# Differential root responses to experimental drought in seedlings from five dominant andean species highlight the importance of ecosystem functional diversity.

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# DIFFERENTIAL ROOT RESPONSES TO EXPERIMENTAL DROUGHT IN SEEDLINGS FROM FIVE DOMINANT ANDEAN SPECIES HIGHLIGHT THE IMPORTANCE OF ECOSYSTEM FUNCTIONAL DIVERSITY

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## ABSTRACT

Seedling establishment is a critical stage for forest turnover and is susceptible to abiotic conditions such as drought events, which are becoming more frequent and severe combined with a drier atmosphere. Seedlings can respond differently to water limitation, depending on their life strategies and specific traits. The root system's response to water limitation is particularly relevant, as it is critical in water uptake, nutrition, and plant growth. Tropical Andean forests are among the most vulnerable ecosystems to climate change because of their limited geographic range and dependence on humidity conditions. However, knowledge about the effects of drought on Andean tree species is limited. In this study, we aimed to assess the root systems' responses to drought conditions during the seedling establishment of five Andean tree species (*Quercus humboldtii*, *Croton magdalenensis*, *Erythrina edulis*, *Meriania nobilis*, and *Clusia sp*.). We subjected seedlings to four soil water levels in the plant-available water (PAW) range: (1) watered to field capacity (control), (2) watered to 80% of PAW, (3) watered to 50% of PAW, and (4) total soil moisture exclusion. Seedlings in the soil moisture exclusion treatment started at field capacity and were allowed to dry down during the experiment, while 80% and 50% of PAW treatments included three successive dry–rewetting cycles. We measured various root morpho-functional traits, relative growth rate, above- vs. below-ground biomass allocation, predawn water potential, and recorded seedling mortality. Our results indicate that drought conditions reduced the species' growth rate and produced species-specific root responses more evident in the plants subjected to successive stress. *E. edulis* and *M. nobilis* were vulnerable species with high mortality percentages (23% and 80%, respectively). These species were associated with root acquisitive resource strategy with higher specific root length, thinner lateral roots, and high taproot branching density. C. magdalenensis, in turn, was a drought-resistant species also related to an acquisitive resource strategy. In contrast, Clusia sp. and Q. humboldtii were drought-resistant species related to a conservative resource strategy with thicker lateral roots, low specific root length, and higher root dry matter content. The results suggest that Andean species have the potential to respond to drought conditions by adjusting their intrinsic morpho-functional root traits. These responses depend on their resource acquisition strategies and highlight the potential impacts of climate change on seedling establishment and the forest composition due to species vulnerability to drought. Our findings could also aid in selecting species resistant to drought with root traits associated with conservative strategies for forest restoration projects.

**Keywords:** drought resistance, drought vulnerability, plant water status, root resource acquisition strategies, dry-rewetting cycles

## 1. INTRODUCTION

The reduction of soil water availability resulting from current and projected drought conditions, along with the increased vapor pressure deficit, can induce plant stress and exacerbate tree mortality in various ecosystems (Arias et al., 2021; Falk et al., 2022; IPCC, 2022; Barkhordarian et al., 2019). Nonetheless, compensating mechanisms for physiological processes enable plants to survive environmental stress, thereby acquiring stress resistance or increase their vulnerability to drought (Lambers & Oliveira, 2019). This plant species ability to respond to stress depends on its plant functional types and its strategies for resource acquisition (Reich, 2014; Zhou et al., 2018; Mitchell et al., 2013). This imply significant variations in the ability of co-existing species to cope with drought (Mitchell et al., 2013), which is relevant for the Tropical Andean Forests ecosystem, where the climate anomaly modifies the environmental conditions to which tree species are locally adapted, such as high humidity, lower temperatures, and the presence and persistence of fog

(Aguirre-Gutiérrez et al., 2022; Arias et al., 2021; Pérez-Escobar et al., 2022), potentially affecting the composition of this ecosystem.

Recent studies have found that drought conditions can severely impact seedling establishment, particularly because seedlings are sensitive to environmental conditions and have relatively shallow roots that can only explore the superficial layers of the soil that are the first to dry (Comita & Engelbrecht, 2014; Herzog et al., 2012). The potential impacts of this climate variation on seedlings have implications not only for the present ecosystem composition but also for its future dynamics, as this life stage is a determinant factor for defining recruitment and potential composition of the forest community (Comita & Engelbrecht, 2014). During the seedling stage, plants are vulnerable to changes in biotic and abiotic conditions. However, they can also respond to stress by adjusting their functional traits to ameliorate the effects of drought, for example (Stanik et al., 2021; Taiz et al., 2015; Violle et al., 2007). These responses, however, can vary among species due to the genetic and adaptive differences of each species that condition their physiological requirements for development and establishment (Comita & Engelbrecht, 2014).

The root system is decisive in seedling establishment, absorbing water, and nutrients necessary to maintain their functioning, providing physical stability to anchor itself to the ground, and sending signals to the rest of the plant when there are threats or environmental disturbances (Chirino et al., 2017; Freschet, Roumet, et al., 2021; Olmo et al., 2014). Roots also contribute to the soil's physical and chemical properties through the continuous renewal of fine roots (<2mm), facilitating the organic matter deposition that contributes to the regulation of biogeochemical cycles such as carbon and increases the porous structure of the medium (Freschet, Roumet, et al., 2021; Lambers & Oliveira, 2019). Studies on seedling responses to water limitation in drought experiments have focused mostly on aerial functional traits, while the root system responses have been little evaluated (Chirino et al., 2017; Lozano et al., 2020, 2022; Olmo et al., 2014). Despite root systems being essential for the metabolism of plants and their potential response to drought (Brunner et al., 2015; Freschet, Roumet, et al., 2021), the response of plant functional root traits has been less studied, due to the difficulty in their measurement and the lack of standardized methods to perform them (Brunner et al., 2015; Freschet, Pagès, et al., 2021). This knowledge gap is particularly critical in Tropical Andean Ecosystems where neither seedling drought vulnerability nor the response of root systems has been systematically assessed (Chirino et al., 2017; Duque et al., 2021; Nottingham et al., 2020). Understanding the potential impacts of this climate variation are critical for the seedling establishment for this system and are crucial to projecting future forest dynamics, as this life stage represents forest turnover.

Experimental studies on drought have shown significant associations between root functional traits and a plant species' resistance to water limitation (Brunner et al., 2015; Lozano et al., 2020; Markesteijn & Poorter, 2009). Some plant species, on one hand, reduce their root diameter to increase longitudinal growth and, thus, explore more volume of soil, and acquire water and nutrients as a strategy that allows them to avoid water limitation (Amissah et al., 2015; Chirino et al., 2017; Larson & Funk, 2016). In contrast, other species produce thicker and more durable roots with longer lifespans, allowing them to store resources (Birhane et al., 2023; Lozano et al., 2020; Olmo et al., 2014). Additionally, plants that invest more biomass in their roots tend to be more drought-resistant than those with greater aerial biomass, as increased root biomass can improve water uptake (Aaltonen et al., 2017; Chirino et al., 2017; Lozano et al., 2020). As the belowground components of plants play an essential role in their overall function, it is crucial to understand the mechanisms by which drought-induced changes in root traits can positively or negatively impact plant performance (Zhou et al., 2018).

In this study, we use an experimental approach to assess root responses to drought in seedlings from five species of dominant trees in the forests of the Northern Andes. We assigned seedlings to four water limitation treatments and measured the species response through several root morpho-functional traits. Additionally, we measured the predawn water potential, relative growth rate, biomass allocation, and we recorded seedling mortality. This experimental approach allowed us to evaluate three hypotheses: (1) what are the differences in intraspecific root response of species to water limitation? (2) How does interspecific root response of Andean seedlings vary in response to water limitation? and (3) which root responses are associated with increased seedling resistance or vulnerability to water limitation? Our results, which use a small subset of Andean tree species diversity, provide new insights that can aid in the overall assessment of ecosystem vulnerability and potential restoration on these important biodiversity hotspots.

## 2. MATERIALS AND METHODS

### 2.1. Species selection

We developed a greenhouse drought experiment with seedlings from five species (Clusia sp, Croton magdalenensis, Erythrina edulis, Meriania nobilis, and Quercus humboldtii) that represent a suite of potential functional responses to disturbance and have different ecological characteristics (Table 1). We obtained a total of 120 seedlings for each species (a total of 600 seedlings in the experiment) from a local nursery that guaranteed that all seedlings came from the same cohort and from the same site (seeds were germinated at the nursery). This allowed us to control any age or population variability that could affect individual responses to drought. We transplanted the seedlings into cylindrical nursery bags (28 cm x 9 cm) using a common potting soil collected from a location in the Central Andes of Colombia, mixed with rice hull to improve soil structure at a ratio of 3:1 (soil to rice hull). The resulting soil was a sandy loam (64% sand, 29% silt and 7% clay) with 24,76% total organic matter (14,36% organic carbon), Ca = 5,95 cmol/kg, Mg = 1,32 cmol/kg, P = 19,29 cmol/kg and, K = 0,75 mg/kg.

### 2.2. Experimental design

We conducted a controlled water limitation experiment from February to May in a greenhouse at the Unidad de Biotecnología of the Universidad CES in Medellín-Colombia. We measured daily air temperature, air relative humidity, and estimated vapor pressure deficit (VPD) with an Arable Mark2®. The greenhouse diurnal mean temperature during the experiment was 23.75°C, and the maximum was 37.1°C. The mean and maximum daytime relative humidity values were 69.5% and 92.7%, respectively. The VPD mean and maximum values were, respectively, 1.014 kPa and 3.06 kPa. We transplanted and watered the seedlings to allow them to acclimate to the greenhouse for two months before starting the drought experiment. We also fertilized all seedlings during the drought experiment with a Wuxal® foliar fertilizer to avoid nutrient deficiency effects.

**Table 1**. Native Tree species selected from the North Andean Forest for the study and their main ecological characteristics.

| **Specie/Family** | **Altitudinal distribution** | **Ecological characteristics** |
|---|---|---|
| *Clusia sp.* Kunth. Clusiaceae  | 1000 - 3500 m.a.s.l. | It is a late secondary slow-growing species, abundant in the northern Andean Forest. It usually has broad-ended oblong leaves, yellow resinous sticky sap, and some species have a hemiepiphytic habit with aerial roots (Medina et al., 2006).<br>We used a species from this genre distributed in the central Andes Mountain ranges at an approximate 2000 msnm, which was not possible to identify to the species level. |
| *Croton magdalenensis* Müll.Arg. Euphorbiaceae  | 1000 – 3000 m.a.s.l. | It is a pioneer fast growing species, endemic and abundant in Colombian Andean Forests. It has red resinous sticky sap and dense coverage of dendritic trichomes (Smith, 2006). |
| *Erythrina edulis* Triana ex Micheli. Fabaceae  | 1000 - 2500 m.a.s.l. | It is a pioneer fast growing species, frequent in the northern Andean Forests. Is a deciduous species with compound trifoliate leaves of chartaceous consistency. It establishes symbioses with nitrogen-fixing bacteria of the genus *Rhizobium* and with AMF (Pérez et al., 2015). |

| | | |
|---|---|---|
| *Meriania nobilis* Triana.<br> | 1500 – 3000 m.a.s.l. | It is a pioneer slow growing species, endemic and abundant in Colombian Andean Forests. Large diplostemone flowers; isophyllous, symmetrical, and petiolate leaves; numerous seeds, sub-quadrangular branches. Relative photosynthetic plasticity (Mendoza-cifuentes, 2021). |
| *Quercus humboldtii* Bonpl.<br> | 1000 - 3300 m.a.s.l. | It is a late secondary medium to slow growing species, and dominant of Andean Forests. Heliophilous in seedling and juvenile stages. It is a deciduous species, and it establishes symbiotic associations with mycorrhizal fungi which makes it efficient in the use of nutrients. It can resist drought conditions due to its evolutionary origin and original distribution (Nixon, 2006). |

The controlled water limitation experiment had four soil water content treatments in the plant-available water (PAW) range. We defined these treatments from the moisture retention curve of the soil used, taking as reference the field capacity and permanent wilting point in tensions of -30 and -1500 KPa, respectively (Weil & Brady, 2017). These treatments were: watered to field capacity (control), watered to 80% of PAW, watered to 50% of PAW, and total soil moisture exclusion (Fig 1A); in which we randomly assigned 30 seedlings per species on each treatment (4 treatments x 30 replicates = 120 seedlings/species). Seedlings in the soil moisture exclusion treatment started at field capacity and were allowed to dry down completely during the experiment (Fig 1A), while treatments of 80% and 50% of PAW included three successive dry–rewetting cycles that started at their respective initial soil volumetric water content. We applied moisture pulses to seedlings in these treatments to restart each dry–rewetting cycle based on a reference of 5 additional seedlings per species, that started at field capacity and dried until the temporary wilting point (10% of PAW), indicating the time to rehydrate the seedlings. We performed this to guarantee that seedlings reached soil volumetric water content below the permanent wilting point (PWP) in 80% and 50% of PAW treatments.

We dried two soil samples using an oven at 105°C for 48h to weigh the dry soil and to calculate the soil bulk density (Db) with the volume of the nursery bags. This information was necessary to convert weekly measurements of gravimetric moisture content to volumetric water content (VWC) of each treatment per species, weighing a sample of 10 pots/species/treatment (Equation 2 in Fig 1B). We manually watered seedlings based on the differences between the correspondent and the checked VWC on each treatment (Equation 3 in Fig 1B). We watered seedlings in the control treatment weekly until reaching field capacity, and the 80% of PAW and 50% of PAW treatments at their respective VWC, when the reference seedlings reached the temporary wilting point. We also watered these reference seedlings to field capacity to restart each dry-rewatering cycle.

The four soil water content treatments allow us to control the soil humidity, but as each species has different hydric requirements and water use, we defined three soil drought metrics based on the dry–rewetting cycles: intensity, severity, and pulsation (Fig 1C), to identify their association with lethality and to which of them seedlings adjust their root responses. We calculated the drought intensity (I) as the differences in soil VWC at the PWP and the minimum VWC reached by seedlings on each dry–rewetting cycle (Equation 4 in Fig 1C). Drought severity was the cumulative number of days (CD) below PWP in all dry–rewatering cycles (Equation 5 in Fig 1C). Drought pulsation was the cumulative number of days (CD) below PWP divided by the time of each dry–rewetting cycle (Equation 6 in Fig 1C). Additionally, we obtained the maximum vapor pressure deficit (VPD) per week in the greenhouse to relate it to soil drying (Fig 3).

