be used for anchorage (Benzing 2000; Takahashi et al. 2022). However, a few recent studies showed that the roots of some tank bromeliads were involved in nutrient and water absorption (Carvalho et al. 2018; Gomes et al. 2021; Leroy et al. 2019a; Silva et al. 2018; Vanhoutte et al. 2017). These recent studies investigated mature tank bromeliads, either collected in the field or from commercial nurseries and relied on short-term experimental approaches. Because they used fully grown plants, these

studies are unable to identify the real quantitative role of the roots and the consequences for plant growth and performance. In addition, at the seedling stage, some tank-forming bromeliads belonging to the genus *Aechmea* were shown to be totally devoid of leaf absorbing trichomes and thus depended entirely on their roots for water and nutrient absorption (Leroy et al. 2019b, 2017; Petit et al. 2014). In that case, the seedlings performed better when growing on an organic-rich substrate than on a mineral-rich substrate, pointing to a significant nutritional role for the roots (Leroy et al. 2017, 2019b). Despite this increasing evidence for a significant role for roots in bromeliad nutrition, little is known about the root structure and the importance of the nutrient content of the substrate for the development and growth of tank-bromeliads. The lasting effects of substrate fertility on tank-bromeliad development (from the seedling to the mature stage) may have a key ecological impact on their performance.

The two objectives of this study were thus to (i) assess the extent to which the fertility of the substrate affects the growth and overall performance of the tank-bromeliad *Aechmea aquilega* and (ii) to identify specific physiological and morpho-anatomical leaf and root responses to substrate fertility. The root system of *A. aquilega* contributes significantly to both nutrient uptake (Leroy et al. 2019a) and seedling performance (Leroy et al. 2019b). Furthermore, in nature, this species can be found growing on terrestrial, lithophytic and epiphytic supports (Leroy et al. 2013). Because at the seedling stage, *A. aquilega* depends entirely on the root system for water and nutrient absorption (Leroy et al. 2017, 2019b) and at the mature stage, on both the leaves and the roots (Leroy et al. 2019a), we hypothesised that the substrate on which the bromeliad grows since germination plays a key role and that the growth and overall performance of plants growing on nutrient-rich substrates is better than that of plants growing on nutrient-poor substrates. In addition, because environmental pressures have profound impacts on leaf and root trait values (Wang et al. 2021) and because *A. aquilega* individuals are found in different habitats (Talaga et al. 2017), we hypothesised that leaf and root trait values vary widely across different substrates allowing high capacity for adaptive phenotypic plasticity. To test these hypotheses, we grew *A. aquilega* from seeds to 15-month-old individuals in three substrates imitating an organic- to mineral-rich substrate gradient using potting soil (i.e., nutrient-rich substrate), white sand (i.e., nutrient-poor substrate), and a mixture of equal volumes of potting soil and white sand. Leaf and root traits related to nutrient acquisition and resources capture were then measured to assess plant response and performance.

Material And Methods

Plant material and growth conditions

Aechmea aquilega (salisb.) Griseb is a tank-bromeliad which belongs to the subfamily Bromelioideae and has crassulacean acid metabolism (CAM) photosynthesis (Crayn et al. 2004). In October 2018, seeds from one mother epiphytic plant growing in natural environment were sowed in a greenhouse at the *campus agronomique* in Kourou (French Guiana) in horticultural seedling trays in three different soils: 100% white sand (nutrient-poor substrate, WS), 100% potting soil (nutrient-rich substrate, P) and a mixture of white sand and potting soil (v:v 50:50, intermediate substrate, WSP). The sand was collected from a location close to Paracou research station (5°16'26"N, 52°55'26"W), while the potting soil was purchased.

Physico-chemical analyses were performed on triplicate samples of each type of substrates to determine their granulometry and Carbon, Nitrogen, Phosphorous contents (PAPC, Toulouse). Results showed significant differences of granulometry and CNP contents (supplementary information, **Fig.S1**).

During their development, individual plants were transplanted in their corresponding substrate into 0.5L, 1L and then 2L pots. The pots were placed randomly on horticultural tables in similar environmental conditions. Plants were irrigated with tap water for ten minutes twice a day at 8:00 am and 6:00 pm. Greenhouse temperature, light intensity and relative humidity were monitored with HOBO probes (model UA-002-64, HOBO Pendant Temp/Light – 64k and model U23-001, HOBO Pro V2 Temp/RH Data logger, Amanvillers, France). The mean relative humidity was 82.9 ± 0.1%, mean air temperature was 27.8 ± 0.1°C, and light intensity was 21016.7 ± 580.6 lux (*ca.* 30% of full external irradiance, corresponding to a mean PAR (photosynthetically active radiation) of 496.5 ± 34.4 $\mu mol.m^{-2}.s^{-1}$).

Sampling

Measurements and samplings were performed on 15-month-old plants. Ten plants per treatment were randomly selected and all measurements and sampling were performed on three to four mature leaves, depending on plant size and the amount of plant material required for chemical analysis.

