Coupled plant traits adapted to wetting/drying cycles of substrates co-define niche multidimensionality

Ulises Rodríguez-Robles[1], J. Tulio Arredondoi[2], Elisabeth Huber-Sannwald[2], Enrico A. Yépez[3], and J. Alfredo Ramos-Leal[2]

[1]Universidad de Guadalajara Centro Universitario de la Costa Sur
[2]Instituto Potosino de Investigacion Cientifica y Tecnologica
[3]Instituto Tecnologico de Sonora

April 28, 2020

Abstract

Theories attempting to explain species coexistence in plant communities have argued in favor of species' capacities to occupy a multidimensional niche with spatial, temporal and biotic axes. We used the concept of hydrological niche segregation to learn how ecological niches are structured both spatially and temporally and whether small scale humidity gradients between adjacent niches are the main factor explaining water partitioning among tree species in a highly water-limited semiarid forest ecosystem. By combining geophysical methods, isotopic ecology, plant ecophysiology and anatomical measurements, we show how coexisting pine and oak species share, use and temporally switch between diverse spatially distinct niches by employing a set of functionally coupled plant traits in response to changing environmental signals. We identified four geospatial niches that turned into nine, when considering the temporal dynamics of the wetting/drying cycles in the substrate and the particular plant species adaptations to garner, transfer, store and use water. Under water scarcity, pine and oak exhibited water use segregation from different niches, yet under maximum drought when oak trees crossed physiological thresholds, niche overlap occurred. The identification of niches and mechanistic understanding of when and how species use them will help unify theories of plant coexistence and competition.

Summary statement

How forest species coexist and prosper in complex substrate and limiting resources? We answered this question, locating the root distibution and identifying the sources of wáter and the time when oak and pine use it. We identified up to nine spatial and temporal hydraulic niches that reduce resource use overlapping and coexistence.

Acknowledgements

We are very grateful to M.C. Gutierrez-Díaz, J.P. Rodas and M.N. Barranco for technical assistance with field studies; L. Yañez for advice on histologic sections in pines and oaks and F. Maestre for comments on a previous version of the manuscript. We thank the Applied Geosciences Division at IPICYT for access to geophysical instruments and tools. Authors thank Copernicus Publications eds. for the permission to use published data (https://www.biogeosciences.net/14/5343/2017/ Creative Commons Attribution 4.0 License).

Type of article **- Original**

Number of words:

Abstract - **199**

Main text - **6785**

Number of references – **52**

Number of tables - **1**

Number of figures - **5**

Supporting Information **- 9 figures**

Introduction

Unveiling the mechanisms of species co-existence in plant communities has been one of the greatest challenges in ecological research (Valladares, Bastias, Godoy, Granda, & Escudero, 2015). Over the past seven decades, many studies have concluded that species coexist by sharing limited resources and occupying different niches (de la Riva, Marañón, Violle, Villar, & Pérez-Ramos, 2017; Peñuelas et al., 2019). An ecological niche is an abstraction of a time-space continuum, where individual species have exclusive access to limited resources thereby sharing them with coexisting species in spatially and / or temporally segregated pools (Gause, 1934; MacArthur, 1968; Noble & Fagan, 2015; Whittaker, 1969). In terms of sharing water soil resources by two or more plant species, Araya et al. (2011) introduced the concept of hydrological niche segregation (HNS), where species specialize in the use of distinct niches as a result of trade-offs between tolerance to aeration stress and tolerance to drying stress (Jonathan Silvertown, Dodd, Gowing, & Mountford, 1999). Hydrological niche segregation has been defined as partitioning of i) space along fine-scale moisture gradients, ii) water as a resource or iii) recruitment opportunities between years when species respond differentially to distinct patterns of temporal variance in water supply (storage effect) (Araya et al., 2011; Jonathan Silvertown, Araya, & Gowing, 2015).

Since all plants require the same principal resources (light, carbon dioxide, water, mineral nutrients) (Jonathan Silvertown, 2004) and acquire them in a highly limited number of ways, as mentioned by Jonathan Silvertown et al. (1999), this might result in frequent niche overlaps. Even though niche segregation among species has being identified as a mechanism to reduce niche overlap in several studies (Araya et al., 2011; Bartelheimer, Gowing, & Silvertown, 2010; Jonathan Silvertown, 2004; Jonathan Silvertown et al., 2015; Jonathan Silvertown et al., 1999; J. Silvertown & Law, 1987), it is still unresolved how physiological, phenological, and anatomical traits of coexisting species may potentially act together, i.e. are coupled, to sense and garner spatially and/or temporally available resources and thereby avoid or reduce competition.

Thus far, species coexistence studies have assumed a certain substrate homogeneity, even though individual niches may actually exhibit complex geological, geomorphological and edaphic properties (Gray, 2004; Kukowski, Schwinning, & Schwartz, 2013). It is this geodiversity that eventually contributes to the spatial and temporal segregation and use of resources and thereby facilitate species coexistence. Critical non-resource factors (soil depth, precipitation patterns, rockiness, etc.) (Maestre, Callaway, Valladares, & Lortie, 2009) may also control resource availability and species adaptations and thereby contribute to an ecological niche. Consequently, it is the spatial, temporal (both referring to resource availability) and biological (trait based) dimensions of a niche that permit differential resource use and therefore species coexistence. Plant functional traits are measurable morphological, anatomical, physiological and phenological features that species employ to effectively capture resources and to adapt and acclimate to and tolerate a wide spectrum of environmental conditions (Adler et al., 2014; Muscarella & Uriarte, 2016). In highly resource limited environments, the coexistence of long-lived species reflects species-specific, selective, complementary plant-resource response spectra spanning all developmental stages. Therefore, adult individuals of co-existing species exhibit distinct plant responses to resource availability, which are controlled by a set of traits (e.g., differential spatial placement of coarse and fine roots with distinct root anatomy access different water sources, etc.) together forming a dynamic biological niche axis along an environmental gradient, such that each interacting species occupies a certain niche along the space and time continuum (axes). Some species traits are fine-tuned sensors (for example, when reaching a physiological threshold) that detect environmental signals of niche quality and thus may induce a switch in niche occupation; for instance, when resources become depleted.

To examine hydrological niche segregation, it is necessary to understand the complexity of the "nichescape" in a real-world setting. With nichescape we refer to the set of traits employed by plants to garner resources, that are spatially and temporally distributed in the soil-rock continuum. The ideal model system to study this should include a minimum set of well-differentiated, measurable spatial niches, which are occupied differentially and/or temporally in a complementary manner by naturally coexisting species. This setting is present in the semi-arid pine-oak forest of the Sierra San Miguelito Volcanic Complex (SSMVC) in Central Mexico. Recent explorations (Rodriguez-Robles, Arredondo, Huber-Sannwald, & Vargas, 2015) identified potential geoecohydrological niches derived indirectly from plant and soil water potential values, where deep roots of oak (*Quercus potosina*) trees apparently explored, acquired and remobilized water stored in belowground rock cracks, while pine (*Pinus cembroides*) trees apparently fulfilled their water demand by root uptake from shallow soil. Given the extreme water scarcity, and the suite of inherently different functional traits of pine and oak (i.e., root distribution, phenology, plant water relations) (Rodriguez-Robles et al., 2015), their coexistence must have evolved in a complex dynamic "nichescape" allowing spatially and temporally complementary water use by the two species.

In this study, we examined whether differential water uptake capacities of coexisting species in response to recurring fine-scale spatial moisture gradients (i.e. occurring within a few cm, generated by water movements in substrates with different water holding capacities) are the main mechanisms of hydrologic niche segregation, or whether tree functional traits as well as the water dynamics in the substrate together control such niche segregation. Based on the HNS concept, we hypothesized that even an apparently simple geological microenvironment (i.e. shallow soil over fractured rock) partitions into a multidimensional niche consisting of spatial soil humidity gradients, temporal variability of water access, and different plant functional traits that effectively exploit each potential water source. We further hypothesized that the humidity gradients and water partitioning between species are coupled to the wetting/drying cycles in this forest ecosystem. Using hydrogeological and geophysical prospection methods (Rodríguez-Robles, Arredondo, Huber-Sannwald, Ramos-Leal, & Yépez, 2017) coupled with intensive monitoring of natural abundance of stable isotope ratios of water, heavy water labelling studies, eco-physiological and anatomical measurements, we describe for the first time the identity, spatial extent and temporality of several niches used by two coexisting forest species, as well as the plant functional traits employed of their occupation and use, jointly forming the multidimensional nature of niches (nichescape) in a semiarid forest ecosystem.