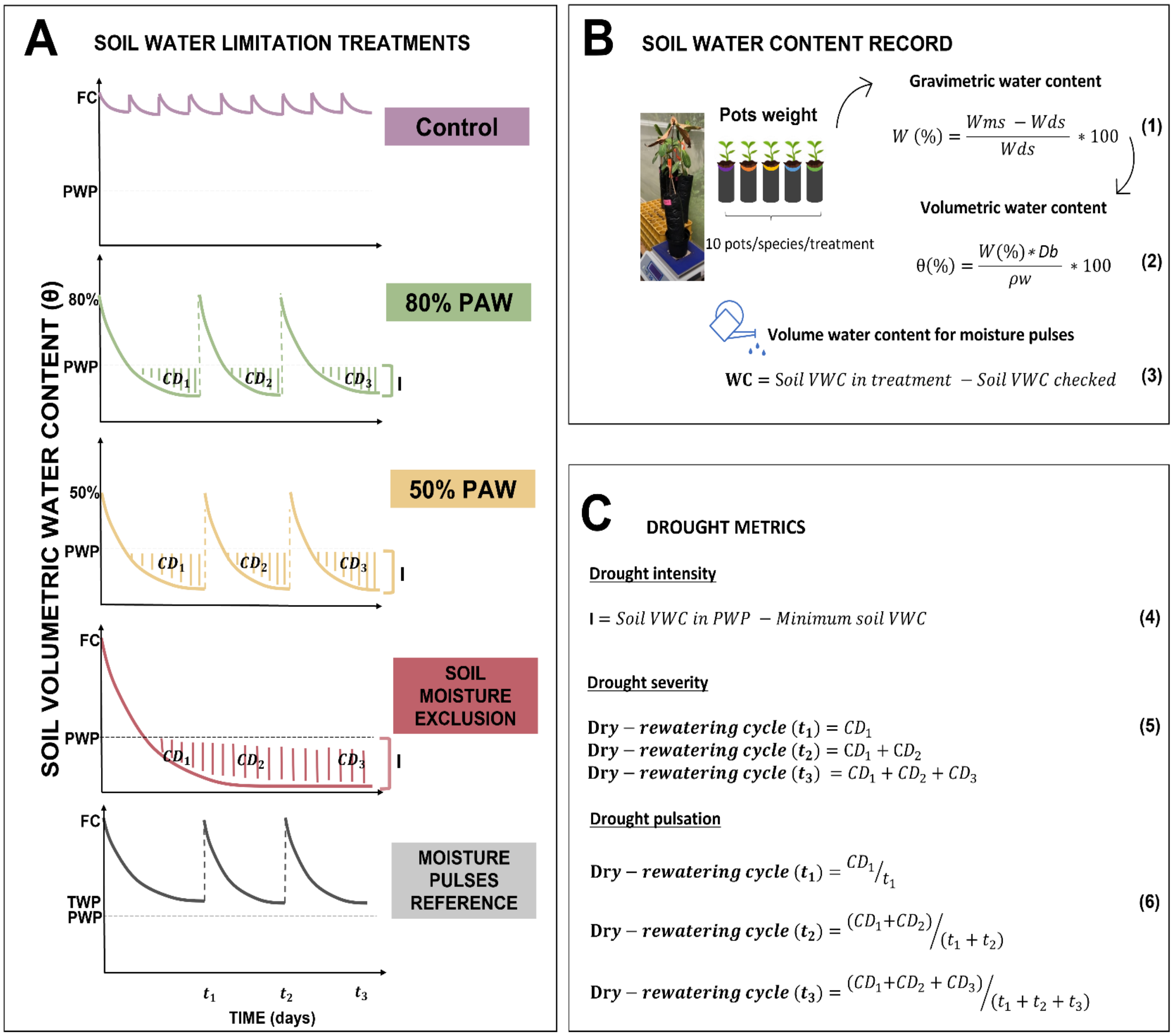

**Figure 1. (A)** Soil water limitation treatments: (1) control watered at field capacity with soil Volumetric Water Content (VWC) of ~48%, (2) 80% of plant-available water (PAW) that started with ~35% of soil VWC, (3) 50% of plant-available water (PAW) that started with ~30% of soil VWC, and (4) the soil moisture exclusion treatment which started at field capacity and we let to dry during the experiment. Additionally, the moisture pulses references five seedling/species that started at field capacity and dried until the temporary wilting point (TWP, at 10% of PAW) and indicated the time (days) to apply moisture pulses in 80% and 50% PAW treatment. Each dry-rewatering cycle is defined as $t_n$. **(B)** Weekly soil water content record. We obtained soil gravimetric water content (W(%), equation 1) weighing the pots samples (weight of the moist soil, Wsw) and operating with the weight of the dry soil (Wds) previously obtained. Then we calculated the volumetric water content ($\theta$(%), equation 2) with the soil gravimetric water content (W(%)), the soil bulk density ($Db$) and the density of water $\rho w$. **(C)** Drought metrics calculation: drought intensity (I), drought severity for each dry-rewatering cycle with the number of Cumulative Days below the Permanent Wilting Point (PWP), and drought pulsation with the number of Cumulative days (CD) below the PWP divided by the total number of days of each dry-rewatering cycle ($t_n$).

## 2.3. Seedling Performance and root functional traits

We harvested ten seedlings per species per treatment on each dry-rewetting cycle before rewatering the rest and recorded the mortality status of seedlings based on the complete desiccation of above-ground biomass. We randomly selected five of these harvested seedlings to measure root functional traits. Then, we separated roots, stems, and leaves, and removed the soil from the root system, washed them gently and obtained fresh weight the same day. We put the roots in an acrylic tray with a thin layer of water to easily organize the roots and prevent them from overlapping. We took the root photos at a standard height of 30 cm from the acrylic tray and used ruler as a calibration scale. We first took pictures of the taproot with the lateral roots visible to quantify the taproot diameter and length with SmartRoot software. Additionally, we identified the lateral roots number in this software with nodes to determine the taproot branching density.  We divided the taproot into three sections, and randomly selected four lateral roots (2 per side) from each section. We took photos of them to measure their diameter and length with Rhizovision Explorer software (see the methods S1 to more detail). We also measured the root nodule area of E. edulis with ImageJ to determine the effect of water limitation in this symbiotic relationship. We calculated total specific root length by multiplying the total root length by the total number of lateral roots, and the root length density based on the volume of the nursery bags (Methods S1). To measure these traits, we followed the procedures described in Freschet et al. (2021). Then, we dried the roots, stems, and leaves in an oven at 60°C until the structures were completely dry (~48 hours) and weighed them to calculate the relative growth rate (RGR) and root-to-shoot ratio with the total seedling biomass. We calculated the RGR for each treatment using biomass measurements from the previous dry-rewetting cycle with the 10 harvested seedlings as (equation 7):

$$RGR\ (biomass) = \frac{(\underline{lnb_n} - \underline{lnb_{n-1}})}{(t_n - t_{n-1})} \qquad \text{Equation 7}$$

where $lnb_n$ and $lnb_{n-1}$ correspond to the means of natural logarithm plant biomass at time $t_n$ and $t_{n-1}$.

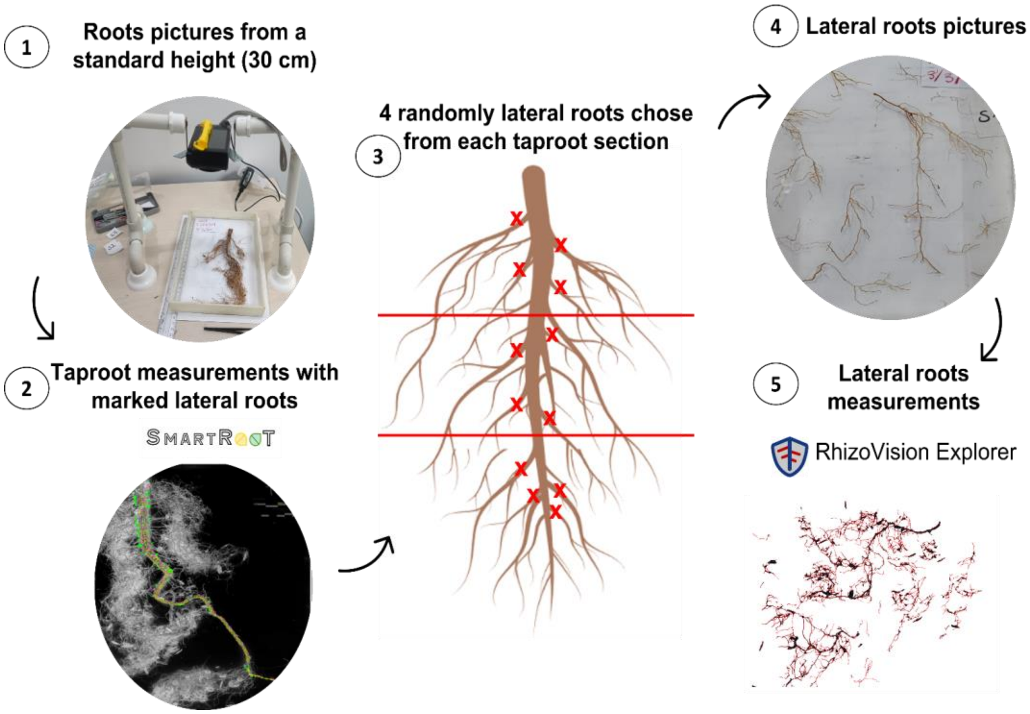

**Figure 2.** Root morpho-functional traits measurement procedure.

In addition, we measured predawn leaf water potential with a Scholander pressure chamber (Model 1000, PMS Instruments) to track the water status of plants during the experiment. We selected one fully developed leaf from 6 of the selected seedlings to harvest (before separating roots, stems, and leaves) for each of the four treatments. We chose these seedlings one day before taking the measurements and kept them in a dark chamber until the next day to avoid the effects of light at dawn. Then, we cut leaves at the petiole inside the dark chamber at 6:00 am and covered them in an aluminized bag to minimize transpiration. We measured leaf water potential following the procedure described by Knipfer et al. (2020). In *M. nobilis* seedlings, we measured water potential in the apex of the seedling shoot that included the completely developed leaves because of their sessile leaves.

### 2.4. Statistical analysis

We tested the effect of treatments, time, and their interaction on root functional traits and biomass allocation of each species using a two-way analysis of variance (ANOVA) to characterize the intraspecific differences in species response to the water limitation treatments. Although the residuals of some variables were not normally distributed, we carried out the ANOVA test because of its robustness. Also, we removed outliers from the analyses based on the z-scores (Table S1). We performed a-posteriori pairwise comparison of means with the Dunn-sidak method when we found significant interactions ($p < 0.05$). Additionally, we used simple linear models to assess the effect of soil drought metrics (intensity, severity, and pulsation) and plant water potential on species performance in terms of their RGR.

We conducted a principal components analysis (PCA) per treatment to identify the species interspecific root response through the relationship between species, root functional traits, seedlings performance and drought metrics. For that, we previously analyzed the relationships between the studied variables by Spearman correlations (Fig S1), from which we selected relevant functional traits and drought variables: taproot diameter, taproot branching density, specific root length, lateral mean root diameter, root dry matter content, root length density, drought pulsation and intensity. Also, we selected the mortality rate, root to shoot ratio and relative growth rate as metrics of plant performance. We analyzed the relationship with the second and third dry-rewetting cycle information because RGR data were only available with respect to the first dry-rewetting cycle.

We used multivariate logistic regression analyses to test the probability of mortality as a function of root traits (specific root length, lateral mean root diameter and taproot branching density), and thus, to determine the species' root responses associated with seedling resistance or vulnerability to water limitation. We selected these traits because of their significant association with mortality rate (Fig S1). We also evaluated the effects of RGR, biomass allocation, and drought metrics on seedling mortality. We performed all the statistical analysis in R studio (v. 4.1.3).

## 3. RESULTS

### 3.1. Experimental plant water limitation

Species in the water limitation treatments took different times to reach the temporary wilting point at the different dry-rewatering cycles (Fig 3). In terms of maximum vapor pressure deficit conditions in the greenhouse, species experience dry atmospheric conditions with an average value of 1.9 kPa, and the highest value reaching up to about 3.2 kPa (Fig 3A). *C. magdalenensis* seedlings were the fastest drying with a total duration of 62 days in the experiment, with each dry-rewatering cycle lasting about 20 days (Fig 3C). The lowest soil VWC values that seedlings of this species experienced were: ~20.5% in the 80% of PAW, ~19% in the 50% of PAW, and ~12% in soil moisture exclusion. The second fastest drying species was *E. edulis*, with 76 days in the experiment and a dry-rewatering cycle duration, on average, of 25 days (Fig 3D). This species had similar minimum soil VWC values to *C. magdalenensis* in water limitation treatments, except in the soil moisture exclusion, with a low value of ~15% of soil VWC (higher than *C. magdalenensis*). *Q. humboldtii* seedlings had an experimental duration of 83 days, with each dry-rewatering cycle duration of about 30 days (similar to *Clusia sp*, Fig 3F). With soil VWC low values of ~22.5% in the 80% of PAW, ~19% in the 50% of PAW, and ~15% in soil moisture exclusion. The slowest species in drying were *Clusia sp* and *M. nobilis*, with an experiment duration of 104 and 97 days, respectively (Fig 3B, E). Because of their high mortality rate, M. *nobilis* experienced only two dry-rewatering cycles (Table S2). Seedlings of *Clusia sp* reached low soil VWC values in water limitation treatments: ~20% in the 80% of PAW, ~16% in the 50% of PAW, and ~9.5% in soil moisture exclusion. *M. nobilis* experienced the following low values: ~22% in the 80% of PAW, ~23% in the 50% of PAW, and ~15% in soil moisture exclusion; indicating that this species experienced less dry soil conditions than the other species; but, despite this, was the most vulnerable. The differences in experimental drought duration between species are potentially associated with differences in transpiration rates (data not shown).