Carbon metabolism traits

Chlorophyll fluorescence— Two chlorophyll fluorescence parameters, the maximum quantum yield of photosystem II ($F_v.F_m$) and the maximum electron transport rate (ETR_{max}, $\mu mol\ photon.m^{-2}.s^{-1}$) were measured with a portable fluorometer (MINI-PAM II, Walz, Effeltrich, Germany). Measurements were made on the adaxial surface of the leaf between 8:00 am and 12:00 am.

To measure $F_v.F_m$, a portion of the leaf was dark acclimated for 30 minutes with a dark leaf clip (DLC-8, Walz). The minimal fluorescence F_0 was measured by exposing the leaf to a low intensity light (< 0.1 $\mu mol\ photon.m^{-2}.s^{-1}$), then a 0.8 second saturating pulse (5000 $\mu mol\ photon.m^{-2}.s^{-1}$) was produced to assess maximal fluorescence F_m. $F_v.F_m$ was calculated as: $F_v.\ F_m = \dfrac{F_m - F_0}{F_m}$

ETR_{max} was calculated using the rapid light curve (Manzi et al. 2022; Rascher et al. 2000) procedure on the same leaf as that used for $F_v.F_m$. For quasi-dark acclimation, the leaf was placed in an opaque plastic bag for 30 seconds (Manzi et al. 2022; Rascher et al. 2000) and left in the bag for the rapid light curve. The leaf was then gradually exposed to increasing PAR values in 12 steps from 50 to 3000 $\mu mol\ photon.m^{-2}.s^{-1}$ each lasting for 30 seconds. The ETR was calculated using the fluorometer and the WinControl-3 software (Walz, Effeltrich, Germany) according to the photoinhibition REG1 function of Platt et al. (1980). ETR_{max} was then extracted from the resulting curves as the highest measured ETR.

Chlorophyll content— Leaf chlorophyll a and b content (Chl, $mg.g^{-1}$) were estimated with a SPAD-502 chlorophyll meter (KONICA MINOLTA, U.S.A). Eight measurements were taken on the apical and median leaf portion and averaged to obtain the average SPAD value of the leaf. SPAD values were then converted

into µg cm^{-2} according to Coste et al. (2010): $Chloro(µg. \, cm^{-2}) = \frac{(117.1 * SPAD)}{(148.84 - SPAD)}$, then converted

into mg.g^{-1} as follows: $Chl = \left(Chloro * 10^{-3} \right) * \left(\frac{1}{LMA * 10^{-4}} \right)$

Gas exchange— Net photosynthesis assimilation (A, µmol CO_2.m^{-2}.s^{-1}) and stomatal conductance (Gs, µmol H_2O.m^{-2}.s^{-1}) were measured on 18 additional plants (N = 6 per treatment) with a CIRAS-3 analyser (PP Systems, Amesbury, U.S.A). All the measurements were made at ten-minute intervals throughout the night from 6:00 pm to 9:00 am the following morning. The CO_2 concentration in the leaf chamber was set to 400 ppm, the temperature to 27°C, and the air flow to 250 µmol.s^{-1} while relative humidity and light were left at ambient conditions. To compare treatments, we calculated the maximum net photosynthesis assimilation (A_{max}, µmol CO_2.m^{-2}.s^{-1}) by averaging the five highest consecutive values and the corresponding G_S values were averaged to obtain the maximum stomatal conductance (G_{Smax}, µmol H_2O.m^{-2}.s^{-1}). In addition, we quantified integrated net photosynthesis assimilation (A_{int}, mmol CO_2.m^{-2} over a 15 h period) by integrating the area under the assimilation curve for the whole night (15 h) using the AUC function in the DescTools R package (Andri Signorell et mult. al. 2021) (**Fig. S2**).

Plant size and growth

Prior to all measurements, leaves were counted (Nb leaves) and the water volume in the tank (Tank capacity, mL) was measured. The total leaf and root dry mass (see below) were used to assess the root to shoot ratio (RS ratio) as follows: $RSratio = \frac{DM_{root}}{DM_{shoot}}$. To evaluate plant growth, the number of new and dead leaves was assessed on ten additional individual plants on each of the three substrates once a month for a year. Growth was estimated as the number of new leaves emitted per year.

Leaf structural traits

The youngest mature leaf was sampled, and its length measured (Leaf length, cm). Eight 10-mm diameter disks were collected with a cork borer from the apical portion of the leaf of plants growing on nutrient-rich and intermediate substrates (P and WSP) while four 8-mm diameter disks were collected from bromeliads growing on nutrient-poor substrate (WS). The leaf area (LA, m²) was then calculated by summing the surface areas of all disks. The thickness of each disk was measured with a micrometric calliper (Digit Outside Micrometre 193 – 101, Mitutoyo, Japan) and averaged to determine leaf thickness (Leaf thickness, mm). Fresh mass (FM, g) was determined by weighing on an electronic balance (AB 204-S Mettler Toledo, Switzerland), while to obtain the turgid mass (TM, g), the disks were stored in distilled water at 4°C in the dark for 48 to 72 h. To determine the dry mass (DM, g), the disks were then dried at 60°C for 72 h. The leaf mass area (LMA, g.m^{-2}) was calculated as DM/LA, while the leaf dry matter content (LDMC, g.g^{-1}) was calculated as DM/FM.