Materials and methods

Study site, geology and forest stand

The study area is a semiarid tropical forest located at the Southern extension of the SSMVC, in central Mexico (Fig. S1; 2350 m.a.s.l.; latitude 22.25° and longitude -101.12°). Climate is semiarid with the main precipitation period occurring between June and September and winter rains between December and February (5-18% of total annual rainfall). For the last 65 years, average annual rainfall was 408 mm (weather station "La Purisima", 22° 5' 22.4", 101° 12' 28.9" and records on site), where in 64% of the years rainfall was below and only in 12% above the average of 500 mm (Rodríguez-Robles et al., 2017). The topography is abrupt and irregular representing "complex terrain". The landscape is characterized by slopes steeper than 30° and elevations ranging between 1900 and 2870 m.a.s.l. According to the classification system of the World Reference Base for Soil Resources (WRB), the extremely shallow soils and fractured rocks of this area correspond to lithic-paralithic Leptosols (LPlip) (FAO, 2006). The average depth of the organic soil horizon ranges from 10 to 25 cm; soil organic matter accumulates in crevices and soil pockets (Pérez, Arredondo, Huber, & Serna, 2014). The dominant native tree species are *Pinus cembroides*Zucc. (1832) and *Quercus potosina* Trel. (1924); they both form pure and mixed forest stands with little understory, while *Quercus* species exhibiting in general dimorphic root systems and *Pinus* species producing shallow superficial root systems (Cermak, Nadezhdina, Meiresonne, & Ceulemans, 2008; Kutschera & Lichtenegger, 2002). Tree roots anchor underneath weathered rocks, from where they obtain mineral resources. Lithological profiles show a high density of vertical roots distributed in rock fractures and soil pockets (Fig. S2). With geophysical prospection tools, we observed that this forest landscape composed of monospecific and mixed pine and oak stands developed over heterogeneous soil/rock profiles consisting of extremely shallow surface soils (< 25 cm) over granulated rock material (regolith; at 25 to 45 cm depth), impermeable volcanic rocks with pronounced fractures and fissures (from 35 to 75 cm depth), rock pockets (at 5 to 38 cm depth), and fresh rock (below 80 cm depth) (Fig.S2).

Experimental plots

We considered comparing pure and mixed stands of oak and pine a suitable approach to identify potential differences in species-specific preferences for occupying (via root placement) certain geologic substrates, rock fractures or soil depths, etc., when growing either in intra- or inter-specific neighborhoods. We established 12 circular experimental plots of 25 m diameter (four plots per stand type) along a 3.5 km long transect running parallel to a narrow watershed, where pine and oak trees were evenly distributed in pure and mixed stands. By simultaneously examining root distribution, plant leaf water potentials and intra-annual soil and rock water availability, we were able to isolate and describe specific geoecohydrological niches.

Monitoring of soil water availability and uptake

In each plot, four soil psychrometer sensors (TSP-55, Wescor Inc. USA; 64 total) were inserted at 12-15 cm depth (depending on the presence of soil pockets) near tree trunks to monitor soil water potential (Ψs). To determine leaf water potential (Ψl) for the same trees, we harvested 2-3 leaf discs from healthy mature leaves and needles exposed to sunlight, allowing 25 minutes for stabilization in C-52 chambers (C-52, Wescor Inc. USA). Soil and leaf Ψ were monitored during the diurnal peaks of water stress (from 11 to 14 hrs) at biweekly intervals for 27 months (from September 2012 to December 2014). To help interpret plant responses to soil water availability, Ψl and Ψs time series were divided into three ecohydrological periods: two dry periods (depletion and recovery) followed by one wet (wet season) period. The depletion period corresponded to the time when soil humidity started declining a few weeks after the rainy season ended; it included the months with the lowest leaf and soil Ψ (October–December). The recovery period started after the leaf and soil Ψ had reached their lowest values and once leaf Ψ initiated recovering; it lasted for 3-4 months until the beginning of the rainy season (January–May). The recovery period was unrelated to precipitation input, stable isotope analysis of water used by plants during this period suggests that water was extracted exclusively from fractured rocks. The wet season corresponded to the months with monsoon precipitation, when leaf and soil Ψ fully recovered (June–September).

Implementation of geophysical methods for spatial niche description

In each of the 12 experimental plots, we determined the vertical extent of soil, regolith, and massive rock, the presence of rock fractures, as well as the horizontal and vertical distribution of different root diameters using a ground penetrating radar (GPR, MALÅ RAMAC X3M –ProEx system coupled to an inspection wheel and shielded antennas: 500 and 800 MHz). We also generated 12 profiles with the electric radar tomography (ERT, SYSCAL KID SWITCH-24, IRIS instruments, with a 24-multi-electrode switch box) (four for each stand type) on four dates (October 2013, December 2013, February 2014 and May 2014). In each ecohydrological period, we monitored the two-dimensional (20 x 5 m area) water distribution at 5 points separated at equal distances along a 25 meter transect considering the different substrate layers with ERT. The geophysical methods employed in this study were described in detail in Rodríguez-Robles et al. (2017). All roots detected in the radar profile along each 25 m transect were grouped into five root diameter classes (< 3.0, 3.0–4.0, 4.0–5.0, 5.0–6.0 and > 6.0 cm). Finally, for calibration purposes, individual roots (total of 76) were excavated to determine their depth and diameter in situ.

Geographic rainfall partitioning

In order to identify all potential water sources in the soil and rocks used by the two tree species, we first generated a sketch map of the Local Meteoric Water Line (LMWL) using the δ^{18}O and δD values of rainwater of each of 52 precipitation events (Fig. 1). Since the Atlantic basin (Golf of Mexico) is closer to the study area than the Pacific basin (351.56 vs 426.44 km, Fig. S1), distinct continental effects resulted in well-defined isotopic signatures of summer rainwater originating from the Pacific and the Atlantic. Between January 2012 (early winter) and December 2014 (late autumn), we tracked each continental ingress of moisture from tropical storms deriving from the Pacific Ocean basin (18 events), the Gulf of Mexico (16 events), and from cold fronts from the North (12 events). Five rain collectors (All-weather rain gauge, Forestry Supplies Inc.) partially filled with mineral oil to avoid evaporative water loss were installed equidistantly in the different forest (pure and mixed pine and oak) stands along the 3.5 km transect. Water from each rain event was collected and stored in glass vials with screw caps to prevent isotopic fractionation; all vials were stored in iced coolers and transported to the laboratory in the Division of Environmental Sciences at the Instituto Potosino de Investigación Científica y Tecnológica, A.C., in San Luis Potosí, Mexico, where vials were refrigerated (6 °C) until later isotopic analysis. To track each meteorological rainfall event, we used forecasts from The National Meteorological Service of Mexico (http://smn.cna.gob.mx/), the weather network from INIFAP (http://clima.inifap.gob.mx/), The Weather Channel (http://weather.com/) and The Tropical Weather Center (http://wx.hamweather.com/tropical/).

Examining spatial and temporal water use and niche utilization

Tree water sources

Spatial niches were identified based on soil/geologic strata characteristics and the temporal changes in the distribution of water stable isotope signatures in these strata. To identify the sources of water taken up by pine and oak trees, we determined the isotopic signatures of each rainfall event, plant water extracted from woody tissue (twigs), soil water and rock water at monthly intervals for a total of 36 months. Xylem samples were obtained from collected twigs that were immediately frozen with dry ice in air-tight tubes for subsequent water extraction using cryogenic distillation (West et al. 2006) and posterior determination of stable isotope ratios for hydrogen (D/H) and oxygen (18O/16O). The bark of four to six 3 cm long twigs was peeled off and the stem stored in 30-mL glass vials sealed with parafilm-lined caps. At each sampling date (72 samples), we collected twigs fully covered with periderm from randomly selected adult trees (four trees, two species and three stands) (n = 16 twig samples per sampling date, with a study total of 1152 twigs). Soil water was obtained through cryogenic distillation of soil core (n= 576) extracted at 15 cm depth and 10-20 cm distance from the tree; at the same time twigs of the same tree were sampled to identify the source of plant water use. In addition, we sampled water from rock fractures accumulating water and from a water spring within the watershed (14 samples total).

Tracing D_2O in xylem tissues

To examine and track the dynamics of water uptake, transport and storage in trees of the two species, we used a marker technique with isotopically labelled water similar to that described by James et al. (2003) and Meinzer et al. (2006) We injected deuterium oxide (99.9 atom% D_2O, Cambridge Isotope Laboratories, Inc.) into the xylem transpiration stream after having drilled four holes at equidistant points (90° apart) around the trunk circumference at 130 cm height. The dosage was kept constant at [?]0.7 g D_2O per cm of sapwood circumference. This assay was performed once during a dry season (spring 2013) and once after a winter rain (winter 2014) to examine the dynamics of water transport and storage in trees. Deuterium injections in three pine trees per plot of pure and mixed stands (total = 24 injected trees) ($\delta D = 366$natural abundance of deuterium) occurred in spring 2013 (March 28 between 11:00 h and 13:30 h). Also, in winter 2014 (February 21 between 10:00 h and 14:00 h), injections of $\delta D = 503$compared to natural abundance of deuterium) deuterium was applied in each of four pine and oak trees per plot in pure and mixed stands (total = 48 injected trees). The presence of D_2O tracer in stem water was determined by periodic collection of twigs oriented N, S, W, E in the tree crown. Twigs (3 cm long) were immediately sealed in borosilicate (VWR®) and then wrapped with Parafilm to prevent evaporation. Prior to D_2O injections stem samples were collected to establish the baseline for hydrogen isotope ratios. After the injection of D_2O (day 0), twigs (total 720 samples) and wood cores (total 216 samples) were collected at noon on the following 10 days (for water extraction method and stable isotope analysis and calculations see below).

Hydraulic lift and water acquisition from rock fractures

In addition to determining the isotopic fingerprints in tree wood and water sources, we implemented a second labelling study, to examine the capability of tree species to explore rock fractures and vertically re-distribute water to the soil surface. We injected labeled water (δD = -88.2fractures next to either oak or pine trees as target plant with a neighboring tree of the respective other species (oak_{target} - $pine_{neighbor}$, $pine_{target}$ - $oak_{neighbor}$) considering three distance ranges (0-2, 2-4 and 4-6 m) between tree pairs (total number of trees = 24 targets and 24 neighbors). For the labelled water injection, we inserted a 1.5 cm diameter hose 30 cm down a rock fracture within 30-50 cm of the target tree. With a syringe (750 ml), labeled water (δD = 485.1deuterium) was slowly injected through the hose to avoid contamination of the surface soil with labeled water. We tracked the D_2O tracer by collecting 4 to 6 twigs from different places of the crown from both the target and neighboring tree, and soil samples next (minimum distance 50 cm) to both trees (0-15 cm depth). After the D_2O injection (day 0), twigs and soil cores ($n = 4$) were collected at noon for the following 10 days.