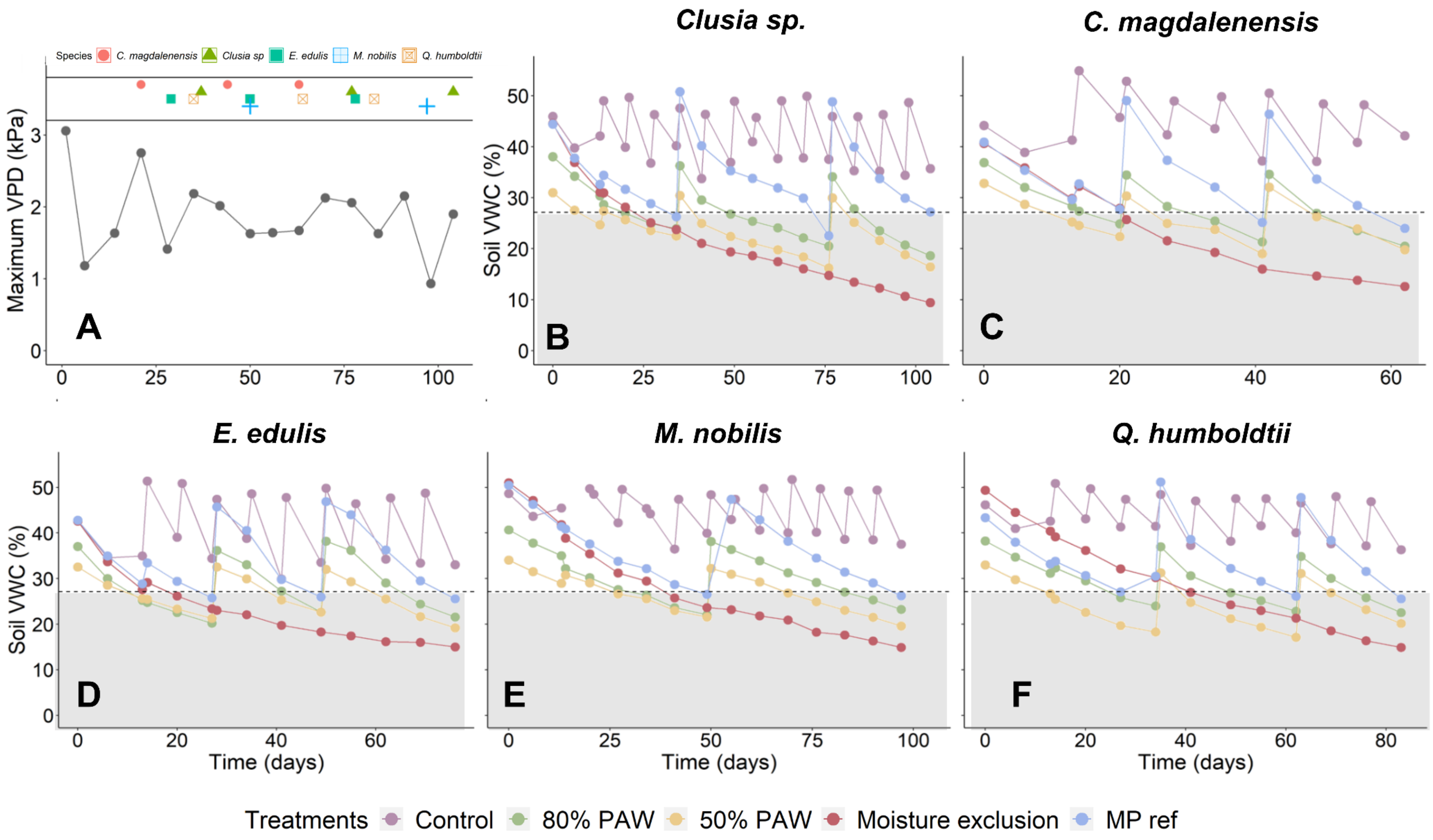

**Figure 3.** Soil volumetric water content of each treatment per species and vapor pressure deficit conditions. (A) Maximum vapor pressure deficit (VPD) values reached in the greenhouse. The upper strip of the graph shows the time (days) when we harvested the seedlings of each species. (B-F) Changes in soil volumetric water content (VWC, %) per species on each treatment with the total days of the experiment, including soil VWC of the pots with the reference seedlings to apply moisture pulses (MP ref) when these reached the temporary wilting point. The dashed lines represent the theoretical permanent wilting point (PWP), and the gray box highlights the soil VWC values below the PWP.

## 3.2. Species intraspecific response to drought

### *Drought metrics and water status effects on plant performance*

We found that, when considering species altogether in the water limitation treatments, drought metrics were significantly related to changes (always negative associations) in relative growth rate (biomass) over species ($p < 0.001$; Table 1). More specifically, drought pulsation (the cumulative exposition to drought) had both the most significant negative association with growth (negative slope with lowest p-value) and the resulting model had the highest explanatory power ($R^2$= 0.24), compared to the other drought metrics. This suggests that seedlings of species here evaluated are more susceptible to drought pulsation than to intensity and severity. However, the pulsation metric implicitly includes both intensity and severity. Water status (indicated by predawn leaf water potential with negatives values), in turn, had a positive significant association with species reduced growth, yet the resulting model had a very low explanatory power ($R^2$= 0.04, one order of magnitude lower than the others).

We separately assessed the association between RGR, drought metrics, and water status on each species to compare the magnitude and direction of the slope (neither looking for significance nor explanatory power, as original data was limited, Table 1). Our findings indicate that *C. magdalenensis* was the most negatively impacted species by drought severity and pulsation, followed by *E. edulis* and *Q. humboldtii*, which exhibited similar sensitivity to drought intensity based on the slope magnitude, implying a higher susceptibility to drought. Conversely, the RGR of *Clusia sp*. and *M. nobilis* was less affected, based on the less negative slope. In water status, *E. edulis*, *Q. humboldtii*, and *Clusia sp*. exhibited similar behavior with decreased growth positively associated with lower water potential values (negative values). On the other hand, C. magdalenensis reduced its growth as water potential became less negative during drought treatments. We did not observe any significant associations between root traits (as explanatory variables) and RGR (Table S3).

**Table 2.** Regression analysis parameters used to explore the relationship between each drought metric and water potential as explanatory variables of the seedlings' relative growth rate (RGR), considering all species together and their individual effects. We denoted significance levels with *, **, and ***, indicating p-values of less than 0.05, 0.01, and 0.001, respectively. Bold numbers denote the most and lower negative slopes.

**Relative growth rate (biomass)**

| | | **Intercept** | **Slope** | $R^2$ | **p value** |
|---|---|---|---|---|---|
| **Drought severity** | **All species** | 8.343e-03 | -1.645e-04 | 0.189 | 1.70e-07 *** |
| | *Clusia sp* | 5.038e-03 | **-8.851e-05** | 0.397 | 1.71e-05 *** |
| | *C. magdalenensis* | 1.728e-02 | **-5.090e-04** | 0.639 | 1.03e-09 *** |
| | *Q. humboldtii* | 9.767e-03 | -2.131e-04 | 0.264 | 9.79e-04 *** |
| | *E. edulis* | 1.074e-02 | -2.356e-04 | 0.390 | 2.78e-05 *** |
| | *M. nobilis* | 6.478e-03 | -1.664e-04 | 0.626 | 3.30e-05 *** |
| **Drought intensity** | **All species** | 8.045e-03 | -7.41e-04 | 0.134 | 1.47e-05 *** |
| | *Clusia sp* | 5.793e-03 | **-5.857e-04** | 0.629 | 1.74e-09 *** |
| | *C. magdalenensis* | 1.428e-02 | -1.034e-03 | 0.302 | 2.89e-04 *** |
| | *Q. humboldtii* | 1.110e-02 | **-1.270e-03** | 0.409 | 1.54e-05 *** |
| | *E. edulis* | 1.155e-02 | **-1.270e-03** | 0.473 | 1.87e-06 *** |
| | *M. nobilis* | 4.064e-02 | -5.904e-04 | 0.339 | 7.07e-03 ** |
| **Drought pulsation** | **All species** | 1.325e-02 | -2.156e-02 | 0.238 | 2.57e-09 *** |
| | *Clusia sp* | 6.294e-03 | **-1.045e-02** | 0.591 | 1.07e-08 *** |
| | *C. magdalenensis* | 1.597e-02 | **-2.426e-02** | 0.382 | 2.73e-05 *** |
| | *Q. humboldtii* | 9.740e-03 | -1.567e-02 | 0.238 | 1.87e-03 ** |
| | *E. edulis* | 1.382e-02 | -2.263e-02 | 0.718 | 1.93e-11 *** |
| | *M. nobilis* | 6.478e-03 | -1.613e-02 | 0.625 | 3.30e-05 *** |
| **Water potential** | **All species** | 4.70e-03 | 1.619e-03 | 0.046 | 1.684e-02 * |
| | *Clusia sp* | 8.376e-03 | 6.463e-03 | 0.534 | 1.27e-07 *** |
| | *C. magdalenensis* | -4.240e-03 | **-8.771e-03** | 0.294 | 3.66e-04 *** |
| | *Q. humboldtii* | 1.378e-02 | 4.664e-03 | 0.459 | 3.01e-06 *** |
| | *E. edulis* | 1.490e-02 | **9.788e-03** | 0.670 | 6.12e-11 *** |

*Root functional traits, symbiotic relationships, and biomass allocation*

We found that species had different responses to successive and prolonged droughts that varied not only among, but also within species depending on the extent of water limitation treatment and the intrinsic root system architecture. We observed that the adjustment of the species in the morpho-functional root traits was divided mainly between those species with greater root diameter (*Clusia sp and Q. humboldtii*), which tend to cope better with drought, and those with thinner roots and high specific root length with most drought vulnerability (such as *M. Nobilis*). Besides, species biomass allocation (root-to-shoot ratio) played an important role in improving water acquisition, as species tend to increase root biomass inversion despite prevailing in an aboveground biomass inversion.

### ***Clusia sp***

*Clusia sp.* had the highest aboveground biomass allocation among all species (root to shoot <1) in all treatments (Fig 4A), with significant interactions between treatment and time ($p<0.05$, Table S4). Seedlings in the 80% of PAW treatment adjusted their biomass allocation in the first and third dry-rewetting cycle with higher root-to-shoot values closer to one. Individuals in soil moisture exclusion treatment also converged to this proportion in the second dry-rewetting cycle but kept aboveground biomass allocation for the third drying cycle (Fig 4A). This aboveground biomass allocation is reflected in an increase in root dry matter content (Figure 4B).

We found significant interactions between treatment and time that influenced specific root length ($p<0.05$, Fig 4C). Individuals in the 50% of PAW increased their biomass investment on root length in the third dry-rewetting cycle compared to the second cycle, with a reduction in their lateral root diameter (Fig 4E). On the contrary, Individuals in the soil moisture exclusion treatment increased their lateral root diameter with time, with low investment in root length and increased taproot branching density (Fig 4D). Finally, we found a significant effect of water limitation and the interaction between treatment and time on the root length density in *Clusia sp* individuals. This suggests that seedlings reduce root-soil exploration in response to water limitation in the second and third dry-rewetting cycles and was lower in individuals in the soil moisture exclusion treatment in the third cycle. In addition, we found that this species did not change the seedlings' taproot diameter nor mycorrhizal colonization percentage.

### *C. magdalenensis*

We did not find significant differences among water limitation treatments in the root-to-shoot ratio in *C. magdalenensis*. Despite this, we observed that this species had values closer to 1 in water limitation treatments for the second and third dry-rewetting cycles (Fig 5A). This proportion suggests that seedlings kept their photosynthetic structures and their root system to maintain or to improve water absorption in response to water limitation. In addition, we found a significant effect of water limitation in root dry matter content, and a significant interaction of treatment and time (Table S4.2). The 80% PAW treatment caused a higher root dry matter content in the first dry-rewetting cycle (Fig 5B). However, in the following dry-rewetting cycles, the moisture pulses possibly increased their root water content. Also, soil moisture exclusion treatment affected seedlings of this species with an increase in their root dry matter content, with higher values than the other treatments in the third dry-rewetting cycle.

Individuals in the total moisture exclusion treatment had lower values of specific root length than the other treatments for the third dry-rewetting cycle (Fig 5C), while individuals in control and water limitation treatments increased their specific root length with time. We found significant effects of treatments for lateral root diameter in the first dry-rewetting cycle and effects of time but with no interaction between factors (Fig 5E). Seedlings in the control treatment had thicker lateral roots than in all the water limitation treatments, possibly due to the new root growth. We found that, in time, individuals in 80% and 50% of PAW reduced their lateral root diameter in the second dry-rewetting cycle and then increased it, which could be related to the fact that seedlings adjusted this trait after the moisture pulses. Additionally, this species did not change seedling taproots branching density.

In soil exploration strategies of plants, we found that treatments and time factors had significant effects on root length density but there was no interaction between them. Individuals in treatments of control, 50% of PAW and 80% of PAW increased their root length density in time while individuals in moisture exclusion treatment reduced soil exploration in the third dry-rewetting cycle. Finally, we found an effect of time with an increase in the colonization percentage in all treatments except in individuals in the total moisture exclusion treatment (Fig 5F). This result suggests that individuals of *C. magdalenensis* maintain their mycorrhizal symbiotic relationship despite water limitations.

### *E. edulis*

In general, *E. edulis* had higher aboveground than belowground biomass allocation, as indicated by the root-to-shoot values, with significant effects of water treatments in the first dry-rewetting cycles (Table S4.3), where individuals increased the root-to-shoot values closer to 1 compared to the control treatment (Fig 6A). Notably there were no other differences over time among treatments. Seedlings in the soil moisture exclusion treatment reached values close to 1 in the second and third dry-rewetting cycles, with significant differences compared to the first cycle. In addition, water limitation treatments, time, and their interaction explained the root dry matter content variation, where soil moisture exclusion treatment was the one that most affected this trait (Fig 6B).

In terms of root biomass inversion, we found significant effects of water limitation treatments and time on the specific root length of seedlings, while the interaction between these two factors was not significant (Table S4.3). Individuals increased their root length in the total moisture exclusion treatment compared to control treatment in the first and third dry-rewetting cycles (Fig 6C). We also found that individuals in 50 and 80% of PAW also had higher root biomass investment in root length than the control, although differences were only significant in the second cycle. This increase in carbon investment in longer roots could be related to the reduction of nodule size in water limitation treatments (Fig 6H) and correlated to thicker lateral root (Fig S2). Water limitation treatments reduced the taproot diameter of seedlings (Fig 6E), while lateral root diameter was affected by treatments, time, and their interactions (Fig 6F). Individuals of *E. edulis* increased soil exploration in time with higher values of root length density (Fig 6G), because of root length increase, without effects of water limitation treatments. Additionally, this species kept their mycorrhizal relationship in time and in all water limitation treatments.