A 1x4 cm portion of each leaf was collected from both the apical and basal part of the leaf and fixed in FAA (5% formalin, 5% glacial acetic acid, 70% ethanol and 20% water) for two weeks and then stored in 70° ethanol. Stomatal density (Nb stomata.mm^{-2}) was measured on the adaxial face of the apical portion and trichome density (Nb stomata.mm^{-2}) and diameter (mm) were measured on both sides of the basal part of the leaf. Stomata and trichomes were observed from imprints made using transparent nail varnish. The imprints were observed with an inverted microscope (Olympus BX51). Four pictures per imprint were acquired with a digital camera (Lumenera LW1135C-IO, Ottawa, Canada) and processed using ImageJ software (Schneider et al. 2012). The number of stomata and trichomes per mm² were recorded for each imprint and averaged. The diameter of 20 trichomes (5 per picture) was measured on ImageJ and averaged to estimate the mean trichome diameter.

Root structural traits

Once all the measurements of the aerial part were completed, the bromeliads were gently unpotted. The root system was carefully washed with tap water and any remaining soil particles were very carefully removed from each adventive root. The roots were dried with a paper towel and weighed on an electronic balance to get the fresh mass (FM$_{root}$, g). The root system was scanned using an office scanner (Xerox DocuMate 4700 5.1) and the basic WinRHIZO software (Instrument Regent, Quebec City, QC, Canada). The roots were then placed in distilled water at 4°C for 48–72 h to get the turgid mass (TM$_{root}$, g) and oven dried at 60°C for another 48 h to get the dry mass (DM$_{root}$, g). From the scanned images we obtained the following morphological root traits: total root length (TRL, cm), number of adventitious roots (Nb roots), number of root tips (Nb tips), average root diameter (ARD, mm), root volume (cm^3), and the total surface area of the root system (cm²). The root tissue density (RTD, g.cm^{-3}) was calculated by dividing DM$_{root}$ by its volume, specific root length (SRL, m.g^{-1}) TRL by DM$_{root}$ and specific tip root average (STRA, tips.g^{-1}) Nb tips by DM$_{root}$.

Leaf and root chemical traits

Carbon, nitrogen and phosphorous were quantified in both leaf tips and roots. The leaf tips and roots were dried in an oven at 60°C for 72 h and ground into fine powder in a mill. About 9–11 mg of leaf and root powder were used to quantify carbon (C, mg.g) and nitrogen (N, mg.g) contents (elemental analyser, Flash 2000 ThermoFisher, NFISO 10694, NF ISO 13878, NF EN 13137). 3–4 mg of leaf and root powder were used to quantify total phosphorus content (P, mg.g; spectrometer, Uvi Light XT5 Secomam, spectrometric method with ammonium molybdate at 880 nm after H2SO4 acid hydrolysis and persulfate oxidation, adapted NFEN 6878).

Statistical analysis

All statistical analyses were carried out in R version 4.0.4 (R Core Team 2021). Graphs were produced using the R package ggplot2 (Wickham 2016). In order to estimate multivariate differences in *A. aquilega* leaf and root trait coordination, we used standardised multiple factor analysis (MFA, Escofier & Pages, 1990). The MFA method enables examination of common structures in datasets with many variables that

can be separated into different groups of variables (i.e., leaf and root traits). MFA was performed with the Factominer package (Lê et al. 2008) on two set of variables: leaf traits (LMA, LDMC, Stomatal density, Trichome density and diameter, C, N, P, Chl, F_vF_m, ETR_{max}) and root traits (STRA, SRL, ARD, RTD, C, N, P). We then plotted all individuals and variables on the two first MFA dimensions and showed the 95% confidence ellipses for all three treatments. To test whether bromeliad traits were significantly affected by the fertility of the substrate, we performed permutational multivariate analysis of variance (perMANOVA).

with the adonis function of the vegan package (Oksanen et al. 2022) using Euclidean distances and 10,000 permutations. A post-hoc test was then conducted with the pairwise.adonis wrapper function in the pairwiseAdonis package (Martinez Arbizu 2022) with pvalues adjusted with Holm's method.

In addition, we examined differences in all traits in the three treatments with the Kruskal-Wallis rank test, which is a non-parametric alternative to one-way analysis of variance (ANOVA) when the assumptions of homogeneity of variance and normality are not met. The Kruskal-Wallis test was performed with the kruskal.test function in base R. When the Kruskal-Wallis associated pvalue was significant (P < 0.05) the post-hoc pairwise Wilcoxon test was used to identify the effects of the treatments on each trait. Finally, we calculated the coefficients of variation (CV, %) of each trait among treatments as $CV = \frac{SD}{mean} * 100$ to describe the extent of phenotypic plasticity of each trait.

