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REGULAR ARTICLE

Coordination of rooting depth and leaf hydraulic traits defines drought-related strategies in the campos rupestres, a tropical montane biodiversity hotspot

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Abstract
Background and aims The survival and coexistence of plants in water-limited environments are related to their ability to coordinate water acquisition and regulation of water loss. To assess the coordination among below and aboveground hydraulic traits and the diversity of water-use strategies, we evaluated rooting depth and several leaf hydraulic traits of 15 species in *campos rupestres*, a seasonally-dry biodiversity hotspot in central Brazil.
Methods We assessed the depth of plant water acquisition by excavating roots and analyzing the stable isotope composition of hydrogen (δD) and oxygen (δ^{18}O) in the xylem and soil water. We also measured mid-morning stomatal conductance, leaf-water potential at turgor loss point (Ψ_{TLP}) and pre-dawn leaf water potentials (Ψ_{PD}) during wet and dry seasons.
Results We demonstrated that rooting depth is a good predictor of seasonal variations in stomatal conductance and Ψ_{PD}. Shallow-rooted plants had greater variation in stomatal conductance and Ψ_{PD} than deep-rooted plants. Woody plants with shallower roots also had lower Ψ_{TLP} than deep-rooted plants, revealing higher drought resistance.
Conclusion We demonstrate that shallow-rooted species, more exposed to variation in water availability, have mechanisms to confer drought resistance through turgor maintenance. Our results support the theory of hydrological niche segregation and its underlying trade-offs related to drought resistance.

Keywords Eco-hydrology · Hydrological niche · Plant community ecology · Stable isotopes · Stomatal conductance · Turgor loss point

Data accessibility
All data used in this manuscript are presented in the manuscript and its supporting information.

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Introduction

Water shortage is an important driver of niche partitioning, coexistence and functional trait differentiation leading to high diversity of water-use strategies in water-limited ecosystems (Bartelheimer et al. 2010; Jackson et al. 1996; Rossatto et al. 2013; Schwinning et al. 2004). In these ecosystems, plants generally exhibit contrasting sets of traits related to drought resistance or avoidance that allow spatial and temporal hydrological partitioning of water allowing the species niche to segregate (Schwinning and Ehleringer 2001; Silvertown et al. 2015). The hydrological niche segregation (HNS) framework proposes that plants within a community should exploit different water sources by specializing on: 1) occupying soil patches with different moisture regimes, for example along a toposequence (see Cosme et al. 2017); 2) having different recruitment opportunities between years, depending on water availability; and 3) having different rooting depths or phenologies (e.g. drought- deciduous and evergreen species with contrasting water demands) (Silvertown et al. 2015). The HNS is predicted to exist in a wide range of ecosystems where species occurrences and individual performance are affected by spatial and temporal variation in water availability. To occupy each niche patch, coordination between hydraulic traits is necessary to insure a positive water balance and avoid a negative carbon balance.

Deep rooting to access water in deep soil layers or groundwater pools is an important trait allowing species survival in water-limited ecosystems (Dawson and Pate 1996; Nepstad et al. 1994; Oliveira et al. 2005a; West et al. 2012). Stable isotopes of hydrogen and oxygen from soil and plant xylem water have been used to describe the spatial differences in water sources used by co-occurring species (Dawson et al. 2002). Because shallow soils are more prone to evaporation and evaporation causes isotope fractionation, enriching the soil water in deuterium (D) and oxygen (^{18}O), shallow-rooted plants tend to show more enriched values of xylem water isotope compared to deep-rooted plants (Dawson et al. 2002). Dimorphic-rooted plants usually exhibit seasonal variation in xylem-water isotope composition, indicating changes in water source from shallow to deep soil (Dawson and Pate 1996; Nie et al. 2011; Quesada et al. 2008; Rossatto et al. 2013). Species-rich communities in environments with seasonal variation in water availability provide an excellent opportunity to understand the mechanisms underlying both spatial and temporal HNS.