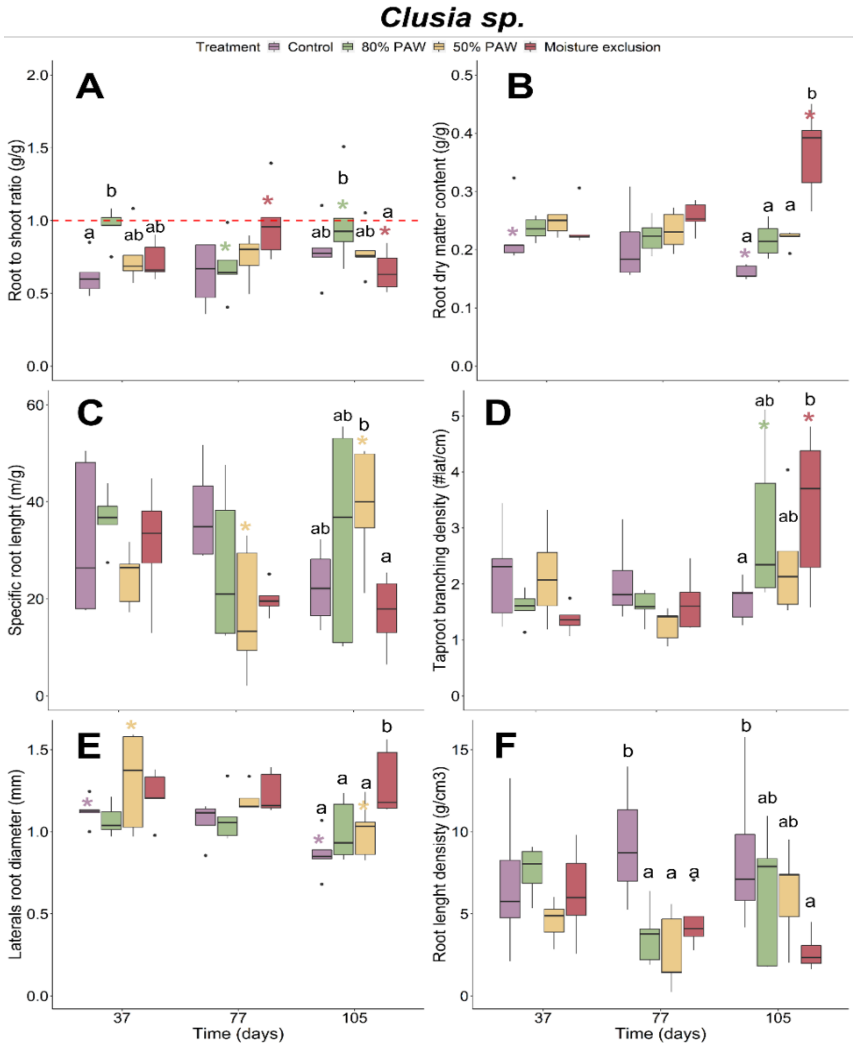

**Figure 4.** Root functional traits variation in seedlings of *Clusia sp*. Different letters correspond to statistically significant differences among treatments ($p < 0.05$), and asterisks colored by treatments identify statistically significant differences in time ($p < 0.05$). The red dashed line indicates the root-to-shoot value of 1 (panel A).

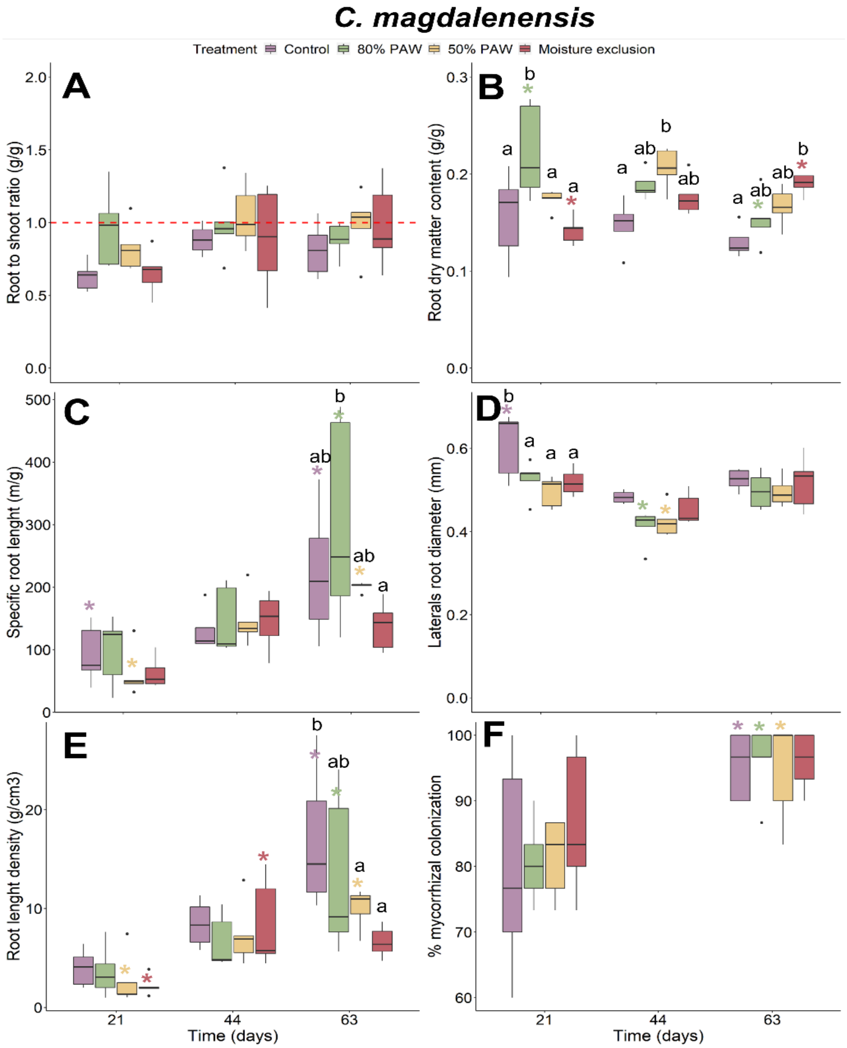

**Figure 5.** Root functional traits variation in seedlings of *C. magdalenensis*. Different letters correspond to statistically significant differences among treatments ($p < 0.05$), and asterisks colored by treatments identify statistically significant differences in time ($p < 0.05$). The red dashed line indicates the root-to-shoot value of 1 (panel A).

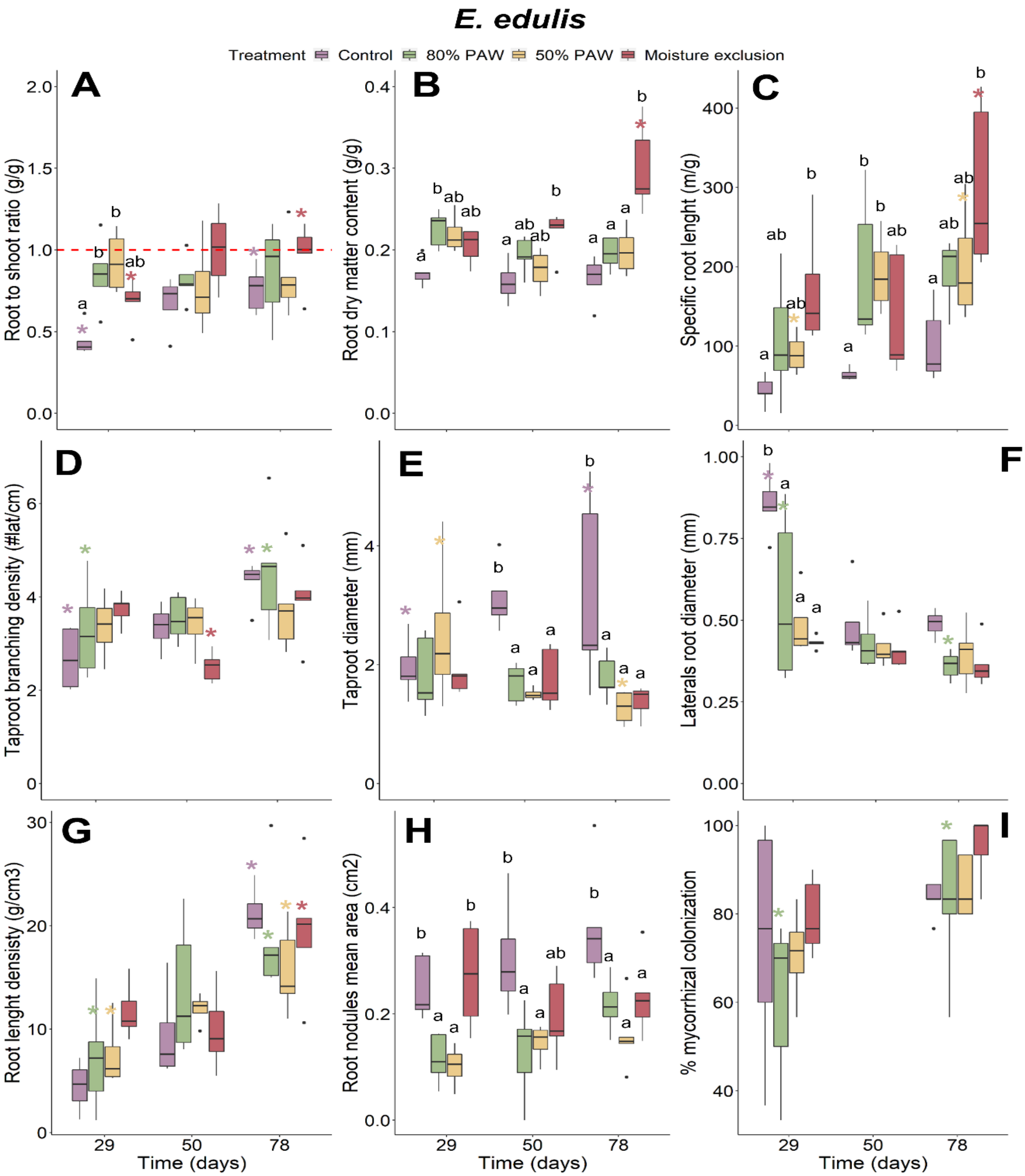

**Figure 6.** Root functional traits variation in seedlings of E. edulis. Different letters correspond to statistically significant differences among treatments ($p < 0.05$), and asterisks colored by treatments identify statistically significant differences in time ($p < 0.05$). The red dashed line indicates the root-to-shoot value of 1 (panel A).

### *M. nobilis*

In the experiment, we only could expose individuals of *M. nobilis* to two dry-rewetting cycles, as about 80% of the seedlings died in this period, whereby we harvested all the replicates. We observed that in general this species had more aboveground biomass allocation. Nevertheless, had higher root-to-shoot values in the 80% of PAW treatment at the second cycle (Fig 7A), which could suggest that with soil moisture pulses, seedlings tried to enhance water absorption by roots. However, this response was not enough to cope with the effects of water limitation, which also affected root dry matter content of individuals with increased values in water limitation treatments (Fig 7B). Seedlings in the 80% PAW treatment increased their taproot branching density over time. This could mean a limited potential for soil exploration, with lower values of root length density compared to the control treatment (Fig 7G), despite having increased biomass investment in long roots (Fig 7C). We found that water limitation treatments and time influenced the taproot and lateral root diameter without significant interaction between these factors in the second dry-rewetting cycle (Table S4.4). The taproot diameter had lower values in water treatments with differences in seedlings of 50% of PAW, while seedlings exposed to soil moisture exclusion treatment reduced their lateral root diameter (Fig 7F). Regarding mycorrhizal colonization, seedlings in control treatment reduced this symbiotic relationship in time, while seedlings in water limitation treatment kept this (Fig 7H).

### *Q. humboldtii*

Unlike the other species, seedlings of *Q. humboldtii* had higher biomass investment in their root system (root-to-shoot ratio > 1). Where seedlings in the 80% of PAW treatment doubled their root biomass allocation compared to aerial structures in the second dry-rewetting cycle, and then reduced this carbon investment to reach root-to-shoot values closer to 1 (Fig 8A). We found that the root dry matter content increased significantly in individuals exposed to water limitation treatments in the third dry-rewetting cycle (Fig 8B), with higher root dry matter content in seedlings of the 50% PAW treatment in the other cycles. In terms of root biomass investment, we found that water limitation, time, and their interaction, influenced the specific root length in this species (Table S3.5). Seedlings exposed to water limitation treatments did not change their specific root length in time, and this made them have shorter roots compared to individuals in the control treatment in the third dry-rewetting cycle (Fig 8C). The seedlings' adjustment with shorter roots correlates with thicker taproots and lateral roots (Fig 8D, Fig S1). However, we did not find an influence over lateral root diameters by water limitation and time (Table S4.5). This suggests that seedlings invested carbon allocation in roots to maintain the lateral root diameter, with a consequent effect on the reduction of soil exploration. Also, we observed that seedlings reduced their root length density in water limitation treatments, and, in time, the values were lower compared to seedlings in control treatments (Fig 8E). Additionally, this species maintained the mycorrhizal relationship over time and water limitation treatments (Fig 8F).