Results

Effects of substrate fertility on A. aquilega size and growth

Plants growing on nutrient-rich substrate (P) had twice as many leaves, three times more adventitious roots, higher total biomass, tank water capacity, a twice higher growth rate, a five times lower R:S ratio, longer and thicker leaves, and total root length was up to up to six times greater than that of plants growing on nutrient-poor substrate (WS; Table 1, Fig. 1). Plants grown on the intermediate substrate (WSP) had either intermediate numbers of roots, total dry mass, tank capacity, growth rate, leaf thickness and total root length compared to plants grown on the two other substrates or the number of leaves, RS ratio and leaf length did not significantly differ from those of plants grown on the nutrient-rich substrate (Table 1, Fig. 1). Total dry mass, root length, tank capacity, RS ratio and number of roots were the traits with the largest coefficient of variation (Table 1).

Trait correlations and overall effect of substrate fertility on A. aquilega

The two first dimensions of the MFA explained 55.6% of the variability of the data: PC1 explained (34.4% and PC2 explained 21.2%). The MFA showed two orthogonal groups of traits (**Fig. 2A**). Most of the traits were correlated with PC1 while RTD, STRA and SRL were correlated with PC2. Multivariate analysis of trait correlations showed that root structural traits were orthogonal to leaf structural and

Table 1

Statistical summary table: For each trait the mean ± standard deviation (SD) is displayed for the three treatments (WS – white sand, WSP – white sand/potting soil, P – potting soil). The global coefficient of variation (CV) is given in %. The associated Kruskal Wallis Chi² and P-values are shown. Significant P-values (< 0.05) are in bold. Letters within brackets indicates significant pairwise differences, Wilcoxon pairwise test ($\alpha < 0.05$). Growth, A_{max}, GS_{max} and A_{int} have been measured on different plants.

Traits	Mean ± SD			CV	Significance	
	WS	WSP	P	%	KW.chi	P.val
Overall plant performance						
Number of leaves	8.7 ± 1.89 (a)	13.9 ± 1.1 (b)	14.2 ± 1.4 (b)	24.00	18.300	**0.000107**
Number of roots	25.7 ± 5.08 (a)	51.5 ± 12.7 (b)	72.3 ± 13.3 (c)	44.40	23.100	**< 0.0001**
Total DM	1.09 ± 0.372 (a)	10.7 ± 1.97 (b)	16.3 ± 3.08 (c)	71.50	25.100	**< 0.0001**
Tank capacity	4.15 ± 1.83 (a)	81 ± 17.2 (b)	145 ± 34.7 (c)	81.40	25.100	**< 0.0001**
Growth	6.98 ± 1.17 (a)	12.6 ± 0.94 (b)	14.1 ± 1.29 (c)	29.20	364.000	**< 0.0001**
RS ratio	17.1 ± 8.18 (a)	4.16 ± 1.87 (b)	3.84 ± 1.22 (b)	93.90	18.600	**< 0.0001**
Leaf length	14.6 ± 1.84 (a)	25.1 ± 1.95 (b)	26.5 ± 2.28 (b)	26.00	20.300	**< 0.0001**
Leaf thickness	6.21 ± 0.262 (a)	7.75 ± 0.272 (b)	8.3 ± 0.356 (c)	12.70	23.600	**< 0.0001**
TRL	328 ± 96.5 (a)	832 ± 436 (b)	1460 ± 464 (c)	67.80	20.900	**< 0.0001**
Leaf traits						
LMA	46.1 ± 3.95 (a)	64.1 ± 16.4 (ab)	76 ± 13.3 (b)	27.90	14.000	**0.000891**
LDMC	0.0859 ± 0.0105	0.0938 ± 0.0243	0.104 ± 0.0193	20.80	5.030	0.081
Stomata density	23.2 ± 3.56 (a)	37.1 ± 5.61 (b)	34 ± 4.04 (b)	23.70	19.200	**< 0.0001**
Trichome density	6.72 ± 0.868 (a)	11.5 ± 1.03 (b)	12.7 ± 0.98 (c)	27.00	21.500	**< 0.0001**
Trichome diameter	0.331 ± 0.0498	0.304 ± 0.031	0.295 ± 0.029	12.80	2.770	0.25
Leaf C	402 ± 11.3 (a)	429 ± 6.2 (b)	427 ± 4.27 (b)	3.47	19.500	**< 0.0001**