Determination of tree stem water content

To examine the volume and use of stored plant water, we determined the relative wood moisture content (WMC). For this, two horizontal wooden cores (1" x 0.5" cylinder) were extracted at breast height from 16 trees with a perforating puncher at the opposite sides of the trunk (four cores per species/plot) at the end of each of five ecohydrological periods (depletion: February 2013 and April 2014; recovery: May 2013; wet: October 2013 and 2014) (n=64). In this case, the number of samples was small to reduce the damage of sap conduction through the trunk. Relative wood moisture content (WMC) was calculated by using the fresh weight (Wfresh) and dry weight (Wdry) (48 hours at 70^{o}C) of each sample:

WMC = (Wfresh – Wdry) / Wdry x 100%

Wood anatomy

To examine whether the forest species exhibit functional anatomic adaptations to explore niche water, we collected stem samples at 0.20, 1.30 and 3.00 m height from 12 trees of each species in mixed stands using a wood core (cylinder 1.5" x 1") (n= 24 samples) to determine vessel elements. From the same trees, we also excavated four fine roots (approximately three millimeters in diameter) close to the stem at 15-25 cm depth (n=24) to determine root anatomical/ structural characteristics. All samples included sap wood and vascular cambium so to examine vessel elements. Slides were prepared to gather data on vessel elements, fiber lengths and tracheids. Once wood samples were collected, they were immediately fixed with FAA (Formaldehyde Alcohol Acetic Acid, 0.1:0.5:0.05 + 0.35 water) (Berlyn & Miksche, 1976) in the field. In the laboratory, samples were washed and stored in GAA (glycerin-95% ethanol-water, 1:1:1). Transverse and radial sections 30 - 40 mm thick including wood and vascular cambium were obtained with a sliding microtome. For each sample, unbleached sections (Ruzin, 1999) were stained with safranin-fast green (Johansen, 1940) and mounted on microscopic slides with synthetic resin. Macerations were prepared using Jeffrey's solution (Berlyn & Miksche, 1976). Temporary slides were prepared to gather data on vessel elements, fiber lengths and tracheids. Measurements were made with the image analyzer BIO7 1.6 (Eclipse Public License) attached to a video camera (Hitachi KP-D51) on a microscope (Olympus BX-50). The terminology used for wood description follows the recommendations of the International Association of Wood Anatomists (IAWA Comittee, 1989).

Examining spatial and temporal water use and niche utilization

Stable isotope analysis

For all stable isotope analysis, water was extracted from soil, stem and twig samples with the cryogenic vacuum distillation technique using an extraction line of 5 ports (West, Patrickson, & Ehleringer, 2006). δD and δ18O values of the woody stems, twigs, soil, precipitation and fracture water were determined using a stable isotope ratio mass spectrometer (Picarro L1102-i water isotope analyzer, PICARRO, INC) at the Instituto Potosino de Investigación Científica y Tecnológica in San Luis Potosi, Mexico. Results are reported in delta values, representing deviations in per mil (and Standard Light Antarctic Precipitation 2 (VSMOW2/SLAP2). Overall, analytical precision of the spectrometer was +-0.1for δD. Additionally, we used GISP (International Atomic Energy Agency) and SSM2 (spring water from SSMVC) as two controls for quality checks of the analysis.

Calculations

Mixing model for the analysis of stable isotopes: spatiotemporal niche identification

Stable isotopic compositions of potential water source pools and plant water provide important information on water sources and water uptake patterns (Dawson, Mambelli, Plamboeck, Templer, & Tu, 2002). Plants may have access to more than one water source pool (e.g. recent rain, soil water, and groundwater) in different proportions. Rooting depth and distribution define the depth and soil volume from where plants potentially extract these water sources. Therefore, water isotopic compositions of plant xylem can be viewed as a mixture of isotopic compositions from different water sources. Vegetation with roots distributed throughout the soil profile, for example, garner water from different soil depths resulting in twig water exhibiting mixed isotopic signature (Cramer, Thorburn, & Fraser, 1999).

A typical formulation using two isotopic signatures (δ1 and δ2) to partition the contributions (f) of three sources (a, b, c) to a mixture (m) is:

$$\delta_m^1 = f_a\delta_a^1 + f_b\delta_b^1 + f_c\delta_c^1$$

$$\delta_m^2 = f_a\delta_a^2 + f_b\delta_b^2 + f_c\delta_c^2$$

$$1 = f_a + f_b + f_c$$

The number of sources that can be partitioned is limited by the number of isotopic signatures employed. For the dual isotope example above, the mixing model is a system of three equations with three unknowns (fa, fb, fc), for which there is a unique solution (Phillips & Gregg, 2003). The relative contributions of different sources to xylem water were estimated by Bayesian mixing modelling using the Stable Isotope Analysis (SIAR) package. Stable isotope mixing models are used extensively for studying food webs but can also be applied to the determination of plant water sources (Beyer, Hamutoko, Wanke, Gaj, & Koeniger, 2018; Evaristo, Jasechko, & McDonnell, 2015; Evaristo & McDonnell, 2017; Voltas, Lucabaugh, Chambel, & Ferrio, 2015). We considered four different sources of water: two soil depths (0–10 cm and 10–25 cm), soil pockets and weathered rock (regolith) and groundwater reservoirs remaining in fractures and fissures within the rhyolitic-rock (see Figure S2) protected from evaporation.

Statistical analysis

To compare the stable isotopic composition (D/H and ^{18}O/^{16}O isotope ratios) considering natural abundance (($\Psi_{\lambda\epsilon\alpha\varphi}$in MPa) and soil ($\Psi_{\sigma o\iota\lambda}$ in MPa) water potentials, we implemented a multifactorial repeated-measures analysis of variance using a mixed model, which included as classification factors, species with two levels (*Q. potosina* and *P. cembroides* , fixed effect), forest stand with two levels (pure and mixed, fixed effect) and time (sampling dates, random effect) with 56 levels (biweekly dates). For the fracture water injection assay, we implemented an analysis of variance to examine the isotopic composition of xylem sap, using a mixed model with species (*Q. potosina* and *P. cembroides* , fixed effect), forest stand (fixed effect), tree distance (0-2, 2-4, 4-6 m; fixed effect) and time (sampling dates, random effect) as classification factors. Also, to compare changes of diameter at breast height and wood moisture content a 2x2 mixed model factorial including as factors species with two levels (pine and oak) and ecohydrological periods with five levels (depletion 2013, recovery 2013, wet 2013, depletion 2014, and wet 2014) was applied to compare changes of diameter at breast height and wood moisture content. In addition, a nested two-way factorial was used to compare treatment effects on root frequency for each diameter class. The model included forest stand with three levels (pure and mixed pine and oak stands; fixed effect) and soil depth with four levels (0-10, 10-20, 20-30 and >30 cm; nested effect). In all cases, we conducted Tukey's *post hoc* mean comparison test. Regarding the labeled water treatment in *P. cembroides* trunks, we analyzed the presence of isotopic label in twigs using a one-way

ANOVA followed by a Tukey's *post hoc* mean comparison test. We ran Type I regression analyses to examine the relationships between wood moisture content and tree diameter at breast height for *P. cembroides* and*Q. potosina* . Polynomial quadratic regression analyses were implemented to examine the relationship between soil water potential ($\Psi_{\sigma o \iota \lambda}$, MPa) and resistivity (ERT, Ω m^{-1}) between October 2013 and May 2014. Before statistical analyses, response variables were examined for normality using the Shapiro–Wilk's test (Shapiro & Wilk, 1965). Spatial analysis of species-specific vertical root distribution at different soil depths and soil electrical resistivity were examined using the Kriging interpolation method (Empirical Bayesian Kriging Simulations). All statistical analyses were run in SAS University Edition (Free Statistical Software) using PROC MIXED for the repeated-measures analysis and PROC GLM for generalized linear model for the univariate analysis. All geostatistical analyses were run with ArcGIS v. 10.1 for Windows (ArcGIS Desktop, ESRI 2011). The relative contributions of different sources to xylem water were estimated by Bayesian mixing models using the Stable Isotope Analysis in R (siar) package (Parnell, Inger, Bearhop, & Jackson, 2010).

Results

Spatial and temporal niche characterization

The forest ground is characterized by exfoliated volcanic rocks (0 to 45 cm high) and rock outcrops. With geophysical imaging (GPR), we visualized differential species-specific vertical root placement in the soil surface, regolith, and rock fractures (Fig. 2). For oak, the finest detectable roots (diameter $<$ 3 cm) occurred preferentially in soil pockets and rock fractures at depths $>$ 25 cm, whereas the thicker roots (5 to 7 cm) were located at the soil surface (differences in vertical root distribution, $P < 0.001$, Figs. 2b; 3d, f). Pine in contrast, concentrated its finest roots ($<$ 3cm) near the soil surface (top 10 cm, $P < 0.001$; Figs. 2a; 3a), however the thicker roots were observed in the regolith between 20 – 30 cm depth ($P < 0.001$; Figs. 2a; 3c).