During periods of water deficits, root restriction in water uptake associated with high atmospheric vapor pressure deficit (VPD) can cause a decline in whole plant water potential. Consequently, stomatal conductance and leaf water potential (Ψ_L) may interact as a feedback mechanism, in which Ψ_L has a key role controlling stomatal guard-cell movement (Brodribb et al. 2003; Klein 2014) and restricting plant water loss. Long term drought cycles might favor species with lower values of leaf water potential at turgor loss point (Ψ_{TLP}), which reflects the ability of the cell to maintain its turgor as soil water potential declines (Bartlett et al. 2012). When the water loss is severe, cell pressure and volume declines until the turgor is completely lost, until Ψ_{TLP} is reached (Ding et al. 2014). Drought avoidance and tolerance are likely to occur through HNS however, with a few exceptions (Pivovaroff et al. 2015), studies explicitly investigating the association of leaf water relations with rooting depth at the community scale are missing.

The *campos rupestres* of Central Brazil occur under seasonally dry climates and over quartzite rock outcrops in mountaintops, conditions that generate periods of significant water deficits for vegetation. The vegetation is dominated by perennial species of small trees, woody shrubs, rosettes, sedges and grasses over impoverished soils (Le Stradic et al. 2015; Oliveira et al. 2015). This ecosystem supports one of the highest levels of plant biodiversity on earth, for instance about 1590 species were recorded in 200 km^2 (Giulietti et al. 1987; Silveira et al. 2016), but little is known about the mechanisms that generate and maintain this diversity. The hydrological niche segregation (HNS) framework proposes that partitioning of water resources is a potential mechanism allowing plant coexistence within species-rich communities (Araya et al. 2011). Here we explore water-uptake strategies and water regulation by evaluating root and leaf hydraulic traits in a *campos rupestres* plant community. Considering the high levels of biodiversity and marked seasonality in rainfall, the *campos rupestres* plant communities represent an excellent model to investigate the existence of a hydraulic trait spectrum integrating rooting depth, stomatal regulation capacities, and traits related to drought resistance for a broad number of species. We expect that plants acquire water through diverse root architectures, exhibiting spatial (along the soil profile) and seasonal partitioning of water. We hypothesize that 1) shallow-rooted species have wider variations in stomatal conductance between rainy and dry seasons than deep-rooted plants in order to maintain a favorable water status during the dry season, i.e. water acquisition traits (rooting depth) are negatively related to stomatal regulation capacity; 2) rooting depth is strongly related to leaf drought resistance, with shallow-rooted species being more exposed to seasonal water deficits and therefore more resistant to water stress (more negative Ψ_{TLP}) than deep-rooted species. Strong empirical evidence linking below- and aboveground hydraulic traits is missing in the literature (but see Pivovaroff et al. 2015). Understanding the diversity of plant hydraulic traits is particularly important to unravel the underlying mechanisms that allow species coexistence in hyper-diverse communities and to predict how communities will respond to longer and more severe droughts (Silvertown et al. 2015).

Materials and methods

Study area

The research was carried out between 2011 and 2014 in a *campos rupestre* site at Serra da Canastra National Park, Minas Gerais, Brazil (20°15′40″S and 46°25′25″ W). The site has an elevation of 1200 m and a seasonal climate. Mean historical annual rainfall (1995–2010) is 1293 mm, falling mostly in the wet season (November to April), and mean annual temperature ranges between 19 °C to 23 °C. Plants at the site are exposed to high ultraviolet radiation and wide daily variations in temperature (mean = 18 °C, max = 27.6 °C and min = 4.5 °C), high vapor pressure deficit (VPD) and strong seasonal water fluctuation (Fig. S1) (Jacobi et al. 2007; Porembski and Barthlott 2000). Quartzite rock outcrops characterize the site, which has shallow soils, comprised of a narrow organic layer covering a sandy soil within coarse gravels from the matrix rocks. *Campos rupestres* soils present low nutrient availability, especially phosphorus and potassium (Oliveira et al. 2015). The dominant plant families are Velloziaceae, Fabaceae, Poaceae and Asteraceae and the estimated number of species for this biodiversity hotspot is higher than 5000 (Silveira et al. 2016). The total number of vascular plants in the *campos rupestres* at Serra da Canastra National Park is 768 species (Romero and Nakajima 1999).