Traits	Mean ± SD			CV		Significance
Leaf N	6.48 ± 0.982 (a)	4.71 ± 0.62 (b)	4.68 ± 0.371 (b)	20.70	18.600	**< 0.0001**
Leaf P	1.27 ± 0.351	1.53 ± 0.246	1.44 ± 0.122	19.20	4.000	0.135
CHL	0.0839 ± 0.0228 (a)	0.15 ± 0.0418 (b)	0.172 ± 0.0343 (b)	37.00	17.300	**0.000174**
F_vF_m	0.726 ± 0.0151	0.738 ± 0.0144	0.732 ± 0.028	2.74	3.700	0.157
ETR_{max}	47.3 ± 6.45	46.1 ± 10.2	42.7 ± 9.94	19.60	1.520	0.468
A_{max}	2.7 ± 0.443 (a)	3.38 ± 0.172 (a)	3.63 ± 0.662 (a)	18.50	7.420	**0.0244**
GS_{max}	22 ± 3.69	24.3 ± 4.03	23.8 ± 4.49	17.00	0.936	0.626
A_{int}	12.7 ± 2.33 (a)	17.5 ± 1.41 (b)	18.3 ± 3.66 (ab)	22.10	9.090	**0.0106**
Root traits						
STRA	5880 ± 3890	3460 ± 803	4840 ± 2120	57.20	4.760	0.0927
SRL	0.6 ± 0.085 (ab)	0.706 ± 0.119 (a)	0.58 ± 0.0625 (b)	16.70	8.880	**0.0118**
ARD	30.9 ± 19.2	21.9 ± 5.64	28.9 ± 14.3	52.40	1.370	0.505
RTD	0.139 ± 0.0451	0.142 ± 0.0368	0.145 ± 0.0502	30.10	0.379	0.827
Root C	352 ± 44.1 (a)	450 ± 18.4 (b)	466 ± 5.94 (c)	13.70	21.100	**< 0.0001**
Root N	3.4 ± 0.53 (a)	4.26 ± 0.536 (b)	4.94 ± 0.715 (c)	20.60	16.000	**0.000336**
Root P	0.856 ± 0.067 (a)	1.03 ± 0.106 (b)	1.39 ± 0.231 (c)	24.70	24.400	**< 0.0001**

chemical traits. Projection of all individuals separated the three treatments along PC1 whereas no segregation appeared on PC2 (**Fig. 2B**). PerMANOVA revealed a significant effect of the substrate on

the trait values (F = 14.832, Df = 2, p < 0.0001). Specifically, all individuals grown on each of the three substrates differed significantly from one another (Pairwise.adonis, Holm adjusted P < 0.001 in all cases).

Figure 2 (**A**) Correlation circle of variables to the first two dimensions of the MFA. Variables are coloured by groups, green for leave and brown for roots. (**B**) Projections of individuals in the first two PCs plan. Individuals are coloured by treatments. Small dots are individuals' projections, big dots the centroid for

each treatment and ellipses, the 95% confidence ellipses. WS – white sand, WSP – white sand/potting soil, P – potting soil

Effects of substrate fertility on plant functioning

Substrate fertility had no significant effect on Gs_{max} (Table 1, **Fig. S3**). Net photosynthesis assimilation (A_{max} and A_{int}) differed significantly between the three substrates with higher values for plants growing on nutrient-rich (P) and intermediate (WSP) substrates than for plants growing on nutrient-poor (WS) substrate (but with marginal non-significant differences for A_{max}, Table 1). There was no significant effect of treatment on chlorophyll fluorescence as $F_v.F_m$ and ETR_{max} values were similar (Table 1, **Fig. S4A-B**). The latter was very stable with a CV of 2.74% (Table 1). Plants growing on nutrient poor (WS) substrate had significantly lower chlorophyll content than plants growing on the two other substrates (Table 1, **Fig. S4C**).

Effects of substrate fertility on leaf structural and chemical traits

Substrate fertility had significant effects on LMA but not on LDMC (Table 1, **Fig. S5**). Plants growing on nutrient-rich substrate (P) had significantly higher LMA than plants growing on nutrient-poor substrate (WS), whereas there was no significant difference between the plants growing on intermediate substrate (WSP) and those growing on the two other substrates (Table 1, **Fig. S5**). Stomatal and trichome densities were *ca*. 1.5 times higher in plants growing on nutrient-rich substrate than in plants growing on nutrient-poor substrate (Table 1, **Fig. S6**). Plants growing on the intermediate substrate had similar stomatal density and significantly lower trichome density than plants growing on nutrient-rich substrate. Substrate fertility did not affect the size of the trichomes (Table 1, **Fig. S6**). Finally, substrate fertility had significant effects on leaf C and N contents but not on the leaf P contents (Table 1, **Fig. S7**). The leaves of plants growing on the nutrient-rich substrate were

characterised by higher leaf C but lower leaf N contents than the leaves of plants growing on the mineral-rich substrate. The C and N contents of the leaves of plants growing on the intermediate substrate were similar to those of the leaves of plants growing on the nutrient-rich substrate. Overall, all the leaf traits displayed moderate variation (12.7%-30.1%), except for leaf C content, which had a particularly low coefficient of variation (3.47%).