We confirmed that oak and pine trees used different water sources throughout the year by comparing monthly stable isotope ratios of xylem water with soil and rainwater; these data provide direct evidence for spatial and temporal niche occupation by the two species. When combining geophysical methods with stable isotope ratio information of water from different soil/rock substrates and xylem of trees, we discovered that trees of both species occupied between one to four spatial niches (Table 1, Fig. 4) distributed in three geologically distinct substrates (Fig. S2). In the regolith layer, in several periods two separate pools of water were apparent, one in the upper regolith at the interface with the shallow soil and the other in the lower regolith within rock pockets at its deeper distribution (Figs. 2, 4).

When considering the soil/rock strata and the wetting/drying cycles in the course of three years, as well as specific tree functional traits employed, we identified nine niche configurations together forming a complex nichescape. With nichescape we refer to the set of traits employed by plants to garner water, that is spatially and temporally distributed in the soil-rock continuum (Table 1, Fig. 4).*Single-niche-sharing* only occurred during the rainy season of September 2012, when oak and pine satisfied all their water needs from surface soil (Table $1_{(\mathrm{A})}$, Figs. 4a and S3.1 i).*Double niche sharing* occurred in the wet season 2013, when oak and pine roots acquired water from both the surface soil and the upper regolith (Table $1_{(\mathrm{D})}$, Figs. 4d, S3.2 f-i), however the two tree species exploited differential proportions from each niche. While pine got most of the water from the surface soil (90%), oak acquired most from the upper regolith (85%). Another case, yet of*indirect double niche sharing* occurred in the dry season (depletion period 2012-2013, Table $1_{(\mathrm{C})}$, Figs. 4c, S3.1 d, S3.2 c), when oak in both stands acquired most water (92%) from rock fractures and a small portion (8%) from the surface soil/upper regolith. In contrast, pine (in mixed stands) took up water from surface soil/upper regolith plus water originating from rock fractures, which had previously been hydraulically lifted by oak roots from the rock fracture to the surface soil, where pine could access it (xylem water δD = -38.6

Oak and pine exhibit remarkable differences in wood and root anatomy. While in pine roots, a parenchyma is surrounding the tracheids, in oak roots this tissue is lacking (Figs. S6g, S4). Oak trunks show a particular structural anatomy of specialized tissue formed by fiber tracheids connecting vessel tissue (Cai, Li, Zhang,

Zhang, & Tyree, 2014) (conductive structures, Fig. S6b, d), which serves for water storage (Fig. S6c). Besides, oak has a considerable number of vessels adapted to xeric conditions, i.e., it has mixture of vessels with different diameters. The placements of oak's finest roots in rock fractures suggests specific adaptations in functional anatomy (Rodríguez-Robles et al., 2017) (Fig. 3f). These roots exhibit a triple layer of epidermal tissue and contain calcium oxalate crystals (druzes), and under extremely dry conditions, oak vessel diameter of roots decreases through the formation of tyloses (Gottwald, 1972) (Fig. S5d). Deep oak roots exhibited 83% more vessels with tyloses and 60% more druzes than surface roots (Fig. S5). Pine roots did not exhibit these kinds of anatomical adaptations.

Discussion

In these semi-arid forest ecosystems on rocky soils with little capacity of water storage, temporal water limitation may last between 3 to 6 months. Trees in these environments do not only search and explore for alternative geological water sources (occupation of alternative spatial/geohydrological and temporally emerging/seasonal niches) but employ specific functional adaptations to cope with the highly variable water availability. Once the rain season ended, we attested a humidity gradient as the dry season progressed. Overall, electric resistivity increased with depth (Fig. 2) with the lower regolith and fresh rock being the most resistive (but see Rodríguez-Robles et al. 2017 ;$\Psi_{\sigma o \iota \lambda}$ and electric resistivity). Still, only oak was able to explore all niches along this humidity gradient, while pine was specialized in using the top soil and upper regolith (Table 1, Fig. 4). Geophysical prospection revealed a spatially complex substrate including up to four niches generating a fine-scale partitioning of humidity (within less than 1 m, Fig. S2). Within this geological context, tree species traits in response to the wetting and drying cycles in the substrate generated up to nine niche configurations of water partitioning together forming a highly dynamic nichescape that has allowed the two tree species to co-exist in this forest. Oak and pine shared water from the same niche only when water was abundant, as occurred at the end of the summer months (Table $1_{(\mathrm{A})}$, Fig. 4a). Later in the year, oak and pine used niches differentially, with pine acquiring water only from the superficial soil and the upper regolith layer stemming either from rain or remobilization by oak from deeper rock fractures to the surface (Table $1_{(\mathrm{C})}$, Fig. 4c).

These species-specific water uptake mechanisms of oak and pine are related to different vertical fine root distribution (Figs. 3a - i). Pine located a greater proportion of thin roots responsible for water uptake in the top 10 cm (Fig. 3a), whereas oak positioned the same thin root types at 20 to 30 cm depth (Fig. 3f). Because of anatomical adaptations of fine roots, oak was able to explore the lower regolith and rock fractures (Figs. 4b, c, e, f, g, i) during the dry depletion periods. However, oak also used water from the upper regolith and surface soil thus exploiting water from a total of four identified spatial niches (Figs. 2, 4). In the case of superficial niches in the soil stratum, where oak and pine shared water use, species accessed different proportions of water from each niche (Figs. 4 c, d, h) likely to reduce competition. In one particular case, during the recovery period in spring 2013 (Fig. 4c), oak supplied water to pine through water remobilization, hence partially facilitating the recovery of the water status of pine. In another case, water stored in oak's trunk acted as a biotic/functional niche (trunk capacitance, Table $1_{(\mathrm{F})}$, Figs. 4f and S3.3 b, c). This trait emerged only after winter rains led to mild dry conditions (in February 2014; Fig. 4f); this unique water storage we interprete as a biotic dimension of a niche. In March-May 2014, oak switched water use from the lower regolith (Table $1_{(\mathrm{E})}$, Figs. 4e, S3.3b, c) to trunk water (trunk capacitance) stored from rain events in December 2013 and January 2014. Pine roots, however, continued taking up water from surface soil and the upper regolith layer as evidenced by the stable isotope signature in the twigs (Table $1_{(\mathrm{F})}$, Figs. 4f and S3.3a, b).

Plant functional mechanisms of species contributing to niche partitioning

Mechanisms of water partitioning between species involved environmental clues and plant sensors, physiological responses and anatomical adaptations. During the dry periods, we identified two environmental cues that were directly linked to tree physiological thresholds and hence induced shifts in niche use by oak. The sharp drop in soil water potential indicating extremely dry environmental conditions after the winter season, was mirrored in equally low leaf water potentials in oak (lowest oak $\Psi_{\lambda\epsilon\alpha\varphi}$ and $\Psi_{\sigma o \iota \lambda}$ measured in the depletion period in January and February 2013, Fig. 5, S3.2a,b). This triggered oak trees to switch water uptake from surface soil to fractured rocks (well water, Fig. S3.2b), however under the same conditions pine remained using surface soil water as showed by the stable isotope signature (δO -11.2, δD -73.6 to δO -9.3, δD -55.4) of precipitation originating from the Atlantic (Figs. S3.2 b-d).

The second environmental cue occurred when oak experienced the highest vapor pressure deficit in the air in March and April 2013 as it started redistributing water from fractured rocks to the surface soil (Rodríguez-Robles et al., 2017) through hydraulic lift. After five months (Dec 2012 to April 2013) of seasonal drought, oak $\Psi_{\lambda\epsilon\alpha\varphi}$ recovered by 1.5 MPa in both stands (Fig. 5) by having accessed water from both soil fractures and surface soil to where water had been remobilized by hydraulic lift. On the other hand, pine $\Psi_{\lambda\epsilon\alpha\varphi}$ recovered by 1.0 MPa yet only in mixed stands, where water had been made available by oak via hydraulic lift. The isotopic δ^{18}O and δD signatures of water extracted from twig xylem of both species was similar to that of groundwater in the dry season. The isotopic enrichment of local surface soil water near oak indicates that oak roots were extracting and vertically redistributing enriched water from deep soil layers and fractured rock and therefore were likely responsible for the observed hydraulic lift (Fig 4c). This response type was consistent with that observed in other studies conducted in the Mediterranean, where most of the roots of *Q. ilex* were in all soil horizons, to maximize the use of topsoil water during most of the year, and groundwater together with hydraulically lifted water (enhancing nutrient supply) during seasonal droughts (Aranda, Ramírez-Valiente, & Rodríguez-Calcerrada, 2014; David et al., 2007).

Oak and pine wood anatomical adaptations exhibit remarkable differences that explain species-specific functional adaptations to both highly variable water availability and rapid local soil and rock water depletion (Hacke, Sperry, & Pittermann, 2005; Sperry, Hacke, & Pittermann, 2006) and thus potentially efficient responses to shifts in niche use (Fig. S6). In pine roots a parenchyma surrounding the tracheids grants highly efficient soil water uptake and conduction to the canopy (von Arx, Arzac, Olano, & Fonti, 2015) (Figs. S6g, f, S4), particularly in response to small precipitation pulses. In contrast, in oak trunks a particular structural anatomy of specialized tissue formed by fiber tracheids connects vessel tissue (Cai et al., 2014) (conductive structures, Fig. S6b, d), which provides a high capacity of water storage (Fig. S6c). Besides, oak has a considerable number of vessels adapted to xeric conditions (*i.e.,* mixture of different diameter vessels) to improve the hydraulic efficiency by water pumping (Thomas Tyree, Salleo, Nardini, Assunta Lo Gullo, & Mosca, 1999) (Fig. S6a, S5c). Unlike pine, oak wood anatomy allows plastic shrinkage and expansion of trunk diameter for water storage or trunk capacitance in response to changing ecohydrological conditions (63 ± 6.1% and 37 ± 4.8% wood moisture content of its weight for oak and pine, respectively, $P < 0.001$, Fig. S7). Oak disposes of adaptations in functional root anatomy; it located its finest roots inside rock fractures (Rodríguez-Robles et al., 2017) (Fig. 3f). These roots exhibit a triple layer of epidermal tissue and contain calcium oxalate crystals (druzes), which facilitate root penetration and biophysical breakup of incipient rocks fractures (Franceschi & Nakata, 2005) (Fig. S5b). Under extremely dry conditions, oak vessel diameter of roots decreases by the formation of tyloses (Gottwald, 1972), which is an outgrowth of parenchyma cells into vessels to reduce water conduction and to prevent cavitation (Spicer, 2014), (Fig. S5d). Deep oak roots exhibited 83% more vessels with tyloses and 60% more druzes than surface roots (Fig. S5). Pine roots did not exhibit these kinds of anatomical adaptations. With the injection of labelled water into the rock fractures (3/4 L, δD = 485not garner water directly from water reservoirs in rock fractures. After a 10-day sampling period, the isotopic tracer was not detected in pine xylem water. In contrast, in the same assay, the oak tree closest to the injection (3.22 ± 0.78 m) presented the tracer in its trunk's xylem water after four days of water injection (Fig. S8).