Data collection

To determine community composition and species relative abundance, we surveyed all the species in six randomized circular plots of 8 m of diameter. The circular plots were placed at least 100 m apart from each other. We measured plant height ($n = 5$ per species) of the fifteen most abundant species that represent the main life forms of the *campos rupestres* community inside the plots (Table 1). To evaluate the root architecture of plants, we excavated the entire root system of 3–4 individuals per species across all plots.

To determine the depth of the water source used by the plants, we analyzed the stable isotope composition of hydrogen (deuterium) (δD_{xyl}) and oxygen ($\delta^{18}O$) of the xylem water of 15 species (Table 1) and soil water at different depths. We collected suberized stem segments from woody plants and junctions between roots and shoots not exposed to evaporation in grasses and rosettes ($n = 2–6$ per species) in the peak of the dry season (September 2011; Dawson and Ehleringer (1998)). High evaporation in shallow soil during peak dry season generates a gradient in the stable isotope composition of soil water along the vertical soil profile (Dawson et al. 2002). Within each species, we only sampled mature individuals within the same size class to avoid possible confounding effects of plant size on rooting depth. In the same period, we collected the soil samples using an auger in six different soil depths (5, 15, 30, 60 and 90 cm) in the center of each plot. Deeper excavation was not possible because the bedrock lies at 1 m depth. During the three-year study, we collected water from twenty-two rainfall events from November 2011 to July 2013. We sealed each vial tightly, wrapped with parafilm® and cooled them to avoid evaporation prior to analysis. We collected soil samples in the same plots and depths used for the isotope analysis to determine the gravimetric soil water content based on the relative difference between fresh and dried soil at 65 °C for 72 h.

To evaluate the temporal variation in the depth of water source used by a subset species, we sampled xylem water of six species with contrasting underground architecture and rooting depths (i.e. *Campomanesia pubescens*, *Eremanthus seidellii*, *Lessingianthus warmingianum*, *Mimosa clausenii*, *Vellozia intermedia*, and *V. nivea*). Soil- and xylem water samples were collected on the same individuals in the wet season (November 2011), in the transition between the wet and the dry season (June 2011) and in the dry season (August 2012) ($n = 3–6$).

We extracted water from soil and plant tissue samples using the cryogenic distillation method (Dawson and

Table 1 List of studied species and the results of underground architecture systems and isotope mixing model showing the proportion of water (mean (standard-deviation)) used from shallow (5–15 cm), intermediate (30–40 cm) and deep (60–90) soil layers in the dry season of September 2011. Root morphotypes are: D- dimorphic, VF- *Velloziaceae* fasciculate, S- sobole, L- lignotuber, F – fasciculate systems. Species are sorted from lower to higher value of δD_{xyl}. The graph symbols are used in Figs. 3 and 5 to denote the respective species

Graph Symbol	Species	Family	Growth Form	Root type	Source proportion (%)		
					5 to 15 cm	30 to 40 cm	60 to 90 cm
□	*Eremanthus seidelii* MacLeish & Schumacher	Asteraceae	Dwarf tree	D	9 (9)	18(21)	72(22)
■	*Mimosa clausenii* Benth.	Fabaceae	Shrub	D	4(4)	7(8)	88(9)
○	*Leandra aurea* (Cham.) Cogn.	Melastomataceae	Shrub	D	11(12)	23(22)	65(14)
●	*Myrsine guianensis* Aubl.	Primulaceae	Dwarf tree	D	25(26)	31(25)	42(25)
Δ	*Vellozia intermedia*	Velloziaceae	Rosette	VF	13(13)	27(22)	59(17)
▲	*Microlicia cuneata* Naudin	Melastomataceae	Shrub	D	13(14)	30(25)	55(15)
◊	*Vellozia nivea* L.B.Sm. & Ayensu	Velloziaceae	Rosette	VF	18(17)	32(25)	48(14)
♦	*Vellozia sp.*	Velloziaceae	Rosette	VF	19(17)	33(25)	47(13)
∇	*Mikania sp.*	Asteraceae	Shrub	L	20(18)	34(24)	45(14)
▽	*Campomanesia pubescens* (Mart. ex DC.) O.Berg	Myrtaceae	Shrub	S	29(17)	35(23)	35(14)
◍	*Lessingianthus warmingianum* Less.	Asteraceae	Shrub	L	29(21)	34(25)	35(17)
▣	*Rhynchospora consanguinea* (Kunth) Boeckeler	Cyperaceae	Graminoid	F	42(15)	38(25)	18(14)
◇	*Grass 1*	Poaceae	Graminoid	F	50(21)	33(26)	15(16)
△	*Loudetiopsis sp.*	Poaceae	Graminoid	F	52(23)	32(12)	14(15)
⟐	*Grass2*	Poaceae	Graminoid	F	55(18)	34(24)	10(11)