Effects of soil fertility on root structural and chemical traits

Substrate fertility had a significant effect on the average root diameter. Plants growing on the intermediate substrate had significantly larger roots than plants growing on the nutrient-rich substrate (Table 1, **Fig. S8**). Soil fertility did not affect STRA, SRL or RTD (Table 1, **Fig. S8**). Root C, N, and P contents were significantly higher in plants growing on the nutrient-rich substrate than in plants growing on the nutrient-poor substrate (Table 1, **Fig. S9**). Plants growing on the intermediate substrate had intermediate values compared to the two other substrates. STRA and SRL had high coefficients of

variation (around 50%) while other root structural (RTD, and ARD) and chemical traits had lower CVs (Table 1).

Discussion

Substrate fertility had a strong effect on the size and growth of *A. aquilega*. Plants growing on the nutrient-poor substrate (white sand, WS) grew twice as slowly and were smaller than plants growing on the nutrient-rich substrate (potting soil, P) had fewer leaves and roots, less total dry mass, smaller leaves and shorter total root length. This overall smaller size reduced the capacity of the tank. Biomass partitioning was also strongly affected by substrate fertility. The RS ratio was *ca*. 4 times higher in *A. aquilega* growing on the nutrient-poor substrate, indicating a higher proportion of biomass in the roots, thus enhancing foraging. Such biomass partitioning is a well-known mechanism by which plants of a wide range of growth forms cope in poor-nutrient environments (e.g., Hermans et al. 2006; Mašková and Herben 2018; Sainju et al. 2017). In addition, even if only by a small margin, plants growing on the nutrient-rich substrate (P) out-performed plants growing on the intermediary substrate (WSP) (e.g., had larger tank capacity, higher total biomass, more and longer roots). The fertility of the substrate on which *A. aquilega* was grown from seed to 15 months strongly affected both the morphology of the rosette and biomass allocation. Such phenotypic plasticity' is relatively frequent in bromeliads, which grow in a broad range of light and water regimes, and different nutrient availability conditions (de Freitas et al. 2003; González et al. 2011; Scarano et al. 2002; Zotz and Asshoff 2010).

Most of the structural and chemical leaf traits were related to substrate fertility. Plants growing on the nutrient-rich substrate were characterised by higher LMA, thicker leaves, higher stomatal and trichome densities, along with higher leaf C and Chl and lower leaf N contents. Concerning LMA, our results disagree with those generally reported in the literature. High LMA values are typical of plants growing in nutrient-poor habitats as this value reflects the cost of a fixed leaf surface area and hence leaf cost efficiency (Delpiano et al. 2020; Kramer-Walter et al. 2016; Wright et al. 2004). However, LMA depends on both leaf tissue density (LD, proxy of the cost) and leaf thickness. As LDMC is closely linked to LD (Shipley and Vu 2002), a variation in LMA with no variation in LDMC is likely to be the result of variations in leaf thickness (Vile et al. 2005). Hence, the variations in LMA between our three treatments do not reflect significant variations in the cost of leaf construction (i.e., variation in biomass investment) as would be expected based on the leaf economic spectrum (LES, Wright et al., 2004) but more likely leaf thickening. The inconsistency between *A. aquilega* response to soil fertility and LES expectations is probably the consequence of its naturally epiphytic lifestyle, which is supported by the recent study by Hietz et al. (2021). Epiphytes have a different trait syndrome from herbaceous plants and trees, and, within epiphytes, bromeliads showed different covariation between different traits (Hietz et al. 2021; Richards and Damschen 2021). In general, epiphytes have lower N and P content in their tissues than ground-rooted herbaceous plants and trees (Hietz et al. 2021; Reich and Oleksyn 2004). Epiphytes are considered slow growing plants whose traits are associated with "slow" species, because of their low foliar nutrient concentrations and long leaf lifespan (Zotz, 1998).