Niche multidimensionality and landscape consequences

The distribution of oak and pine trees at the landscape level in pure and mixed stands, appears to be controlled by the same suite of highly specialized functional traits (anatomy, morphology and vertical placement of roots; stem parenchyma with water storage capacity) adapted to an apparently simple geodiversity consisting of four geologic strata (shallow soil, upper and lower regolith and fractured rock), however at the microscale level these strata are highly complex considering the high temporal dynamics of water availability, small-scale spatial water gradients and the suite of plant traits adapted to garner water. These interacting biotic and abiotic elements have generated a complex environment with multidimensional niches together forming a nichescape controlled by a highly dynamic pattern of water distribution and availability, to which two coexisting tree species have adapted to and simultaneously co-shaped these niches to fully exploit the highly limited and growth controlling resource elucidating the mixed stands of these forests (Table 1, Figs. 4). The hydrological dynamics of these ecosystems are highly unpredictable and ultimately driven by extremely variable seasonal, inter-seasonal, and interannual water availability, high temporal variability of seasonal and non-seasonal droughts, heterogeneous geospatial distribution of water stocks, and temporally variable direct and/or indirect access, uptake, storage and use of water. Next to the multidimensional configuration of the nichescape in mixed stands, our data from the pure oak and pine stands reveal an apparent niche segregation of tree communities at the landscape scale. Sites with greater vertical micro-geodiversity favor the co-existence of both tree species in mixed stands (Figs. S9i,j,k,l), whereas less geodiverse microsites are covered by pure stands. Pure pine stands occur where slightly deeper soils (˜30 cm) have developed over little fragmented rock (Figs. S9a,b,c,d). The absence of oak trees on this apparently more favorable substrate suggests a strong competitive exclusion mechanism and a suitable niche for pure pine stand formation. On the other hand, pure oak stands occurred only on poorly developed surface soil (> 10 cm deep) over rhyolitic rocks (Figs. S9e,f,g,h). The absence of pine under these conditions suggests that beyond the minimum soil depth of 20 cm over fractured rocks, oak trees find a suitable niche for pure stand formation.

These results significantly modify the current paradigm of water use by plants, as water stored between rocks has not been regarded as a potentially vital source sustaining mixed forest ecosystem in a semiarid region (but see Schwinning 2010; Jackson, Moore, Hoffmann, Pockman, & Linder*,* 1999) Here, we demonstrate for the first time that rocky soils generate a high degree of spatiotemporal heterogeneity in water distribution against which adaptive tree physiological and anatomical responses present a strong offset. Incorporating the multidimensional nichescape as a major component of ecosystem structure and function may not only mechanistically clarify species coexistence and other species interactions (i.e., competition, community diversity), but also shed new insight on patterns of the progress of landscape phenomena such as forest mortality.

Author contributions

URR and JTAM. designed the study; U.R-R., J.T.A-M., and E.H-S. wrote the manuscript; J.A.R-L and E.Y. provided expertise in geophysical methods and isotopic analysis; U.R.-R., J.T.A.-M. and J.A.R-L. ran the field experiments. U.R-R. and E.Y. executed the stable isotope analyses; U.R-R., J.T.A-M., E.H-S., J.A.R-L, and E.Y. analyzed the data.

Conflict of interest

All authors declare not to have conflict of interests in this study.

Data and materials availability

We declare the data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Adler, P. B., Salguero-Gomez, R., Compagnoni, A., Hsu, J. S., Ray-Mukherjee, J., Mbeau-Ache, C., & Franco, M. (2014). Functional traits explain variation in plant life history strategies. *Proc Natl Acad Sci U S A, 111* (2), 740-745. doi:10.1073/pnas.1315179111

Aranda, I., Ramírez-Valiente, J., & Rodríguez-Calcerrada, J. (2014). Características funcionales que influyen en la respuesta a la sequía de las especies del género Quercus: variación inter- e intra-específica. *Ecosistemas, 23* , 27-36. doi:10.7818/ECOS.2014.23-2.05

Araya, Y. N., Silvertown, J., Gowing, D. J., McConway, K. J., Linder, H. P., & Midgley, G. (2011). A fundamental, eco-hydrological basis for niche segregation in plant communities. *New Phytol, 189* (1), 253-258. doi:10.1111/j.1469-8137.2010.03475.x

Bartelheimer, M., Gowing, D., & Silvertown, J. (2010). Explaining hydrological niches: the decisive role of below-ground competition in two closely related Senecio species.*Journal of Ecology, 98* (1), 126-136. doi:DOI 10.1111/j.1365-2745.2009.01598.x

Berlyn, G. P., & Miksche, J. P. (1976). *Botanical Microtechnique and Cytochemistry* . USA: Iowa State Univ.

Beyer, M., Hamutoko, J. T., Wanke, H., Gaj, M., & Koeniger, P. (2018). Examination of deep root water uptake using anomalies of soil water stable isotopes, depth-controlled isotopic labeling and mixing models. *Journal of Hydrology, 566* , 122-136. doi:https://doi.org/10.1016/j.jhydrol.2018.08.060

Cai, J., Li, S., Zhang, H., Zhang, S., & Tyree, M. T. (2014). Recalcitrant vulnerability curves: methods of analysis and the concept of fibre bridges for enhanced cavitation resistance. *Plant Cell Environ, 37* (1), 35-44. doi:10.1111/pce.12120

Cermak, J., Nadezhdina, N., Meiresonne, L., & Ceulemans, R. (2008). Scots pine root distribution derived from radial sap flow patterns in stems of large leaning trees.*Plant and Soil, 305* (1-2), 61-75. doi:10.1007/s11104-007-9433-z

Cramer, V. A., Thorburn, P. J., & Fraser, G. W. (1999). Transpiration and groundwater uptake from farm forest plots of Casuarina glauca and Eucalyptus camaldulensis in saline areas of southeast Queensland, Australia. *Agricultural Water Management, 39* (2), 187-204. doi:https://doi.org/10.1016/S0378-3774(98)00078-X

David, T. S., Henriques, M. O., Kurz-Besson, C., Nunes, J., Valente, F., Vaz, M., . . . David, J. S. (2007). Water-use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought. *Tree Physiol, 27* (6), 793-803. doi:10.1093/treephys/27.6.793

Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H., & Tu, K. P. (2002). Stable Isotopes in Plant Ecology. *Annual Review of Ecology and Systematics, 33* (1), 507-559. doi:10.1146/annurev.ecolsys.33.020602.095451

de la Riva, E. G., Marañón, T., Violle, C., Villar, R., & Pérez-Ramos, I. M. (2017). Biogeochemical and Ecomorphological Niche Segregation of Mediterranean Woody Species along a Local Gradient. *Front Plant Sci, 8* , 1242. doi:10.3389/fpls.2017.01242

Evaristo, J., Jasechko, S., & McDonnell, J. J. (2015). Global separation of plant transpiration from groundwater and streamflow. *Nature, 525* (7567), 91-94. doi:10.1038/nature14983

Evaristo, J., & McDonnell, J. J. (2017). Prevalence and magnitude of groundwater use by vegetation: a global stable isotope meta-analysis. *Sci Rep, 7* , 44110. doi:10.1038/srep44110

FAO. (2006). *World Reference Base for Soil Resources* (103). Retrieved from Rome:

Franceschi, V. R., & Nakata, P. A. (2005). Calcium oxalate in plants: formation and function. *Annu Rev Plant Biol, 56* , 41-71. doi:10.1146/annurev.arplant.56.032604.144106

Gause, G. F. (1934). *The struggle for existence* : Baltimore: The Williams & Wilkins company.

Gottwald, H. P. J. (1972). Tyloses in fibre tracheids. *Wood Science and Technology, 6* (2), 121-127. doi:10.1007/bf00350825

Gray, M. (2004). *Geodiversity: Valuing and Conserving Abiotic Nature* : Wiley.

Hacke, U. G., Sperry, J. S., & Pittermann, J. (2005). 16 - Efficiency Versus Safety Tradeoffs for Water Conduction in Angiosperm Vessels Versus Gymnosperm Tracheids. In N. M. Holbrook & M. A. Zwieniecki (Eds.), *Vascular Transport in Plants*(pp. 333-353). Burlington: Academic Press.

IAWA Comittee. (1989). *List of microscopic features for hardwood identification* : Int. Assoc. Wood Anatomists Bull.