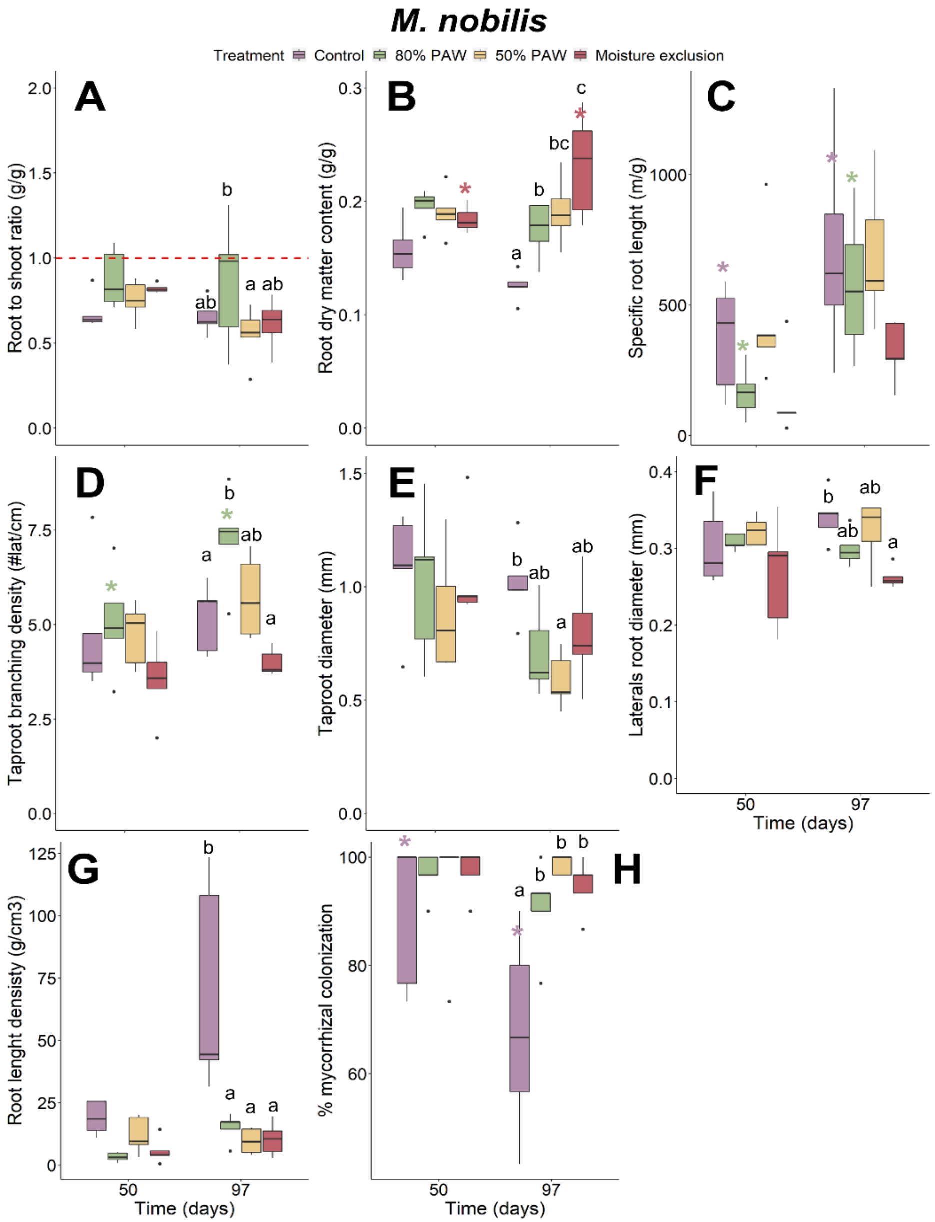

**Figure 7.** Root functional traits variation in seedlings of *M. nobilis*. Different letters correspond to statistically significant differences among treatments ($p < 0.05$), and asterisks colored by treatments identify statistically significant differences in time ($p < 0.05$). The red dashed line indicates the root-to-shoot value of 1 (panel A).

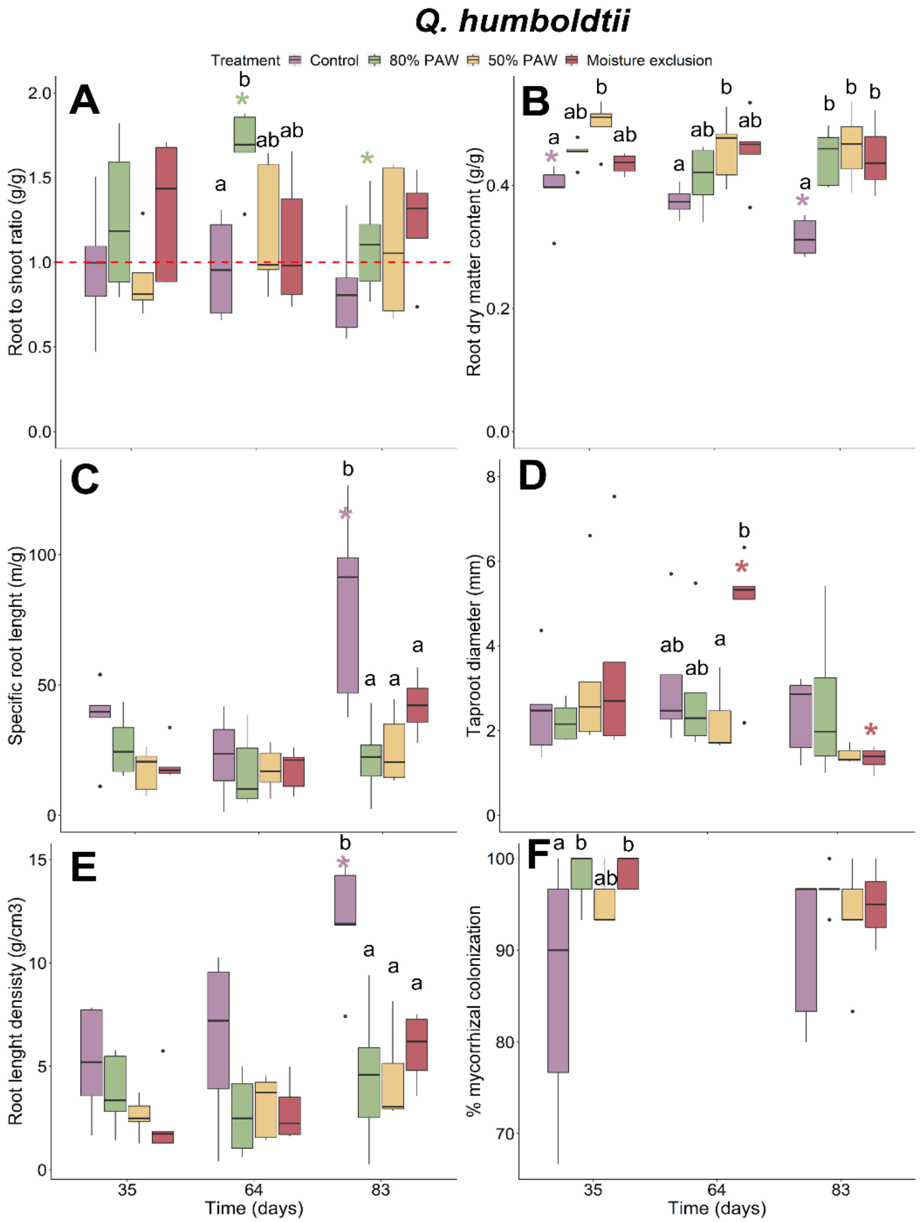

**Figure 8.** Root functional traits variation in seedlings of *Q. humboldtii*. Different letters correspond to statistically significant differences among treatments ($p < 0.05$), and asterisks colored by treatments identify statistically significant differences in time ($p < 0.05$). The red dashed line indicates the root-to-shoot value of 1 (panel A).

### 3.3. Interspecific root morpho functional traits response to drought

The association between root functional traits, biomass allocation, and mortality varied among the different levels of water limitation and, in turn, differed with the control treatment (Figure 9), highlighting the potential plastic responses of these species to water limitation. In the control treatment, the first two components of the PCA explained 53% of the total variance (Fig 9A). In the PCA of 80% PAW treatment, these components explained 57% of the total variation in the dataset (Fig 9B). In PCA of 50% PAW the axes explained 63% of the data variance (Fig 9C), and in PCA with soil moisture exclusion treatment, explained 53% of the total variance (Figure 9D). We observed that the PCA of 50% PAW (Fig 9C) treatment exhibited further separation of species with a contrasting response, followed by the PCA of 80% PAW treatment (Fig 9B). This result indicates a higher species seedling response to successive drought (dry-rewatering cycles) that started with low PAW.

The cluster formed by species in PCA analysis allows us to determine their interspecific response to drought based on their intrinsic root system architecture differences. This adjustment was divided mainly between those species with thicker roots, higher root dry matter content, and higher biomass investment in their root system (R:S, *Clusia sp*, and *Q. humboldtii*); and those with thinner and more branched roots with a high specific root length (*C. magdalenensis, E. edulis,* and *M. nobilis*).

In PCA of 50% PAW and soil moisture exclusion, we did not include *M. nobilis* due to the lack of water potential data. However, this species is related to the same variables as *E. edulis*.

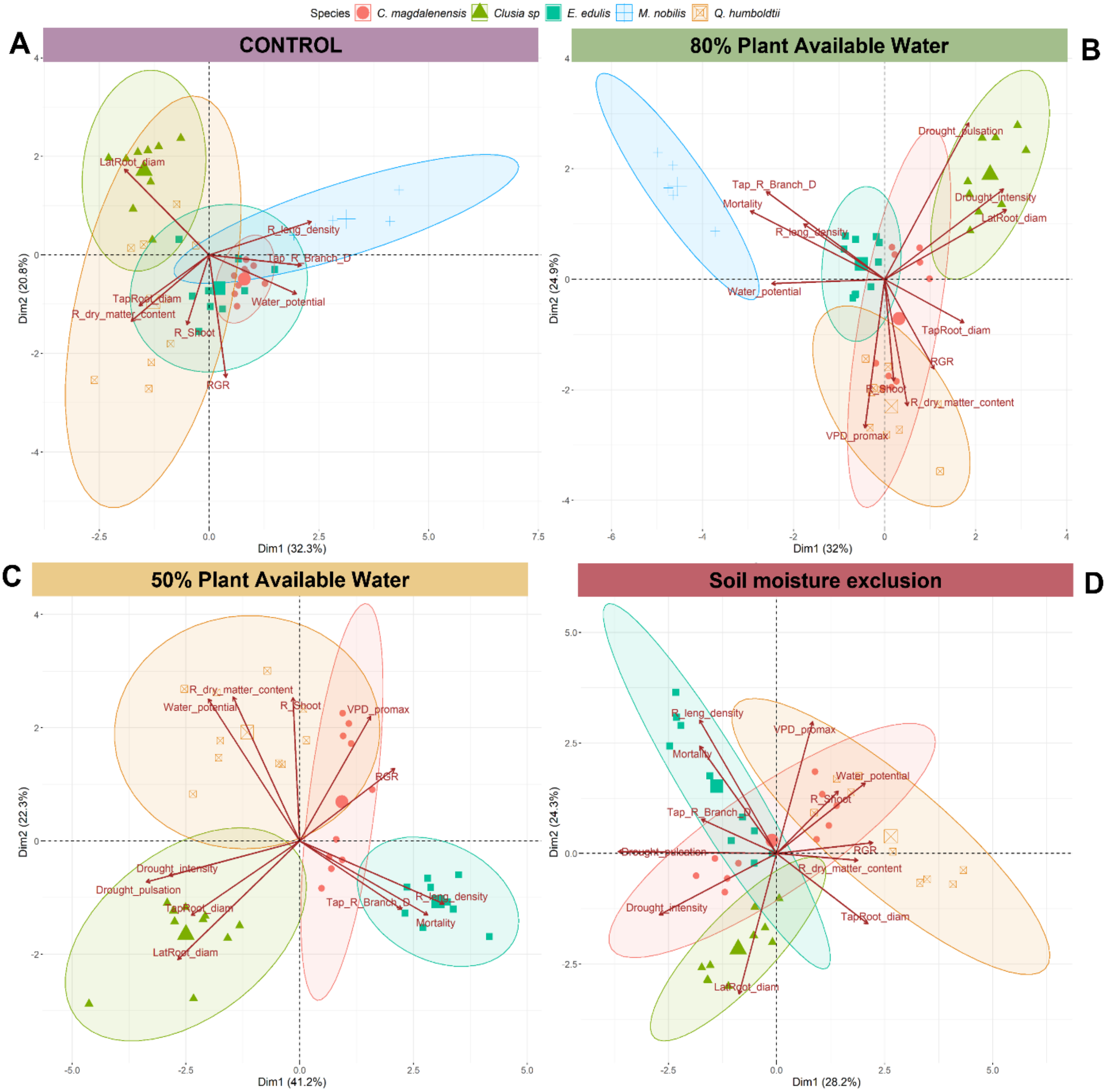

**Figure 9.** Principal Component Analysis (PCA) per treatment: (A) species associations with root traits, biomass allocation and predawn water potential in control treatment. (B) Species associations with root traits, biomass allocation, predawn water potential, maximum VPD and drought metrics in the 80% and 50% of PAW treatments. C) Species associations with root traits, biomass allocation, predawn water potential, maximum VPD and drought metrics in the 50%. (D) Species associations with root traits, biomass allocation, predawn water potential, maximum VPD and drought metrics in the soil moisture exclusion treatment. Abbreviations: relative growth rate (RGR), root-to-shoot ratio (R_Shoot), root dry matter content (R_dry_matter_content), taproot and lateral root diameter (TapRoot_diam and LatRoot_diam), taproot branching density (Tap_R_Branch_D).

### 3.4. Root morphological traits and drought vulnerability

Species' vulnerability to water limitation varied depending on their root morpho functional traits. The PCA analysis (Fig 9) revealed a positive correlation between species mortality and root traits, specifically SRL and TRBD, with *M. nobilis* and *E. edulis* exhibiting these traits. *M. nobilis* seedlings showed a higher mortality rate, with approximately 80% of the seedlings dying in all water limitation treatments by the end of the experiment (Table S2), which indicates that this species is highly vulnerable to slight variations in plant available water. Similarly, 23% of *E. edulis* seedlings died in the soil moisture exclusion treatment that simulated a protracted drought. Our analysis with a multivariate logistic regression model (Table 4) indicates that species with higher specific root lengths, taproot branching density, and smaller lateral root diameters have higher probability to die (Table 4). Thus, species with these characteristics in their root traits are potentially more vulnerable to drought in terms of their mortality. Interestingly, we did not observe any significant explanations associated with water limitation variables, relative growth rate (RGR), or biomass allocation (Table S5).

**Table 4.** Multivariate logistic regression with the relationship between root traits as explanatory variables of seedlings mortality. We selected specific root length, taproot branching density and lateral root diameter based on their significant correlation with seedlings mortality (Fig S1). *, ** and *** significance at the $p < 0.05$, 0.01 and 0.001 levels, respectively.