In our study, *A. aquilega* had similar low leaf N values in all three treatments, as already reported in other bromeliad species (Hietz et al. 2021; Wagner et al. 2021; Wanek and Zotz 2011). We showed that plants growing on the nutrient-poor substrate had the highest leaf N content and lowest leaf P contents compared to plants growing on the nutrient-rich substrate. Our finding concerning leaf N was surprising as in nutrient-poor habitats, species are generally known to have low leaf nutrient concentrations (Lambers and Poorter 1992; Wright et al. 2004). In bromeliads, the high amount of nitrogen in the leaves of plants growing on the nutrient-poor substrate could be the result of luxury consumption, which is common in epiphytes (Zhang et al. 2021; Zotz 2016b). However, when considering the whole plant (i.e. the sum of the leaves and roots) we found no difference in total N content between treatments, suggesting that plants growing on the nutrient-poor substrate allocated N to their leaves. Surprisingly, N allocation was not directed toward chlorophyll as plants growing in nutrient-poor substrate had lower chlorophyll content. We hypothesise that N was allocated to other parts of the photosynthetic apparatus such as proteins, or to defence compounds and other non-photosynthetic processes (Evans 1989; Takashima et al. 2004). Despite the lower chlorophyll content of plants growing on nutrient-poor substrate, we found similar ETR_{max} and F_vF_m across treatments. This result suggests that the substrate fertility did not affect photosystem functioning in *A. aquilega*. Nutrient deficiency usually strongly influences the structure and functions of the photosynthetic apparatus with some damage to PSII, resulting in a reduction in $F_v.F_m$ and ETR (Kalaji et al. 2014, 2018; Wu et al. 2008). Conversely, in other studies, N supply was reported to have no effect on photochemical efficiency (Cruz et al. 2003; Shrestha et al. 2012). These divergent results could be due to several factors (e.g., plant form and species, age of the plant, growing conditions, N recycling mechanisms). Nevertheless, concerning carbon assimilation, we found higher net photosynthesis assimilation (A_{int}) in *A. aquilega* growing on the nutrient-rich substrate than in plants growing on the nutrient-poor substrate. Our results show that the light harvesting (photosystem and electron transport) and CO_2 fixation (Calvin cycle and Rubisco) processes of photosynthesis responded differently to substrate fertility. The light harvesting process was not sensitive to our treatments while it did affect CO_2 fixation. Such contrasting responses may result from a trade-off in N allocation to different components (e.g., thylakoids which are important for the electron transport capacity or soluble proteins which are important for the Calvin cycle, Evans, 1989) .

Concerning P content, *A. aquilega* had a higher concentration than that usually reported for other bromeliad species (e.g., Gonzalez et al. 2011; Wanek & Zotz, 2011). In addition, leaf and root P contents were linked to soil fertility with higher P storage in plants growing on nutrient-rich substrates. Ostertag (2010) showed that plants increased leaf P to a greater degree than leaf N in response to fertilisation. This preferential leaf P accumulation points to P storage, which may have evolved as a common strategy to cope with intermittent or low P supplies in tropical plants. Moreover, when Wanek & Zotz (2011) added fertilizers, they obtained similar P content to that we observed in our study. This suggests that our high P content resulted from higher P availability than in natural conditions and luxury consumption by *A. aquilega*. Further studies are needed to better understand the respective contribution of N and P to the efficiency of photosynthesis as well as to identify their specific critical threshold.

Quantitative data on structural and chemical root traits in bromeliads and in epiphytes in general are only very rarely reported in the literature. In our study, we found that chemical root traits were linked to the fertility of the substrate, whereas this was not the case for structural root traits. Plants growing on the nutrient-rich substrate had higher C, N, and P contents in their roots than plants growing on the nutrient-poor substrate. In ground-rooted species, specific tip root average, root length and root tissue density are expected to be linked with soil resources and properties (Freschet et al. 2021). The second axis of the MFA showed clear co-variation between root structural traits (SRL, STRA, ARD and, RTD) but failed to distinguish between our three treatments, which was confirmed by the non-significance of the pairwise comparisons. The marked variability explained by this axis (21%) seems to result from soil properties or other variables that were not accounted for in our study rather than substrate fertility. The negative relationships between SRL and ARD was observed in another bromeliad species (*Lutheria splendens*, Leroy et al., 2022) and such negative relationships have been widely observed in the context of interspecific variations of mature plants (Bergmann et al. 2020; Kong et al. 2014; Roumet et al. 2016; Spitzer et al. 2021; Wagner et al. 2021). SRL and RTD were negatively correlated in our study, in contrast to what has been reported in vascular epiphytes (Wagner et al. 2021) and in *L. splendens* regarding ontogenetic effects (Leroy et al. 2022). SRL and RTD co-variation across species is not clear, as some studies found a negative correlation (Bergmann et al. 2020; Garbowski et al. 2021), some found a positive correlation (Holdaway et al. 2011; Kong et al. 2014) while still others found no correlation (Kramer-Walter et al. 2016; Valverde-Barrantes and Blackwood 2016). Such variability in root trait combinations may be due to trade-offs between different root functions. Indeed, roots fulfil a wide range of functions comprising physical anchoring, resource storage, and resource capture via the interface with soil bacterial and fungal symbionts (Freschet et al. 2021). Anchorage is likely to be a dominant function in the roots of epiphytes (Wagner et al. 2021) which could mask the structural root trait response to substrate fertility. In addition, in our study, *A. aquilega* were potted in different substrates and this might have affected the structural root traits even if the root systems were not constrained by the pot (pers. obs.).