Jackson, R. B., Moore, L. A., Hoffmann, W. A., Pockman, W. T., & Linder, C. R. (1999). Ecosystem rooting depth determined with caves and DNA. *Proc Natl Acad Sci U S A, 96* (20), 11387-11392. doi:10.1073/pnas.96.20.11387

James, S. A., Meinzer, F. C., Goldstein, G., Woodruff, D., Jones, T., Restom, T., . . . Campanello, P. (2003). Axial and radial water transport and internal water storage in tropical forest canopy trees. *Oecologia, 134* (1), 37-45. doi:10.1007/s00442-002-1080-8

Johansen, D. A. (1940). *Plant microtechnique* . New York: McGraw-Hill.

Kukowski, K. R., Schwinning, S., & Schwartz, B. F. (2013). Hydraulic responses to extreme drought conditions in three co-dominant tree species in shallow soil over bedrock. *Oecologia, 171* (4), 819-830. doi:10.1007/s00442-012-2466-x

Kutschera, L., & Lichtenegger, E. (2002). *Wurzelatlas mitteleuropäischer Waldbäume und Sträucher* : Stocker.

MacArthur, R. H. (1968). *The theory of the niche* . Syracuse University Press.

Maestre, F. T., Callaway, R. M., Valladares, F., & Lortie, C. J. (2009). Refining the stress-gradient hypothesis for competition and facilitation in plant communities.*Journal of Ecology, 97* (2), 199-205. doi:10.1111/j.1365-2745.2008.01476.x

Meinzer, F. C., Brooks, J. R., Domec, J. C., Gartner, B. L., Warren, J. M., Woodruff, D. R., . . . Shaw, D. C. (2006). Dynamics of water transport and storage in conifers studied with deuterium and heat tracing techniques. *Plant Cell and Environment, 29* (1), 105-114. doi:10.1111/j.1365-3040.2005.01404.x

Muscarella, R., & Uriarte, M. (2016). Do community-weighted mean functional traits reflect optimal strategies? *Proceedings of the Royal Society B: Biological Sciences, 283* (1827). doi:10.1098/rspb.2015.2434

Noble, A. E., & Fagan, W. F. (2015). A niche remedy for the dynamical problems of neutral theory.*Theoretical Ecology, 8* (1), 149-161. doi:10.1007/s12080-014-0240-x

Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L. (2010). Source partitioning using stable isotopes: coping with too much variation. *PLoS One, 5* (3), e9672. doi:10.1371/journal.pone.0009672

Peñuelas, J., Fernández-Martínez, M., Ciais, P., Jou, D., Piao, S., Obersteiner, M., . . . Sardans, J. (2019). The bioelements, the elementome, and the biogeochemical niche.*Ecology, 100* (5), e02652. doi:10.1002/ecy.2652

Pérez, S., M., Arredondo, M., J. T., Huber, S., E., & Serna, P., A. (2014). Forest structure, species traits and rain characteristics influences on horizontal and vertical rainfall partitioning in a semiarid pine-oak forest from Central Mexico.*Ecohydrology, 7* (2), 532-543. doi:10.1002/eco.1372

Phillips, D. L., & Gregg, J. W. (2003). Source partitioning using stable isotopes: coping with too many sources. *Oecologia, 136* (2), 261-269. doi:10.1007/s00442-003-1218-3

Rodriguez-Robles, U., Arredondo, J. T., Huber-Sannwald, E., & Vargas, R. (2015). Geoecohydrological mechanisms couple soil and leaf water dynamics and facilitate species coexistence in shallow soils of a tropical semiarid mixed forest.*New Phytol, 207* (1), 59-69. doi:10.1111/nph.13344

Rodríguez-Robles, U., Arredondo, T., Huber-Sannwald, E., Ramos-Leal, J. A., & Yépez, E. A. (2017). Technical note: Application of geophysical tools for tree root studies in forest ecosystems in complex soils. *Biogeosciences, 14* , 5343-5357. doi:10.5194/bg-14-5343-2017

Ruzin, S. E. (1999). *Plant Microtechnique and Microscopy* . New York: Oxford University Press.

Schwinning, S. (2010). The ecohydrology of roots in rocks. *Ecohydrology, 3* (2), 238-245. doi:10.1002/eco.134

Shapiro, S. S., & Wilk, M. B. (1965). An Analysis of Variance Test for Normality (Complete Samples).*Biometrika, 52* (3/4), 591-611. doi:10.2307/2333709

Silvertown, J. (2004). Plant coexistence and the niche. *Trends in Ecology & Evolution, 19* (11), 605-611.

Silvertown, J., Araya, Y., & Gowing, D. (2015). Hydrological niches in terrestrial plant communities: a review. *Journal of Ecology* , DOI: 10.1111/1365-2745.12332 doi:10.1111/1365-2745.12332

Silvertown, J., Dodd, M. E., Gowing, D. J., & Mountford, J. O. (1999). Hydrologically defined niches reveal a basis for species richness in plant communities. *Nature, 400* (6739), 61-63.

Silvertown, J., & Law, R. (1987). Do plants need niches? Some recent developments in plant community ecology.*Trends Ecol Evol, 2* (1), 24-26. doi:10.1016/0169-5347(87)90197-2

Sperry, J. S., Hacke, U. G., & Pittermann, J. (2006). Size and function in conifer tracheids and angiosperm vessels. *American Journal of Botany, 93* (10), 1490-1500. doi:10.3732/ajb.93.10.1490

Spicer, R. (2014). Symplasmic networks in secondary vascular tissues: parenchyma distribution and activity supporting long-distance transport. *J Exp Bot, 65* (7), 1829-1848. doi:10.1093/jxb/ert459

Thomas Tyree, M., Salleo, S., Nardini, A., Assunta Lo Gullo, M., & Mosca, R. (1999). Refilling of embolized vessels in young stems of laurel. Do We need a new paradigm?*Plant physiology, 120* (1), 11-22.

Valladares, F., Bastias, C. C., Godoy, O., Granda, E., & Escudero, A. (2015). Species coexistence in a changing world. *Front Plant Sci, 6* , 866. doi:10.3389/fpls.2015.00866

Voltas, J., Lucabaugh, D., Chambel, M. R., & Ferrio, J. P. (2015). Intraspecific variation in the use of water sources by the circum-Mediterranean conifer Pinus halepensis.*208* (4), 1031-1041. doi:10.1111/nph.13569

von Arx, G., Arzac, A., Olano, J. M., & Fonti, P. (2015). Assessing Conifer Ray Parenchyma for Ecological Studies: Pitfalls and Guidelines. *Front Plant Sci, 6* .

West, A. G., Patrickson, S. J., & Ehleringer, J. R. (2006). Water extraction times for plant and soil materials used in stable isotope analysis. *Rapid Commun Mass Spectrom, 20* (8), 1317-1321. doi:10.1002/rcm.2456

Whittaker, R. H. (1969). Evolution of diversity in plant communities. *Brookhaven Symp Biol, 22* , 178-196.

Table 1. Characterization of the nine identified spatio/temporal niches occupied by *Pinus cembroides* (pine) and*Quercus potosina* (oak) in a semi-arid forest ecosystem in San Luis Potosi, Mexico during different ecohydrological periods (depletion, recovery and wet) between summer 2012 and winter 2014, total rainfall received on a certain number of days in the ecohydrological period, source of water used by oak and pine roots, values of leaf water potential, geoecohydrological mechanisms exhibited by oak and pine trees, and the type of niche use. The nine panels in Figure 4 (a-i) correspond to the description of each niche (A-I) of the Table (see first column).

Spatio/ temporal niches (see Figure 4)	**Ecohydrological period (see Figure 4)**	**Total rainfall (mm / days)**	**Origin/source of plant water used in period**	**Origin/source of plant water used in period**	**Leaf water potential (MPa) minimum / maximum**	**Leaf water potential (MPa) minimum / maximum**	**Geoecohydrological mechanism**	**Geoecohydrolo mechanism**	**Ty ni**
			pine	**oak**	**pine**	**oak**	**pine**	**oak**	
A	Wet *at the end of summer 2012*	40 / 45	Pacific and Atlantic rain	Pacific and Atlantic rain	-1.24 -3.15	-0.98 -2.85	Water acquisition (wa) from top soil and upper regolith	Water acquisition (wa) from top soil and upper regolith	Sh

Spatio/ temporal niches (see Figure 4)	**Ecohydrological period (see Figure 4)**	**Total rainfall (mm / days)**	**Origin/source of plant water used in period**	**Origin/source of plant water used in period**	**Leaf water potential (MPa) minimum / maximum**	**Leaf water potential (MPa) minimum / maximum**	**Geoecohydrological mechanism**	**Geoecohydrolo mechanism**	**Ty ni**
B	Dry / depletion *early autumn 2012 to late winter 2013*	33 / 136	Pacific and Atlantic rain	Rock fracture	-3.15 -5.95	-2.85 -4.83	wa from top soil and upper regolith	wa from fractured rock	Seg
C	Dry /recovery *throughout spring 2013*	11 / 92	Rock fracture, Pacific and Atlantic rain	Rock fracture	-5.95 -4.84	-4.83 -3.41	wa from top soil and regolith	Hydraulic lift	Fac
D	Wet *early summer 2013 to early autumn 2013*	408 / 153	Pacific and Atlantic rain	Pacific and Atlantic rain	-4.84 -1.25	-3.41 -1.05	Preferential wa from top soil	Preferential wa from regolith	Pa sha
E	Dry / depletion *early autumn 2013 to late winter 2014*	105/92	Pacific and Atlantic rain	Pacific and Atlantic rain, cold fronts	-1.25 -1.51	-1.05 -1.33	wa from upper regolith	wa from low regolith. decoupled from topsoil	Seg
F	Dry / depletion *late winter 2014*	14 / 59	Pacific and Atlantic rain	Cold fronts	-1.51 -3.75	-1.33 -1.21	wa from top soil and regolith	Use of water from trunk	Seg
G	Dry / depletion *late winter 2014 to late spring 2014*	6 / 30	Cold fronts	Rock fracture	-3.75 -4.83	-1.21 -1.24	Preferential wa from regolith	wa from fractures	Seg
H	Wet *late spring 2014 to early autumn 2014*	521 / 184	Pacific and Atlantic rain	Pacific and Atlantic rain	-4.83 -2.23	-1.24 -0.42	Preferential wa from top soil	wa from regolith	Pa sha