**Seedlings mortality**

| | **Estimate** | **p value** |
|---|---|---|
| **Intercept** | -2.052639 | 0.349 |
| **Specific root length** | 0.008638 | 0.000524 *** |
| **Taproot branching density** | 0.659318 | 0.013051 * |
| **Lateral root diameter** | -10.32328 | 0.022400 * |

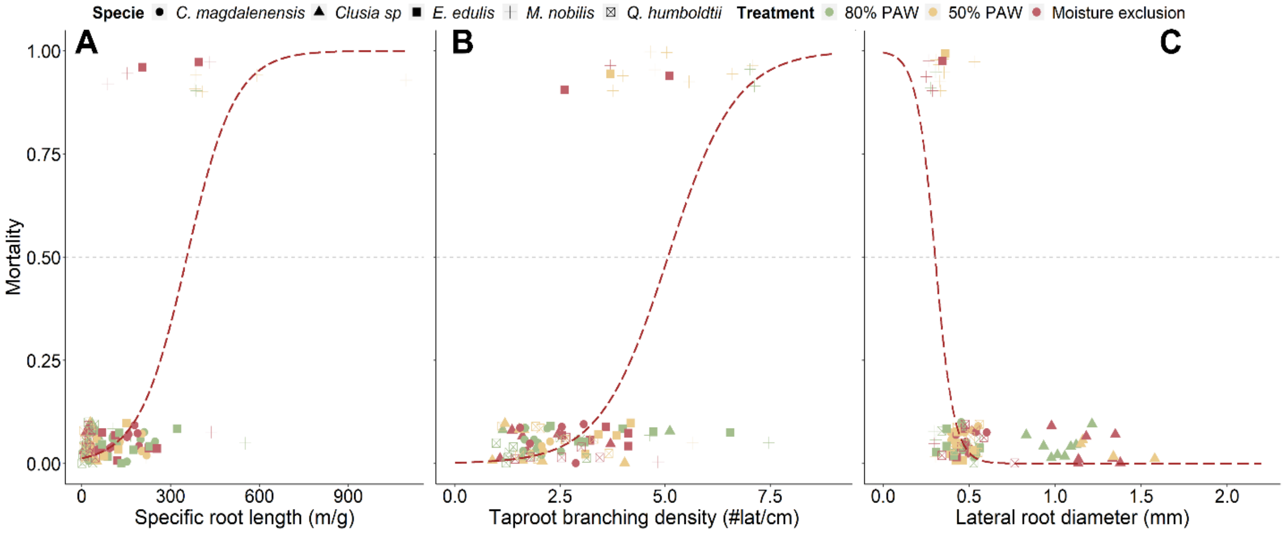

**Figure 11.** Relationship between root morphological and functional traits and seedlings mortality: (A) specific root length, (B) Taproot branching density, (C) Lateral root diameter. The gray horizontal line highlights the 50% mortality probability.

## 4. Discussion

Although the experimental approach to apply dry-rewetting cycles has been previously used to study plant responses to soil water deficit, mostly in crop plants (Abdalla & Ahmed, 2021; Canarini & Dijkstra, 2015; Marchin et al., 2020; Zhan et al., 2015), this is the first study to show how species from native Andean Forest respond to water limitation through their root traits adjustment. We improved the conventional dry-down experiments by exposing plants to successive droughts through the application of three dry-rewatering cycles with the combination of moisture pulses with different levels of soil VWC. This type of approach also allowed us to experimentally create a simulated protracted level of intermediate stress to evaluate the potential plastic responses to changes in the amount and seasonality of water limitation. This new way to assess drought responses goes beyond the conventional approach that focuses on mortality, to evaluate plasticity and species-specific responses in one of the most important biodiversity hotspots in the world (Duque et al., 2021).

### *Species water status and growth restriction*

Drought experimental studies under greenhouse and field conditions reported that plants reduced their growth in the face of water limitation (Bauman et al., 2022; Cusack et al., 2021; Hammond et al., 2022; Larson & Funk, 2016), which is linked with our results that showed a species lower their relative growth rate in response to drought (Table S3.1). This growth reduction is a strategic response related to the regulation of water use, by decreasing transpiration from stomatal closure. However, as a tradeoff, carbon assimilation is also reduced (Amissah et al., 2015; Chirino et al., 2017; Choat et al., 2018). According to the differential species performance declines that we observed, this response could be attributed to the species intrinsic growth rate (Mitchell et al., 2013), as the fast-growing species were more affected (*C. magdalenensis* and *E. edulis*) compared to those with slow-growing rates (*M. nobilis* and *Clusia sp*).

### *Species intraspecific root morpho-functional response*

In terms of overall biomass allocation, the Andean species used in this experiment consistently invested more biomass in aboveground than in belowground structures (Fig 4,5,6, and 7, panel A). However, they tended to increase root biomass allocation in some water limitation treatments while maintaining the overall trend of higher above- than below-ground biomass allocation, except in *Q. humboldtii* that had higher biomass investment in their root system (Fig 8A). This behavior could be explained under Brouwer's hypothesis that postulates that species privilege biomass allocation for acquiring the most limiting resource (soil water in this case; Amissah et al. (2015)). Thus, an increase in root biomass allows species to improve water acquisition (Brunner et al., 2015; Cusack et al., 2021; Markesteijn & Poorter, 2009; Taiz et al., 2015). However, although proportional biomass allocation to roots generally grew with water limitation, there were some interspecific differences related to root architectural attributes.

Several studies that exposed plants to a water limitation regime identified root allocation patterns in morpho-functional root traits related to increasing root length (Harrison & LaForgia, 2019; Lozano et al., 2020; Olmo et al., 2014) or increased root diameter (Larson & Funk, 2016). We found that *C. magdalenensis*, *E. edulis*, and *M. nobilis* were the species with higher root biomass investment in root elongation (Fig 5, 6, and 7, panel C), which it is related to studies that linked this elongation as a response to drought with efficient soil exploration (Brunner et al., 2015; Freschet, Roumet, et al., 2021; Olmo et al., 2014) and together with the high taproot branching density of these species (Fig 5 and 6), supported the localized exploration of soil resources and water absorption (Freschet, Pagès, et al., 2021; Zhan et al., 2015). In contrast, the strategies of *Clusia sp.* and *Q. humboldtii* converged to have thicker roots (despite Clusia sp also increased its specific root length in some water limitation treatments (Fig 4C)), that provide the advantage of conducting more water per cross-section, along with their longer lifespan that ensures resource acquisition over time (Lozano et al., 2020).

The species root adjustment has metabolic costs that differ among species (Lozano et al., 2020). In the case of *E. edulis*, we observed that this compensation for root biomass investment in root elongation was done through a reduction in root nodule construction and in taproot diameter (Fig 6E and 6H). However, a greater specific root length does not necessarily imply a high root distribution in the soil as, for example, in the case of *M. nobilis*, which had a lower root length density, which potentially influenced a reduction of resource uptake by roots (Fig 7G) and could be related to its higher vulnerability to drought. Conversely, *Q. humboldtii* had higher root biomass investment in response to drought, with thicker lateral roots, shorter roots, and higher root dry matter content. This investment in denser roots implies higher lignification and suberisation that minimize water loss and cell dehydration (Brunner et al., 2015; Freschet, Roumet, et al., 2021). For instance, Suseela et al. (2020) found that two species of genera *Quercus* exposed to drought conditions did not change their lignin content, which improves the integrity of xylem walls. This type of strategy maximizes resource uptake by facilitating water transport in longer lasting root tissue that is protected from pathogens and is resistant to environmental deterioration (Freschet, Roumet, et al., 2021).

### *Root species interspecific strategies responses and drought vulnerability*

Our results indicate that species-specific responses were amplified in the successive intermediate drought conditions compared to protracted drought (in the water exclusion treatment). This is particularly relevant to understanding the potential responses of Andean Forests, where intermediate and temporary water limitations are potentially more common than protracted drought as the result of expected amplified climate variability (Ortega et al., 2021). This amplified response also coincides with the observed increased sensitivity to drought frequency, to which the species adjusted their root traits, performance, and biomass allocation (Zhou et al., 2018). These results coincide with previous findings (Aaltonen et al., 2017), which argue that species exposed to previous droughts increase their change to acclimate through, for example, water status regulation and growth rate regulation, both potentially mediated by root responses.

Notably, these responses varied among species and related to (1) their survival rates and (2) their performance. More specifically, we found that *Clusia sp* and Q. humboldtii were drought-resistant species with thicker and denser lateral roots, root traits related to conservative strategies of resource acquisition (Fig 9, Weemstra et al., 2016). Particularly, these attributes are relating to increase mycorrhizal colonization (Lozano et al., 2020; Suseela et al., 2020), as Restrepo et al. (2022) found that these two species showed higher mycorrhizal colonization even under water limitation conditions, which potentially increase species' water acquisition after moisture pulses. On the other hand, we found that *E. edulis* and *M. nobilis* were species with higher specific root length, thinner and branched roots, that are associated with acquisitive resource strategies (Fig 9, Weemstra et al., 2016), and were the only two species that exhibited mortality in water limitation treatments (Table S2). In fact, previous studies have found that species with rapid water transport and storage are more susceptible to dying in dry conditions, since it implies higher metabolic costs when there is a shortage of resources (Chaturvedi et al., 2021; Reich, 2014). Nevertheless, this is not a generalized result, as we also found that *C. magdalenensis* is a drought-resistant species with root traits associated with an acquisitive resource strategy (Fig 9), opening the potential to new explanations in drought vulnerability. In fact, Harrison & LaForgia (2019) and Olmo et al. (2014) also found greater specific root length in species that survive drought. Nevertheless, as Valverde-Barrantes et al. (2017) discuss, non-only the species-specific root adjustment defined the species response to drought, the phylogenetic history of the different species also influenced their resistance and vulnerability to drought conditions.

Studies argue that the resistance of species in scarce conditions such as drought is mediated by the life form (Hooghiemstra et al., 2022; Valverde-Barrantes et al., 2017). In the case of the drought-resistant species of this study, species of *Clusia sp* have a hemiepiphytic growth form for which they produce adventitious roots adapted to an intermittent water supply (Zotz et al., 2021). In the case of *Q. humboldtii*, it is a species that resists warm and drought conditions allowing it to follow its migratory pathways that confer higher flexibility to survive a wide range of environmental conditions (Hooghiemstra et al., 2022). While *C. magdalenensis* is a recognized species for a wide distribution range (Smith, 2006), that possibly confers a greater plasticity response to environmental conditions. On the other hand, the drought-vulnerable species, *M. nobilis* being species distributed in an altitude range with high water availability across the year and low temperatures ([9-17 °C], Mendoza-cifuentes (2021)) could experience plant stress by a combination of soil water scarcity and dry atmosphere. Despite all species being in the same dry conditions, the *M. nobilis* rapid mortality rate could be related to a limited physiological response to rapid changes in the face of drought and high evaporative demand (Choat et al., 2018; Marchin et al., 2020). Regarding the seedling mortality of *E. edulis* exposed to prolonged drought (soil moisture exclusion treatment) this could be related to its loss of carbon, a commonly documented drought response mechanism, that affected its symbiotic association with *Rhizobium cowpea* (a nitrogen-fixing bacteria, Escamilo, 2012), reducing its capacity to promote plant growth by manipulating plant hormones in the face of water limitation (Cusack et al., 2021; Poudel et al., 2021).

Our results showed important insights in terms of the Andean species strategies to cope or fail in drought conditions through the plant root system. This is relevant in the Root Economic Spectrum (RES) difficult to frame the species with the conventional classification of conservative and acquisitive strategies based on the root attributes (Valverde-Barrantes & Blackwood, 2016; Weemstra et al., 2016). We found that in Andean species the root traits are not matched with leaf traits, overwise in the acquisitive strategies, particularly in *M. nobilis*, a species considers with conservative strategies because of its slow growth, but with root traits associated with faster resource acquisition that potentially increase the vulnerability of species to drought. Additionally, despite previous studies found that species with resource conservation strategies have the advantage to survive in low resource conditions because they grow slowly and have less water use (Chaturvedi et al., 2021; Reich, 2014), we observed that species with root traits associated with acquisitive strategies also have others mechanism that underlying their possibilities to cope with drought.

In synthesis, our results show how seedlings that span a gradient of functional strategies among the dominant species in wet Andean forests react to successive and prolonged drought conditions through a variety of functional responses in their root systems. Importantly, these responses were more evident when plants were subjected to successive stress, indicating a plasticity in their response. The effectiveness of these responses varied among species, that depends on their intrinsic morphological root traits, their acquisition strategies, and their ecological characteristics. These results provide information for forest restoration processes with interest in including resistant species to drought, thus, species with thicker and denser roots are more resistant to this climate variation.  Besides, these results highlight a differential Andean species vulnerability to drought that could result in composition shifts for these important ecosystems. Our results which are pioneer in both evaluating root system response to drought and in using seedlings to test this, highlight the potential impacts of climate change on seedling establishment, such that frequent droughts could affect forest composition and function of Andean ecosystems and highlight the importance of functional diversity to preserving these biodiversity hotspots.

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# SUPPLEMENTARY MATERIAL

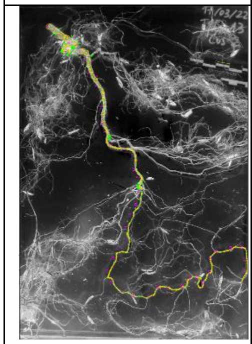
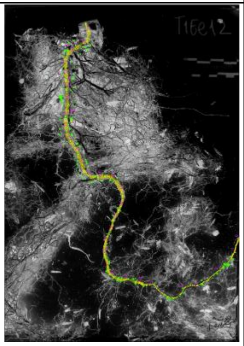
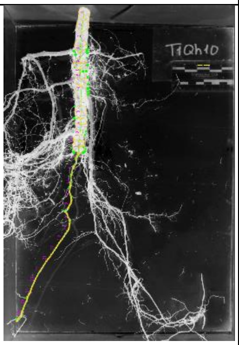
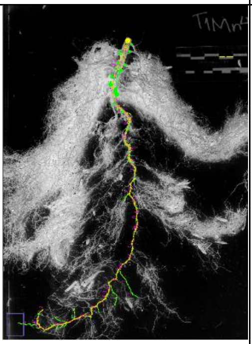
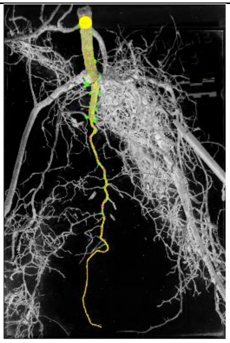
## Methods S1.

In this section we show some pictures of the experiment setup and explain in detail the methodology that we followed to measure the morpho-functional root traits.

- Greenhouse installation with the seedling's aleatory distribution and roots sample pictures.