While the role of the roots in nutrient absorption has already been demonstrated for some bromeliad species (e.g., Gomes et al. 2021; Leroy et al. 2019a), this study is the first to show that the fertility of the substrate on which the bromeliad *A. aquilega* grows following germination has a strong and lasting effect on overall plant morphology and performance. Because of the lack of leaf-absorbing trichomes and of a water tank, *A. aquilega* seedlings can only rely on the root system for mineral absorption (Leroy et al. 2017, 2019b). In a close congeneric species (*A. mertensii*), leaves became larger to form wells and the density of leaf-absorbing trichomes increased as the plant grew (Petit et al. 2014). In *A. aquilega*, leaf-absorbing trichomes and wells appeared at an age of 4–6 months (pers. obs.). With broader leavers, double the number of leaf-absorbing trichomes at the base of the lamina, and a 30-fold higher tank capacity, *A. aquilega* plants growing on a nutrient-rich substrate may benefit from higher nutrient supply compared to plants growing on a nutrient-poor substrate. We have shown that the fertility of the substrate during the germination and establishment stages in *A. aquilega* is of the utmost importance as it also affects performances at later ontogenetic stages. Given the high diversity of substrate (soil, bark or rocks)

used by wild *A. aquilega*, this could have important ecological repercussions. Specifically, large plants with high tank capacity may have ecological advantages such as a greater diversity and abundance of aquatic macro- and microorganisms that echo the bromeliad nutrition (Leroy et al. 2016) and a higher external water storage to resist drought stress (Males 2016).

Overall, we found that 70% of measured traits linked to plant performance responded significantly to the experimental treatments, indicating that *A. aquilega* response to nutrient availability is a combination of physiological processes and leaf and root structure and chemistry. The strong effect of substrate fertility on overall plant size and performance confirmed the importance of the root system in the establishment and growth of *A. aquilega*. This study and others showed that the roots of some bromeliad species are able to absorb nutrients in addition to the leaf trichomes. In our study, we further demonstrate that the nutrients absorbed by the roots determine the growth, size and performance of the plants which likely reflects bromeliad ecology. It thus appears that we need to reconsider the functional role of roots in bromeliad nutrition and the existence of different degrees of dependence on the substrate in the bromeliad family with probably significant variations both between and within species along ontogeny. Given the extraordinary diversity of bromeliads, future studies need to cover a much wider range of species, spanning both phylogenetic and ecological diversity.

Declarations

Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Author contributions: TLR: investigation, methodology, data curation, formal analysis, visualization, writing – original draft. SC : methodology, writing – review & editing. JYG : investigation, methodology. FJ : investigation, methodology. CS : methodology, writing – review & editing. CL : conceptualisation, funding acquisition, investigation, methodology, visualization, writing – original draft.

Data availability: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1

Effects of substrate fertility (**A**) number of green leaves, (**B**) number of adventive roots, (**C**) total dry mass (g), (**D**) tank water capacity (mL), (**E**) growth (nb leaves.year^{-1})), (**F**) RS ratio, (**G**) leaf length (cm), (**H**) leaf

thickness (mm) and, (**I**) total root length (cm). Different letters indicate significant differences between treatments, pairwise Wilcoxon test (α<0.05) after significant Kruskal-Wallis (α<0.05). WS – white sand, WSP – white sand/potting soil, P – potting soil
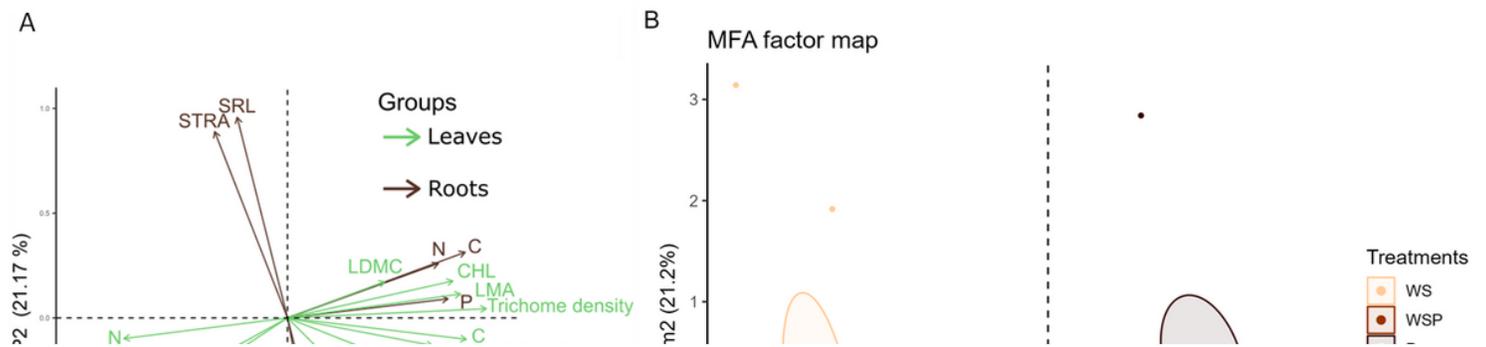

Figure 2

(**A**) Correlation circle of variables to the first two dimensions of the MFA. Variables are coloured by groups, green for leave and brown for roots. (**B**) Projections of individuals in the first two PCs plan. Individuals are coloured by treatments. Small dots are individuals' projections, big dots the centroid for each treatment and ellipses, the 95% confidence ellipses. WS – white sand, WSP – white sand/potting soil, P – potting soil

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