Spatio/ temporal niches (see Figure 4)	**Ecohydrological period (see Figure 4)**	**Total rainfall (mm / days)**	**Origin/source of plant water used in period**	**Origin/source of plant water used in period**	**Leaf water potential (MPa) minimum / maximum**	**Leaf water potential (MPa) minimum / maximum**	**Geoecohydrological mechanism**	**Geoecohydrolo mechanism**	**Ty ni**
I	Dry / depletion *early autumn 2014 to early winter 2014*	44 / 61	Pacific and Atlantic rain	Rock fracture	-2.23 -3.29	-0.42 -0.46	wa from upper regolith	Preferential wa from fractures	Seg

Table 1.

Figure captions

Figure 1. Meteoric Waterlines (WMWL) generated with hydrogen and oxygen isotopic composition of all rainfall events originating from the Pacific or Atlantic Ocean during the study period between 2012 and 2014. The line for water stored and flowing within volcanic rock fractures is also shown (water-rock interaction). WMWL represents the global world meteoric water line of Craig (1961). LMWL represents the local meteoric water line of Sierra San Miguelito Volcanic Complex (SSMVC) in San Luis Potosi, Mexico. Inset shows the routes of the different meteorological events that influenced the research site during the 2012-2014 study period.

Figure 2. Combined electrical resistivity tomograms (ETR) and ground penetrating radargrams (GPR) taken in October 2012 after 10 days of rain (86 mm) in **(a)** *Pinus cembroides* (pine),**(b)** *Quercus potosina* (oak) and **(c)** mixed pine-oak forest stands. ERT profiles reveal a close linkage between the position of roots (diameter > 2.5 cm), soil resistivity (lower resistivity implies greater water availability), and rock fractures. The soil corresponds to the top 20 cm, the layer underneath the soil (regolith) includes soil pockets and rock fractures and is depicted by the dotted band, and below the solid line the fresh bedrock extends to great depth. Open circles of increasing size depict roots of increasing diameter. Black circles indicate roots used for calibration of the ground penetrating radar (GPR). Trees marked with X indicate the location of soil psychrometer sensors inserted at 12 cm soil depth.

Figure 3. Spatial analysis of root distribution for eight root diameter classes of *Pinus cembroides* and *Quercus potosina* , in pure and mixed stands, using the Kriging interpolation method at three different soil depths: 0-10 cm, 10-20 cm, and 20-30 cm. Transversal profiles are the graphical representation of the ERT (Figure 1). Symbols within the circular plots mark pine (left), oak (middle), and co-occurring oak and pine (right) tree positions. Prediction error (average standard error): **(a)** 0.23, **(b)** 0.28,**(c)** 0.32, (d) 0.27, (e) 0.19, **(f)** 0.25, **(g)**0.27, **(h)** 0.20, **(i)** 0.26.

Figure 4. Spatiotemporal niches identified by the value of soil and xylem water isotope composition (δ^{18}O, δ^{2}H) in oak and pine trees. The size of arrows denotes the proportion of water used by each species; a cross denotes uncoupling between soil and xylem water. Colors indicate isotopic signatures of different water sources; the warmer the color the more enriched the isotopic value of the substrate (i.e. yellow - orange - green); winter rain (red - violet); fracture water (blue). Average δ^{18}O and δ^{2}H are the isotopic values of water in different soil layers, xylem water, and the groundwater. Identified ecohydrological periods include

the following: **(a)**wet at the end of summer 2012, **(b)** dry / depletion period early autumn 2012 to late winter 2013, **(c)** dry /recovery period throughout spring 2013, **(d)** wet/ from early summer 2013 to early autumn 2013, dry / **(e)** depletion period early autumn 2013 to late winter 2014, **(f)** dry / depletion period in late winter 2014, **(g)** dry / depletion period from late winter 2014 to late spring 2014, **(h)** wet / from late spring 2014 to early autumn 2014 and **(i)** dry / depletion period early autumn 2014 to early winter 2014. For better interpretation please refer to Table 1.

Figure 5. **(a)** Leaf ($\Psi_{\lambda\epsilon\alpha\varphi}$) and**(b)** soil ($\Psi_{\sigma o\iota\lambda}$) water potentials associated with *Pinus cembroides* and *Quercus potosina* in pure and mixed stands in a semiarid forest ecosystem in San Luis Potosí, Mexico. Closed bars present total monthly precipitation recorded in the study site between September 2012 and December 2014. The shaded and clear areas indicate the length of ecohydrological periods defined in this study. Each point represents the mean ±1 SE (n=4).

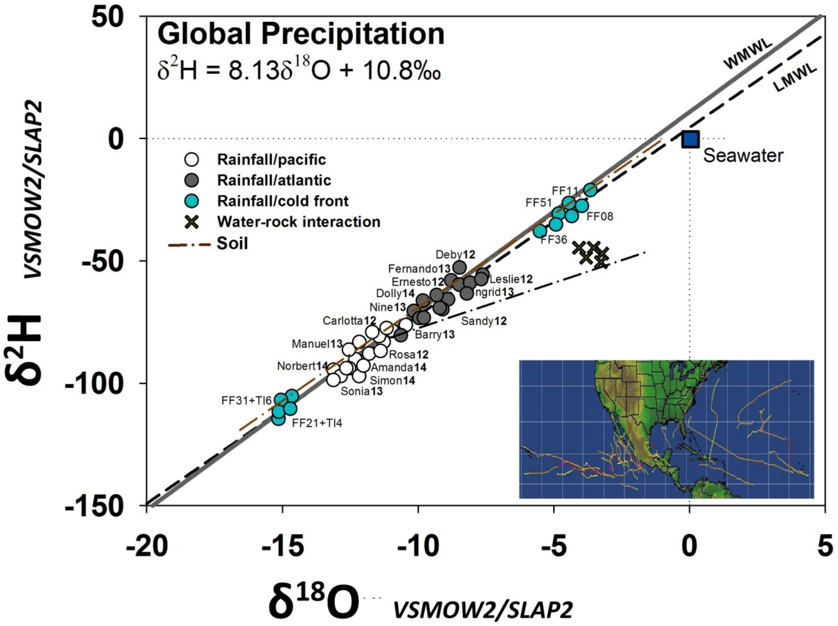

Figure 1.

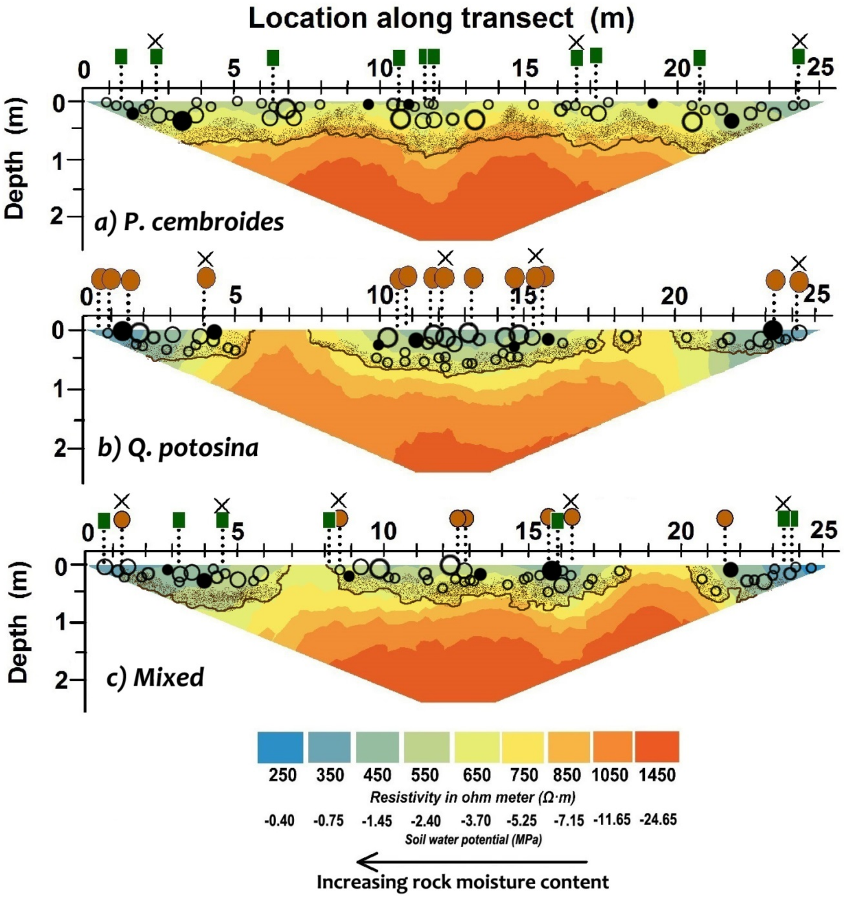
Location along transect (m)
0
5
10
15
20
25
Depth (m)
0
1
2
a) P. cembroides
b) Q. potosina
c) Mixed
250
350
450
550
650
750
850
1050
1450
Resistivity in ohm meter (Ω·m)
-0.40
-0.75
-1.45
-2.40
-3.70
-5.25
-7.15
-11.65
-24.65
Soil water potential (MPa)
Increasing rock moisture content

Figure 2.