Ehleringer 1998) at the Laboratory of Isotope Ecology, CENA - University of São Paulo. The water samples from rain events were analyzed at the Stable Isotope Ratio Facility for Environmental Research at the University of Utah. The oxygen isotopic ratio ($\delta^{18}O = {}^{18}O/{}^{16}O$) and deuterium isotopic ratio ($\delta D = {}^{2}H/H$) were determined using a high temperature elemental analyzer coupled with a mass spectrometer (Thermo Finningan Delta Plus XL). The data were expressed in delta (δ) whose notation is ‰ relative to international Vienna mean ocean water standard (V-SMOW) (Gonfiantini 1978).

To characterize temporal physiological adjustments in the species studied, we measured xylem pre-dawn water potential (Ψ_{pd}) and leaf stomatal conductance (g_s) from June to August 2012 ($n = 5$ individuals/species/month). In the evening before the Ψ_{pd} measurements, we wrapped the leaves with plastic bags to avoid nocturnal transpiration and to ensure that leaf Ψ_{pd} would approximately represent soil water potential where roots were placed (Donovan et al. 2001; Donovan et al. 2003). We measured Ψ_{pd} between 2 to 6 AM using a Scholander pressure bomb (PMS, model 1000, USA). We measured the mid-morning g_s between 8:30 to 10:30, which is the theoretical period of maximum g_s, in two leaves per individual in five individuals per species, using a steady-state porometer (Delta T Devices, Cambridge, England).

We constructed pressure-volume (P-V) curves using the bench drying technique in May 2014 (Turner 1988) to estimate the water potential at turgor loss point (Ψ_{TLP}) in five expanded terminal leaves per species. We recut the leaves under water and let them rehydrate inside a black plastic bag during 2 h before the start the P-V curve construction. We first weighted the hydrated leaves in a precision balance (0.0001 g) to obtain the weight at full turgor and immediately placed them in a pressure chamber (PMS, model 1000, USA) to get the initial water potential. We repeated this process several times while leaves were dehydrating under ambient conditions (20 °C). After all the procedure, we dried the leaves at 65 °C for 72 h before we measured leaf dry weight.

Statistical analysis

We performed all statistical analysis using R version 3.3.2 (R Core Team 2012). We used linear mixed effect models and compared statistical significance between two types of model. We compared a model including soil depth, species and season as fixed effects, with a null model, which used no fixed effects. Random effects in both models included plants and repeated measures within plants. We did a multiple comparisons of means using Tukey contrasts to identify differences between soil depths, species and season differences among the subset of six species of plants with the lsmeans package (Bates et al. 2015; Lenth 2016). For soil analyses, we grouped the samples from 5 to 15 cm to represent superficial soil and 60 to 90 cm to represent the deeper soil profile. We used an ANCOVA to identify differences in slopes of relationship between $\delta^{18}O$ and δD for the global, regional, plant and soil meteoric water lines.