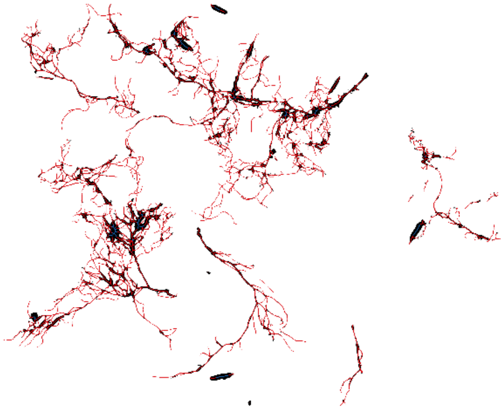
## Morphological root traits measurements and calculations:

- Tap root morphological traits:

| *C. magdalenensis* | *E. edulis* | *Q. humboldtii* | *M. nobilis* | *Clusia sp.* |
|---|---|---|---|---|
|  |  |  |  |  |

$$Tap\ root\ branching\ density\ [\#/cm] = \frac{Total\ lateral\ root\ number}{Tap\ root\ length}$$

- Lateral roots morphological traits:



$$Specific\ Root\ Length\ [m/g] = \frac{(Total\ laterals\ root\ length\ * Total\ lateral\ root\ number)}{Peso\ seco\ raíces}$$

$$Lateral\ root\ median\ diameter\ [mm]\ = Mean\ of\ the\ median\ values$$

$$Root\ Length\ density\ [m/cm^3] = \frac{Total\ laterals\ root\ length + Tap\ root\ length}{Soil\ volume}$$

**Table S1.** Root morpho-functional traits and biomass allocation outliers removed from the ANOVA test on each species based on the higher z-score (highlighted in bold letters).

| Species | Days | Soil water treatment | Root trait and biomass allocation | Values | Z-score |
|---|---|---|---|---|---|
| *Clusia sp* | 105 | Control | Specific root length | **91.2** | **1.74** |
| | | | | 17.5 | -0.595 |
| | | | | 26.8 | -0.300 |
| | | | | 13.6 | -0.718 |
| | | | | 32.2 | -0.127 |
| | 105 | Control | Root length density | **41.0** | **1.71** |
| | | | | 7.86 | -0.473 |
| | | | | 6.36 | -0.572 |
| | | | | 4.17 | -0.716 |
| | | | | 15.8 | 0.0475 |
| | 77 | 50% PAW | Lateral root diameter | **2.12** | **1.76** |
| | | | | 1.34 | -0.110 |
| | | | | 1.15 | -0.558 |
| | | | | 1.15 | -0.555 |
| | | | | 1.16 | -0.533 |
| *E. edulis* | 78 | Control | Root length density | 24.9 | -0.121 |
| | | | | 18.7 | -0.656 |
| | | | | 20.1 | -0.535 |
| | | | | **46.5** | **1.75** |
| | | | | 21.2 | -0.441 |
| *M. nobilis* | 97 | 50% PAW | Lateral root diameter | 0.25 | -1.11 |
| | | | | 0.354 | -0.0896 |
| | | | | 0.329 | -0.332 |
| | | | | 0.352 | -0.100 |
| | | | | **0.528** | **1.63** |
| *Q. humboldtii* | 35 | 50% PAW | Root-to-shoot ratio | 0.804 | -0.513 |
| | | | | 0.696 | -0.592 |
| | | | | **3.90** | **1.76** |
| | | | | 0.820 | -0.501 |
| | | | | 1.29 | -0.157 |

**Table S2.** Cumulative mortality rate of *M. nobilis* and *E. edulis* individuals.

| | | **Cumulative mortality rate** | | | |
|---|---|---|---|---|---|
| **Species** | **Dry-rewetting cycle** | **Control** | **80% PAW** | **50% PAW** | **Moisture exclusion** |
| ***E. edulis*** | $t_2$ | - | 3.3 % | 10 % | - |
| | $t_3$ | - | 3.3 % | 16 % | 23.3 % |
| ***M. nobilis*** | $t_1$ | - | 20 % | 30% | 13.3% |
| | $t_2$ | - | 83.3% | 83.3% | 76.6% |

**Table S3.1.** Simple regression analysis parameters used to explore the relationship between some morphologic root traits as explanatory variables of the seedlings' relative growth rate (RGR), considering all species together and their individual effects. We denoted significance levels with *, **, and ***, indicating p-values of less than 0.05, 0.01, and 0.001, respectively. We did not consider the effect of lateral root diameter, despite being significative, by their low percentage explanation ($R^2$ =0.04).

**Relative growth rate (biomass)**

| | **Intercept** | **Slope** | $R^2$ | **p value** |
|---|---|---|---|---|
| Specific Root Length | 0.0049612 | -1.465e-06 | 0.001671 | 0.592 |
| Tap root branching density | 1.728e-02 | -0.0004381 | 0.008027 | 0.24 |
| Laterals root diameter | 0.004319 | -0.004376 | 0.04619 | 0.012981 * |

**Table S4.1.** *Clusia sp*. outcomes of the analysis of variance (two-way ANOVA) applied in root morpho-functional traits and mycorrhizal colonization in relation to time, water limitation treatments, and their interactions. *, ** and *** significance at the p < 0.05, 0.01 and 0.001 levels, respectively.

| **Specie** | **Factor** | **Df** | **F value** | ***p*-value** |
|---|---|---|---|---|
| *Clusia sp* | **R_Shoot** | | | |
| | Time | 3 | 2.319 | 0.0875 |
| | Treatment | 2 | 0.311 | 0.7344 |
| | Time x Treatment | 6 | 2.784 | 0.0212 * |
| | **R_dry_matter_content** | | | |
| | Time | 3 | 13.214 | 2.20e-06 *** |
| | Treatment | 2 | 0.417 | 0.661 |
| | Time x Treatment | 6 | 6.393 | 5.67e-05 *** |
| | **Specific_Root_Lenght** | | | |
| | Time | 3 | 1.691 | 0.1820 |
| | Treatment | 2 | 1.258 | 0.2940 |
| | Time x Treatment | 6 | 2.368 | 0.0447 * |

|  |  |  |  |  |
|---|---|---|---|---|
|  | **Tap_R_Branch_D** |  |  |  |
|  | Time | 3 | 0.192 | 0.901659 |
|  | Treatment | 2 | 8.389 | 0.000767 *** |
|  | Time x Treatment | 6 | 2.948 | 0.015895 * |
|  | **TapRoot_diam** |  |  |  |
|  | Time | 3 | 0.217 | 0.884 |
|  | Treatment | 2 | 0.047 | 0.954 |
|  | Time x Treatment | 6 | 1.226 | 0.310 |
|  | **SecRoot_diam** |  |  |  |
|  | Time | 3 | 6.377 | 0.00105 ** |
|  | Treatment | 2 | 3.706 | 0.03219 * |
|  | Time x Treatment | 6 | 1.556 | 0.18174 |
|  | **R_leng_density** |  |  |  |
|  | Time | 3 | 5.786 | 0.00192 ** |
|  | Treatment | 2 | 0.460 | 0.63436 |
|  | Time x Treatment | 6 | 2.476 | 0.03696 * |
|  | **% Mycorrhizal colonization** |  |  |  |
|  | Time | 3 | 0.599 | 0.6202 |
|  | Treatment | 1 | 3.626 | 0.0659 |
|  | Time x Treatment | 3 | 1.185 | 0.3309 |

**Table S4.2.** *C. magdalenensis* outcomes of the analysis of variance (two-way ANOVA) applied in root morpho-functional traits and mycorrhizal colonization in relation to time, water limitation treatments, and their interactions. *, ** and *** significance at the p < 0.05, 0.01 and 0.001 levels, respectively.

| **Specie** | **Factor** | **Df** | **F value** | ***p*-value** |
|---|---|---|---|---|
| *C. magdalenensis* | **R_Shoot** |  |  |  |
|  | Time | 3 | 2.371 | 0.0824 |
|  | Treatment | 2 | 3.743 | 0.0310 * |
|  | Time x Treatment | 6 | 0.824 | 0.5569 |
|  | **R_dry_matter_content** |  |  |  |
|  | Time | 3 | 7.886 | 0.000231 *** |
|  | Treatment | 2 | 3.055 | 0.056604 |
|  | Time x Treatment | 6 | 4.935 | 0.000547 *** |
|  | **Specific_Root_Lenght** |  |  |  |
|  | Time | 3 | 2.433 | 0.0767 |
|  | Treatment | 2 | 19.795 | 5.81e-07 *** |
|  | Time x Treatment | 6 | 1.399 | 0.2352 |
|  | **Tap_R_Branch_D** |  |  |  |
|  | Time | 3 | 0.893 | 0.4520 |
|  | Treatment | 2 | 4.252 | 0.0201 * |
|  | Time x Treatment | 6 | 1.258 | 0.2951 |

| | | | | |
|---|---|---|---|---|
| | **TapRoot_diam** | | | |
| | Time | 3 | 2.859 | 0.04686 * |
| | Treatment | 2 | 5.395 | 0.00778 ** |
| | Time x Treatment | 6 | 2.302 | 0.04976 * |
| | **SecRoot_diam** | | | |
| | Time | 3 | 7.375 | 0.000379 *** |
| | Treatment | 2 | 22.756 | 1.23e-07 *** |
| | Time x Treatment | 6 | 1.231 | 0.307738 |
| | **R_leng_density** | | | |
| | Time | 3 | 2.895 | 0.045 * |
| | Treatment | 2 | 23.361 | 9.04e-08 *** |
| | Time x Treatment | 6 | 1.855 | 0.109 |
| | **% Mycorrhizal colonization** | | | |
| | Time | 3 | 0.373 | 0.773 |
| | Treatment | 1 | 23.273 | 3.31e-05 *** |
| | Time x Treatment | 3 | 0.262 | 0.852 |

**Table S4.3.** *E. edulis* outcomes of the analysis of variance (two-way ANOVA) applied in root morpho-functional traits, mycorrhizal colonization, and root nodule area in relation to time, water limitation treatments, and their interactions. *, ** and *** significance at the $p < 0.05$, 0.01 and 0.001 levels, respectively.

| **Specie** | **Factor** | **Df** | **F value** | ***p*-value** |
|---|---|---|---|---|
| *E. edulis* | **R_Shoot** | | | |
| | Time | 3 | 4.757 | 0.00578 ** |
| | Treatment | 2 | 2.366 | 0.10544 |
| | Time x Treatment | 6 | 1.728 | 0.13641 |
| | **R_dry_matter_content** | | | |
| | Time | 3 | 17.779 | 9.37e-08 *** |
| | Treatment | 2 | 4.321 | 0.019207 * |
| | Time x Treatment | 6 | 5.486 | 0.000249 *** |
| | **Specific_Root_Lenght** | | | |
| | Time | 3 | 10.483 | 2.38e-05 *** |
| | Treatment | 2 | 10.176 | 0.000226 *** |
| | Time x Treatment | 6 | 1.922 | 0.097802 |
| | **Tap_R_Branch_D** | | | |
| | Time | 3 | 0.788 | 0.506964 |
| | Treatment | 2 | 9.668 | 0.000322 *** |
| | Time x Treatment | 6 | 1.876 | 0.105998 |
| | **TapRoot_diam** | | | |
| | Time | 3 | 6.506 | 0.000945 *** |
| | Treatment | 2 | 0.179 | 0.836317 |

|  |  |  |  |  |
|---|---|---|---|---|
|  | Time x Treatment | 6 | 2.856 | 0.019213 * |
|  | **SecRoot_diam** |  |  |  |
|  | Time | 3 | 11.925 | 7.14e-06 *** |
|  | Treatment | 2 | 16.210 | 4.99e-06 *** |
|  | Time x Treatment | 6 | 3.042 | 0.0139 * |
|  | **R_leng_density** |  |  |  |
|  | Time | 3 | 0.958 | 0.421 |
|  | Treatment | 2 | 29.374 | 7.89e-09 *** |
|  | Time x Treatment | 6 | 1.699 | 0.144 |
|  | **% Mycorrhizal colonization** |  |  |  |
|  | Time | 3 | 0.373 | 0.773 |
|  | Treatment | 1 | 23.273 | 3.31e-05 *** |
|  | Time x Treatment | 3 | 0.262 | 0.852 |
|  | **R_nodules mean area** |  |  |  |
|  | Time | 3 | 14.255 | 1.15e-06 *** |
|  | Treatment | 2 | 3.397 | 0.0423 * |
|  | Time x Treatment | 6 | 1.347 | 0.2566 |

**Table S4.4.** *M. nobilis* outcomes of the analysis of variance (two-way ANOVA) applied in root morpho-functional traits and mycorrhizal colonization in relation to time, water limitation treatments, and their interactions. *, ** and *** significance at the $p < 0.05$, 0.01 and 0.001 levels, respectively.

| **Specie** | **Factor** | **Df** | **F value** | ***p*-value** |
|---|---|---|---|---|
| *M. nobilis* | **R_Shoot** |  |  |  |
|  | Time | 3 | 3.065 | 0.0420 * |
|  | Treatment | 1 | 4.230 | 0.0479 * |
|  | Time x Treatment | 3 | 0.878 | 0.4625 |
|  | **R_dry_matter_content** |  |  |  |
|  | Time | 3 | 12.517 | 1.4e-05 *** |
|  | Treatment | 1 | 0.013 | 0.90892 |
|  | Time x Treatment | 3 | 4.733 | 0.00764 ** |
|  | **Specific_Root_Lenght** |  |  |  |
|  | Time | 3 | 4.096 | 0.014384 * |
|  | Treatment | 1 | 13.659 | 0.000816 *** |
|  | Time x Treatment | 3 | 0.442 | 0.724543 |
|  | **Tap_R_Branch_D** |  |  |  |
|  | Time | 3 | 7.303 | 0.000729 *** |
|  | Treatment | 1 | 7.702 | 0.009135 ** |
|  | Time x Treatment | 3 | 1.273 | 0.300219 |
|  | **TapRoot_diam** |  |  |  |

|  |  |  |  |  |
|---|---|---|---|---|
|  | Time | 3 | 2.999 | 0.04503 * |
|  | Treatment | 1 | 9.528 | 0.00416 ** |
|  | Time x Treatment | 3 | 0.595 | 0.62272 |
|  | **SecRoot_diam** |  |  |  |
|  | Time | 3 | 4.922 | 0.00654 ** |
|  | Treatment | 1 | 0.258 | 0.61475 |
|  | Time x Treatment | 3 | 0.805 | 0.50065 |
|  | **R_leng_density** |  |  |  |
|  | Time | 3 | 12.121 | 1.83e-05 *** |
|  | Treatment | 1 | 10.371 | 0.00294 ** |
|  | Time x Treatment | 3 | 5.593 | 0.00336 ** |
|  | **% Mycorrhizal colonization** |  |  |  |
|  | Time | 3 | 0.373 | 0.000864 *** |
|  | Treatment | 1 | 23.273 | 0.039556 * |
|  | Time x Treatment | 3 | 0.262 | 0.037399 * |