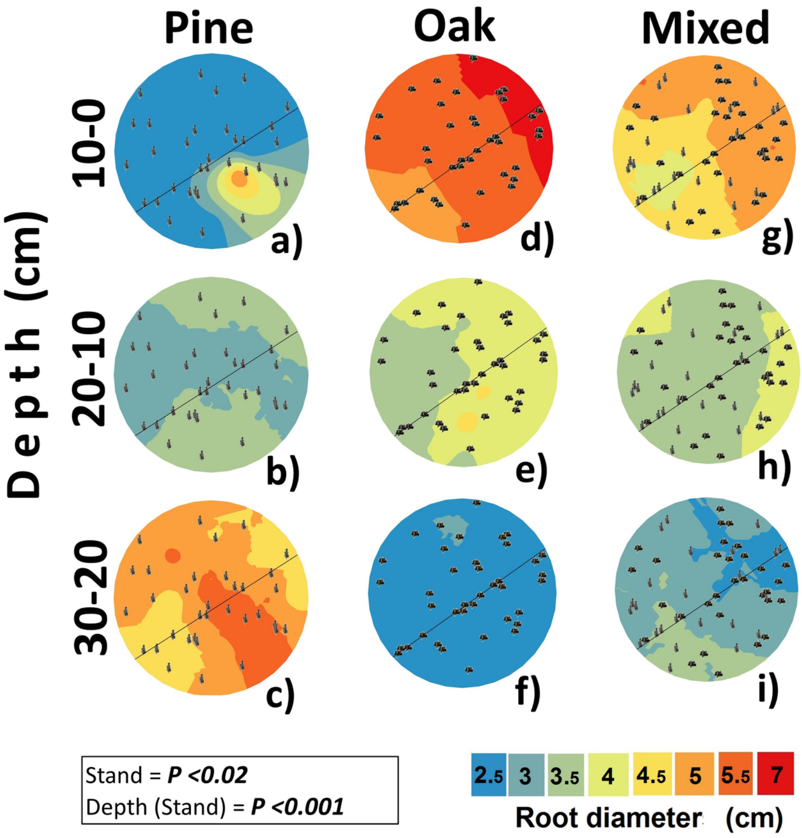
Pine
Oak
Mixed
Depth (cm)
10-0
20-10
30-20
a)
b)
c)
d)
e)
f)
g)
h)
i)
Stand = *P <0.02*
Depth (Stand) = *P <0.001*
2.5
3
3.5
4
4.5
5
5.5
7
Root diameter (cm)

Figure 3.

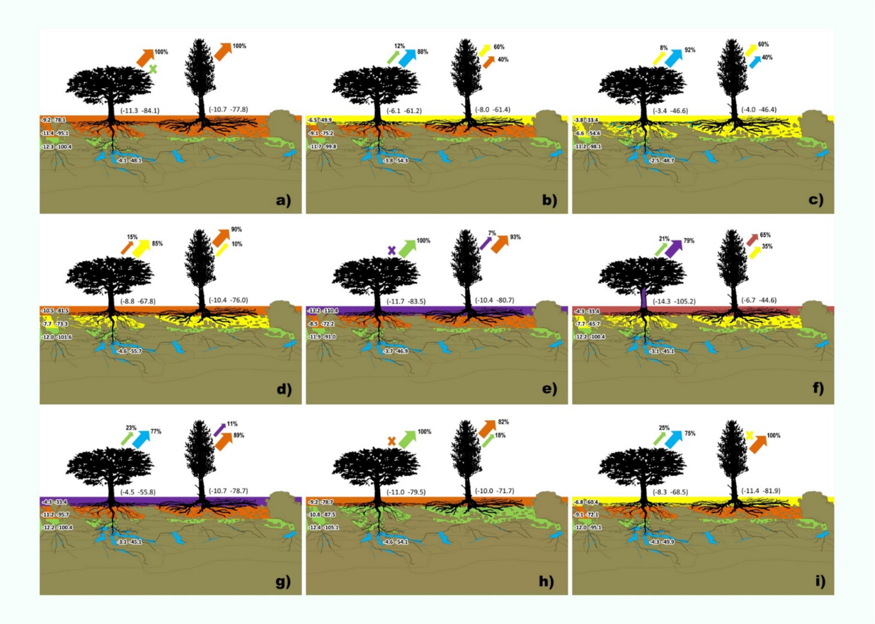
100%
100%
(-11.3 -84.1)
(-10.7 -77.8)
-9.2 -78.1
-11.4 -95.1
-12.3 -100.4
-4.1 -48.1
a)
12%
88%
60%
40%
(-6.1 -61.2)
(-8.0 -61.4)
-6.5 -49.9
-9.1 -75.2
-11.7 -99.8
-3.8 -54.3
b)
8%
92%
60%
40%
(-3.4 -46.6)
(-4.0 -46.4)
-3.8 -33.4
-6.6 -54.6
-11.2 -98.1
-2.5 -48.7
c)
15%
85%
90%
10%
(-8.8 -67.8)
(-10.4 -76.0)
-10.5 -81.5
-7.7 -73.3
-12.0 -101.6
-4.6 -55.7
d)
100%
7%
93%
(-11.7 -83.5)
(-10.4 -80.7)
-13.2 -110.4
-8.5 -72.2
-11.9 -91.0
-3.7 -46.9
e)
21%
79%
65%
35%
(-14.3 -105.2)
(-6.7 -44.6)
-4.3 -33.4
-7.7 -65.7
-12.2 -100.4
-3.1 -45.1
f)
23%
77%
11%
89%
(-4.5 -55.8)
(-10.7 -78.7)
-4.3 -33.4
-11.2 -95.7
-12.2 -100.4
-3.1 -45.1
g)
100%
82%
18%
(-11.0 -79.5)
(-10.0 -71.7)
-9.2 -78.7
-10.8 -87.5
-12.4 -105.1
-4.6 -54.1
h)
25%
75%
100%
(-8.3 -68.5)
(-11.4 -81.9)
-6.8 -60.4
-9.1 -72.1
-12.0 -95.1
-4.3 -49.9
i)

Figure 4.

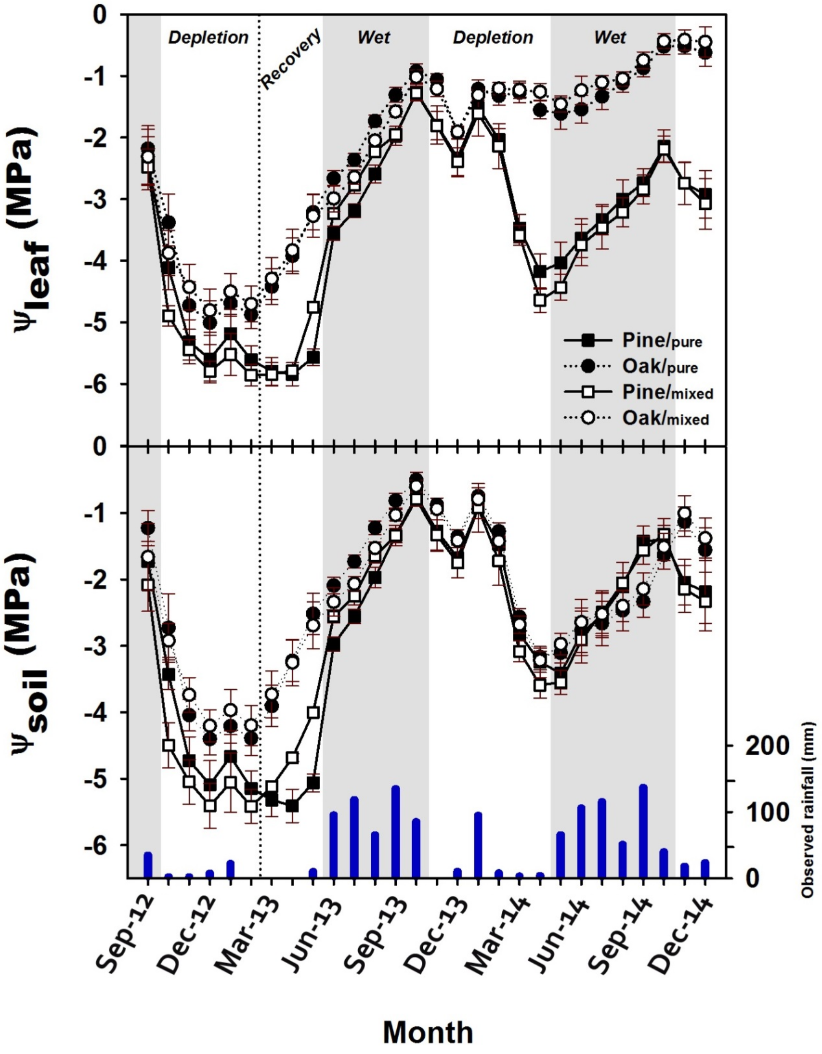

Figure 5.

Hosted file

`Rodriguez et al_2020_PC&E_Figures.docx` available at https://authorea.com/users/310161/articles/440965-coupled-plant-traits-adapted-to-wetting-drying-cycles-of-substrates-co-define-niche-multidimensionality