We used the siar package (Parnell and Jackson 2013) to compute an isotope-mixture model that takes the isotope data and fits into a Bayesian framework and fit a mean water uptake depth from the xylem water content by analyzing the distribution of isotopic concentration in soil water. The model assumes that each target value comes from a Gaussian distribution with an unknown mean and standard deviation (Parnell et al. 2013). We tested if rooting depth, estimated by δD_{xyl}, was a good predictor of the variation in Ψ_{pd}, *gs*, average of plant height and Ψ_{TLP} using linear regression models. We calculated the difference between average Ψ_{pd} measured in June (wettest month in the study) and August (driest month), the delta Ψ_{pd} ($\Delta\Psi_{pd}$). We used the coefficient of variation (CV) of g_s calculated by CV = $(M/SD)*100$, (M = mean, SD = Standard Deviation) as an index of seasonal variation in stomatal conductance and tested if there was a linear relationship between rooting depth (δD_{xyl}) and this variable. The CV provides a measure of data dispersion around the mean allowing us to compare variation between groups with different magnitude of values of g_s. We also performed a quantile regression between plant height and rooting depth (δD_{xyl}) (Cade and Noon 2003) fixed the $\tau = 0.75$ using the quantreg package (Koenker 2017). Finally, in order to detect general group trends based on rooting depth and each trait studied (δD_{xyl}, $\delta^{18}O_{xyl}$, $\Delta\Psi_{pd}$, Cvg_s, Ψ_{TLP}, and plant height), we submitted the bivariate matrix to a hierarchical cluster analysis based on the Euclidean distance between species. After that, we organized the data to a single matrix with six leaf hydraulic traits to find the overall functional clustering as a function of rooting depth and the coordination of leaf traits.

Results

Diversity of root morphologies

We found a wide variety of root morphologies and grouped them into five main categories (Fig. S2; Table 1, Fig. 1): (1) Classical dimorphic (D) root system, with distinct tap and lateral roots, having access to deep water, found on five of our study species; (2) the *sobole* (S) type, also known as underground tree, stem-tuber or underground stem, was found only for *Campomanesia pubescens* (Myrtaceae). This root type grows parallel to the soil surface with no taproot, and only has access to shallow-soil water (Fig. S2); (3) lignotuber (L) systems with a large numbers of fine roots growing at different angles in shallow soil layers (found in the following species: *Lessingianthus warmingianum* and *Mikania* sp., both Asteraceae); (4) fasciculate (F) fibrous type was found in grasses, sedges and (5) fasciculate *Velloziaceae* (VF) species type. In grasses and sedges, fine roots grow from small rhizomes and bulbs and only reached shallow soils, while in *Velloziaceae* species, the fasciculate roots are thicker than in grasses and are able to grow into intermediate soil layers (Fig. S2; Fig. 1).

δD and $\delta^{18}O$ from rainfall, soil and xylem water

The $\delta^{18}O$ of rainfall events collected at Serra da Canastra varied from −16.1 to 0.6 ‰, whereas δD ranged from −119.8 to 20.6 ‰. The local meteoric water line (LMWL) showed little deviation from global meteoric water line (GMWL), the slope of LMLW was 8.40, only 0.36 higher than GMWL (ANCOVA; $t = 1.98$; $p = 0.054$). There was a complete overlap between soil and xylem isotope water lines with similar slope values between them (soil = 4.56 and xylem = 4.65; ANCOVA; $t = 0.21$; $p = 0.64$). The slope between soil and xylem water line was about 3.5 lower than the regional water line (ANCOVA; $t = -6.38$; $p < 0.001$) indicating a strong evaporative loss and very low relative humidity during the sampling work (Fig. 1).

The δD and $\delta^{18}O$ of bulk soil water in the dry season (September 2011) showed a marked vertical gradient,