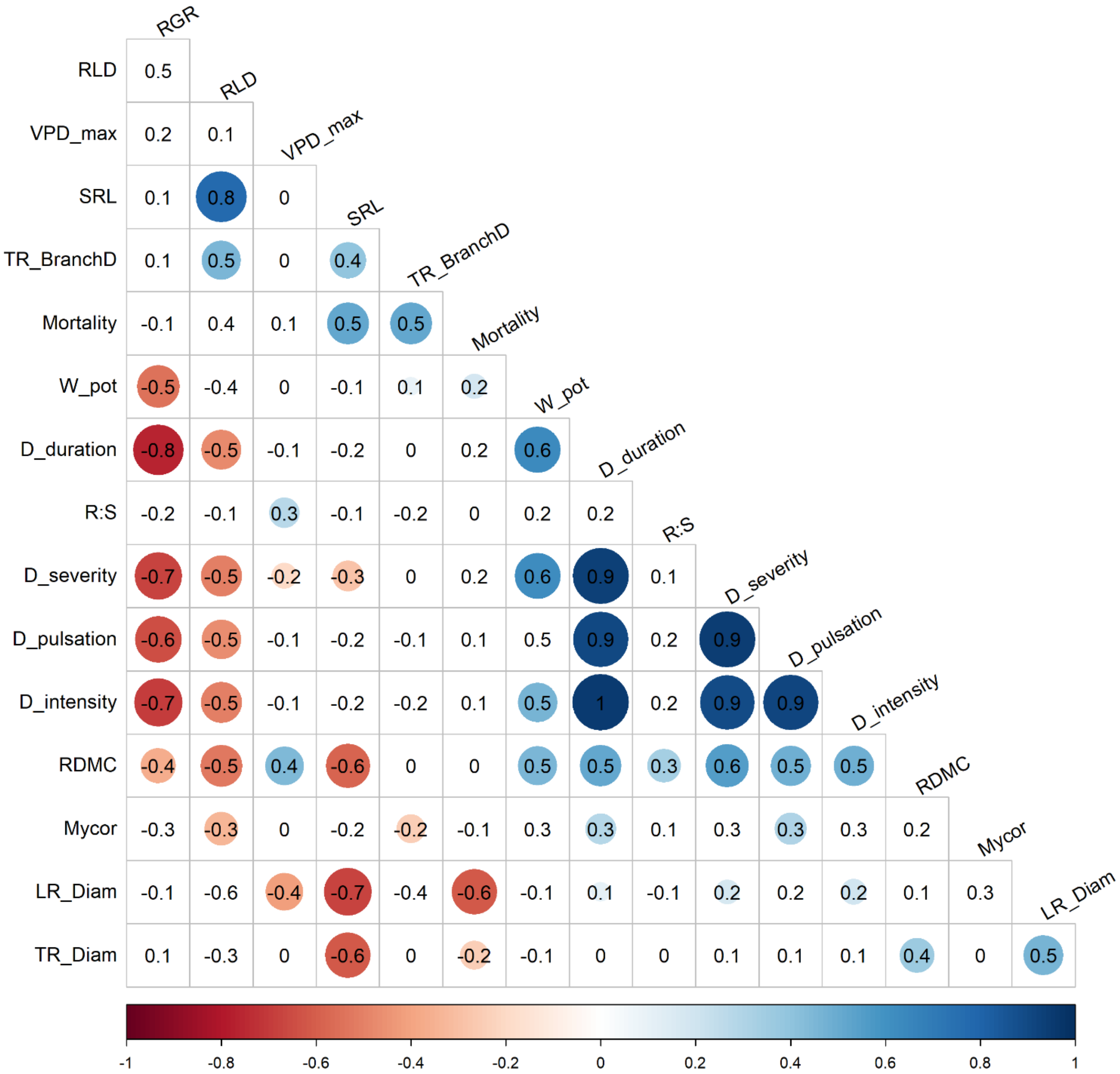
**Table S4.5.** *Q. humboldtii* outcomes of the analysis of variance (two-way ANOVA) applied in root morpho-functional traits and mycorrhizal colonization in relation to time, water limitation treatments, and their interactions. *, ** and *** significance at the p < 0.05, 0.01 and 0.001 levels, respectively.

| **Specie** | **Factor** | **Df** | **F value** | ***p*-value** |
|---|---|---|---|---|
| *Q. humboldtii* | **R_Shoot** |  |  |  |
|  | Time | 3 | 3.531 | 0.0221 * |
|  | Treatment | 2 | 1.146 | 0.3271 |
|  | Time x Treatment | 6 | 1.214 | 0.3168 |
|  | **R_dry_matter_content** |  |  |  |
|  | Time | 3 | 16.346 | 2.27e-07 *** |
|  | Treatment | 2 | 1.184 | 0.315 |
|  | Time x Treatment | 6 | 1.684 | 0.146 |
|  | **Specific_Root_Lenght** |  |  |  |
|  | Time | 3 | 9.303 | 6.4e-05 *** |
|  | Treatment | 2 | 10.297 | 0.000202 *** |
|  | Time x Treatment | 6 | 3.224 | 0.009969 ** |
|  | **Tap_R_Branch_D** |  |  |  |
|  | Time | 3 | 3.876 | 0.0149 * |
|  | Treatment | 2 | 3.079 | 0.0556 |
|  | Time x Treatment | 6 | 0.680 | 0.6664 |
|  | **TapRoot_diam** |  |  |  |
|  | Time | 3 | 1.531 | 0.2190 |
|  | Treatment | 2 | 3.977 | 0.0255 * |
|  | Time x Treatment | 6 | 1.895 | 0.1021 |
|  | **SecRoot_diam** |  |  |  |

| | | | |
|---|---|---|---|
| Time | 3 | 0.851 | 0.473 |
| Treatment | 2 | 0.507 | 0.606 |
| Time x Treatment | 6 | 1.753 | 0.130 |
| **R_leng_density** | | | |
| Time | 3 | 12.094 | 5.82e-06 *** |
| Treatment | 2 | 10.691 | 0.000154 *** |
| Time x Treatment | 6 | 1.713 | 0.139426 |
| **% Mycorrhizal colonization** | | | |
| Time | 3 | 3.856 | 0.0187 * |
| Treatment | 1 | 0.054 | 0.8185 |
| Time x Treatment | 3 | 0.722 | 0.5467 |

**Table S5.** Univariate logistic regression with the relationship between each drought metric, relative growth rate and biomass allocation (observed with the root-to-shoot variable) as explanatory variables of seedlings mortality. *, ** and *** significance at the $p < 0.05$, 0.01 and 0.001 levels, respectively. We did not consider the effect of drought severity and the root-to-shoot ratio, despite being significative, by their low percentage explanation ($R^2 = 0.05$).

| **Seedlings mortality** | | |
|---|---|---|
| | **Estimate** | **p value** |
| **Drought pulsation** | 1.3896 | 0.201 |
| **Drought intensity** | 0.06769 | 0.198 |
| **Drought severity** | 0.028014 | 0.00324 ** |
| **Relative growth rate** | -62.5967 | 0.0976 |
| **Root to shoot ratio** | -2.34770 | 0.00916 ** |



| | RGR | RLD | VPD_max | SRL | TR_BranchD | Mortality | W_pot | D_duration | R:S | D_severity | D_pulsation | D_intensity | RDMC | Mycor | LR_Diam |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RLD | 0.5 | | | | | | | | | | | | | | |
| VPD_max | 0.2 | 0.1 | | | | | | | | | | | | | |
| SRL | 0.1 | 0.8 | 0 | | | | | | | | | | | | |
| TR_BranchD | 0.1 | 0.5 | 0 | 0.4 | | | | | | | | | | | |
| Mortality | -0.1 | 0.4 | 0.1 | 0.5 | 0.5 | | | | | | | | | | |
| W_pot | -0.5 | -0.4 | 0 | -0.1 | 0.1 | 0.2 | | | | | | | | | |
| D_duration | -0.8 | -0.5 | -0.1 | -0.2 | 0 | 0.2 | 0.6 | | | | | | | | |
| R:S | -0.2 | -0.1 | 0.3 | -0.1 | -0.2 | 0 | 0.2 | 0.2 | | | | | | | |
| D_severity | -0.7 | -0.5 | -0.2 | -0.3 | 0 | 0.2 | 0.6 | 0.9 | 0.1 | | | | | | |
| D_pulsation | -0.6 | -0.5 | -0.1 | -0.2 | -0.1 | 0.1 | 0.5 | 0.9 | 0.2 | 0.9 | | | | | |
| D_intensity | -0.7 | -0.5 | -0.1 | -0.2 | -0.2 | 0.1 | 0.5 | 1 | 0.2 | 0.9 | 0.9 | | | | |
| RDMC | -0.4 | -0.5 | 0.4 | -0.6 | 0 | 0 | 0.5 | 0.5 | 0.3 | 0.6 | 0.5 | 0.5 | | | |
| Mycor | -0.3 | -0.3 | 0 | -0.2 | -0.2 | -0.1 | 0.3 | 0.3 | 0.1 | 0.3 | 0.3 | 0.3 | 0.2 | | |
| LR_Diam | -0.1 | -0.6 | -0.4 | -0.7 | -0.4 | -0.6 | -0.1 | 0.1 | -0.1 | 0.2 | 0.2 | 0.2 | 0.1 | 0.3 | |
| TR_Diam | 0.1 | -0.3 | 0 | -0.6 | 0 | -0.2 | -0.1 | 0 | 0 | 0.1 | 0.1 | 0.1 | 0.4 | 0 | 0.5 |

**Figure S1.** Spearman's correlation matrix between the studies variables in all species. Square color and size indicate the direction and significance of correlation coefficients. Abbreviations: relative growth rate (RGR), root length density (RLD), maximum vapor pressure deficit values (VPD_max), specific root length (SRL), taproot branching density (TR_branchD), mortality rate (Mortality), leaf predawn water potential (W_pot), drought duration (D_duration), drought pulsation (D_pulsation), drought severity (D_severity), drought intensity (D_intensity), root-to-shoot ratio (R:S), root dry matter content (RDMC), mycorrhizal colonization (Mycor), lateral root diameter (LR_Diam).

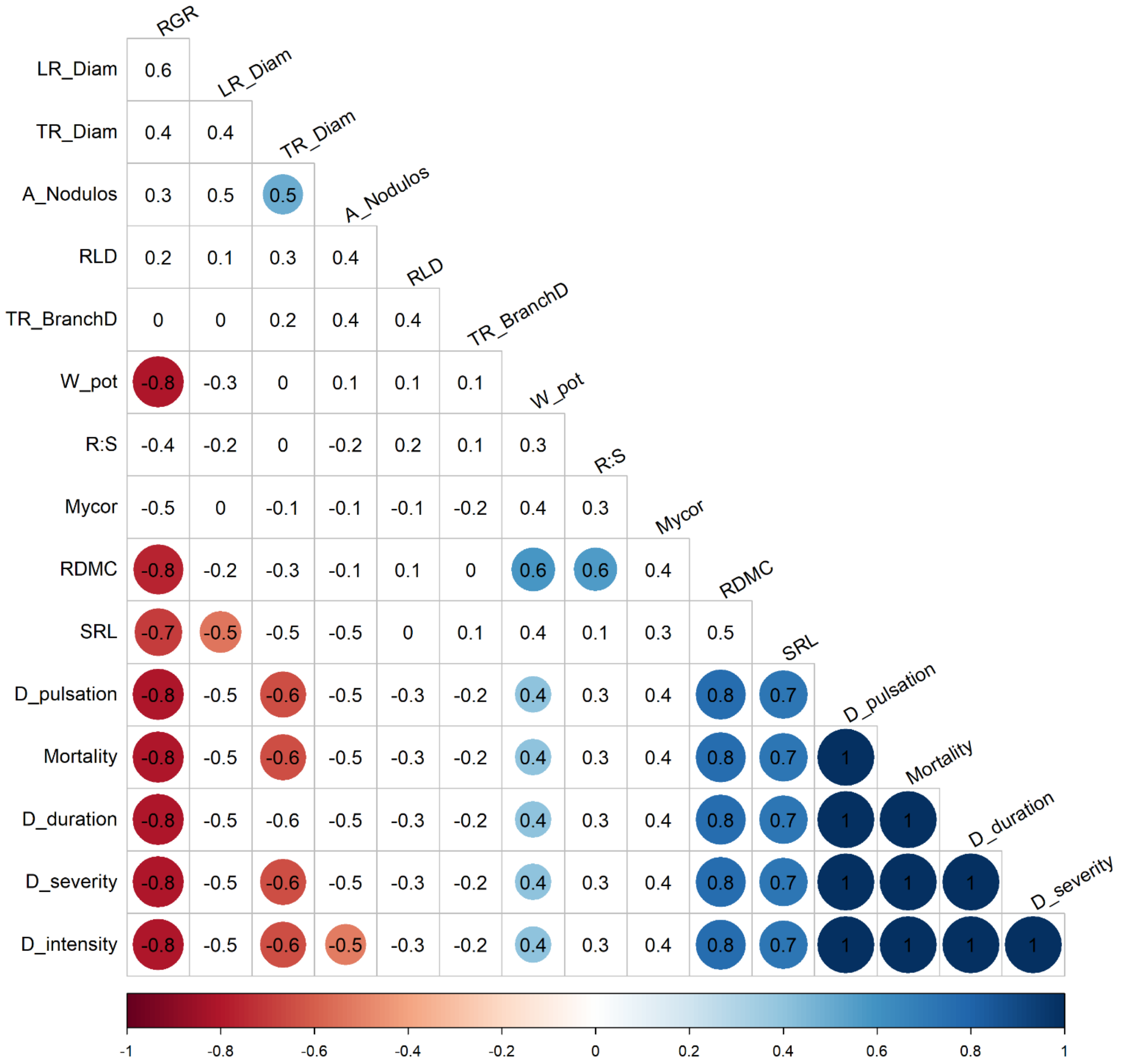


**Figure S2.** Spearman's correlation matrix between the studies variables in *E. edulis* that include the mean root nodule area. Square color and size indicates the direction and significance of correlation coefficients. Abbreviations: mean root nodule area (A_nodule), relative growth rate (RGR), root length density (RLD), specific root length (SRL), taproot branching density (TR_branchD), mortality rate (Mortality), leaf predawn water potential (W_pot), drought duration (D_duration), drought pulsation (D_pulsation), drought severity (D_severity), drought intensity (D_intensity), root-to-shoot ratio (R:S), root dry matter content (RDMC), mycorrhizal colonization (Mycor), lateral root diameter (LR_Diam).