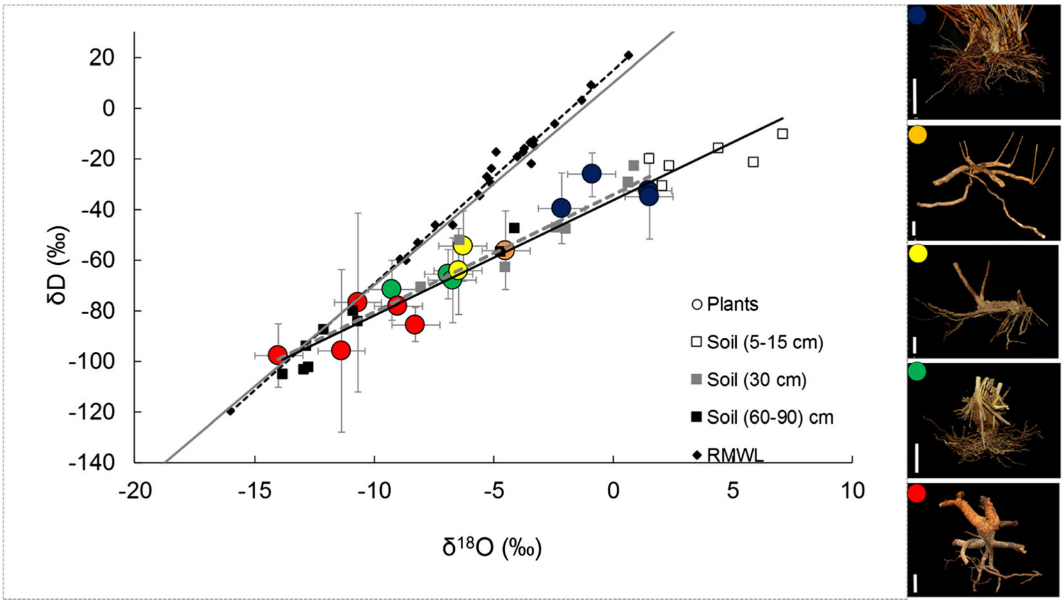

Fig. 1 Relationship between hydrogen (δD) and oxygen ($\delta^{18}O$) stable isotopes from rainfall, soil samples and xylem water collected from September 2011 to July 2013 at Serra da Canastra National Park. Grey continuous line represents global meteoric water line; black diamonds represent rainfall events; dashed black line the regional meteoric water line (RMWL). Squares and continuous black line represents soil depth and soil water line. Circles and dashed gray line represent species and species water line. Grey bars indicate the standard deviation around mean value of xylem water isotopic composition. The images on right side are the most representative roots types found in this survey. From bottom up root type classified as dimorphic root (*red circles*), Velloziaceae fasciculate (*green circles*), lignotuber/xylopodium (*yellow circles*), stem tuber (*orange circle*) and fasciculate grasses (*blue circles*). The white bars in the roots images represent a scale of 5 cm

with an increase of the heavier isotope in shallow soil (Figs. 1 and 2; Table 1). The bulk-soil water δD values ranged from −105.6 to −10.7‰ and $\delta^{18}O$ from −13.8 to 7.1 ‰. The average δD was less negative (−20.8 ± 6.9‰) in shallow soils (15 cm) than in deep soils (−78.3 ± 23.7‰, at 90 cm) (Multiple Comparisons with Tukey contrast, Z value = −5.672, $p < 0.001$). Average $\delta^{18}O$ was also higher in shallow (3.9 ± 2.3‰) than in deep soils (−8.9 ± 4.7‰) (Multiple Comparisons with Tukey contrast, Z value = −6.272, $p < 0.001$). An increase in soil volumetric water content in deep soils accompanied the gradient of decreasing heavy water isotopes with depth (Fig. 2a, b). The xylem water isotopic composition ($\delta^{18}O_{xyl}$ and δD_{xyl}) overlapped with the soil isotopic composition gradient in the dry season (Fig. 1; see dashed gray and black continuous lines). The average δD_{xyl} ranged from −97‰ for the *E. seidelli* to −26‰ for *Grass 2* and the average $\delta^{18}O_{xyl}$ from −13‰ for *M. clausenii* to 1.5‰ for *Loudetiopsis* sp.

Grasses and a species of sedge with fasciculate fibrous roots (Grass 1 and 2, *Loudetiopsis* sp., *Rhynchospora cosanguinea*) were the shallowest-rooted species, with the less negative average δD_{xyl} ranging from −26‰ to −39‰, and the main water source from 15 cm of soil (Table 1). The fasciculate roots in Velloziaceae species were able to uptake water from intermediate to deep soil and they used a deeper water source, less enriched in D in the dry season (*V. nivea*, δD_{xyl} = −73 ± 1‰, *V. intermedia*, δD_{xyl} = −67 ± 4‰) than in the wet season (*V. nivea*, δD_{xyl} = −29 ± 7‰/ *V. intermedia*, δD_{xyl} = −43 ± 8‰) (Fig. 3). The *sobole* type (*C. pubescens*) was only able to uptake water from shallow soils in both wet (δD_{xyl} = −40 ± 4‰) and dry seasons (δD_{xyl} = −48 ± 3‰; Table 1, Fig. 3). *C. pubescens* showed significantly higher dry-season δD_{xyl} (−56 ± 15‰) than in *E. seidelli* (−96 ± 32‰), the deepest-rooted plants (Multiple Comparisons with Tukey contrast, Z value = −4.809, $p < 0.001$). Lignotuber-bearing species *L. warmingianum* and *Mikania* sp. had shallower root systems then *E. seidelli* and other dimorphic-rooted species (Multiple Comparisons with Tukey contrast, Z value = −4.809, $p < 0.001$). Lignotuber root systems had many buds and large numbers of fine roots growing at different angles in shallow soil layers. The dwarf tree *E. seidelli* and the

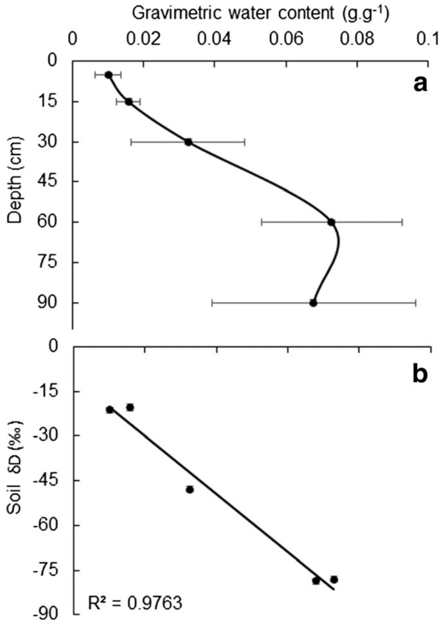

Fig. 2 **a** Gravimetric water content in different soil depths and **b** relationship between soil water hydrogen stable isotope composition (δD) and gravimetric water content where shallow soil showed less water content and were more enriched in deuterium. Bars indicate standard deviation around mean. Samples were collected in the same day as isotope sampling in September 2011 at Serra da Canastra National Park, Brazil

shrubs *Mimosa clausenii, Leandra aurea, Microlicia cuneata* and *Myrsine guianensis* were the deepest-rooted species. They had a classical dimorphic root system with distinct tap and lateral roots, having access to deep water. They had the most negative values of δD_{xyl} with values ranging from −95‰ to −78‰, indicating they accessed water deeper than 60 cm (Table 1). Both *E. seidelli* and *M. clausenii* used deuterium-depleted water from deep soil layers during the dry season and deuterium-enriched water during rainy season (Fig. 3). Variations to a less enriched water source in D and ^{18}O in the wet season, similar to the isotopic signature of rain, suggest the ability of dimorphic-rooted species to seasonally change their water source.

The two deepest-rooted species *E. seidelli* and *M. clausenii* presented the most conspicuous contrast between plant heights. Even though both plants were capable of acquiring water from deepest soil, the average height of the small three *E. seidelli* (157 cm) was seven times greater than *M. clausenii* (21 cm), indicating plant height was not related to depth of water uptake (δD_{xyl}, Fig. 4d; $R^2 = 0.12$; $F = 1.83$; $p = 0.19$). However, when we explored the quantile regression we showed that plants with contrasting heights (small and tall plants) used deeper soil water (Fig. 4d), while shallow soil was only explored by small plants (below 1 m).

Physiological parameters

The Ψ_{PD} ranged from −0.75 to −0.1 MPa in June, the wettest month, −1.4 to −0.15 in July and −1.6 to −0.15 in August, the driest month. Eight species in this study did not show significant reductions in Ψ_{PD} during the transition to the dry season transition, when soil volumetric water content declined (see Supplementary material S1, S3 and S4). These eight species, with exception of one grass (*Loudetiopsis* sp., upward-pointing grey triangle, Fig. 4a), had xylem water contributions from deep soil water (from 60 to 90 cm depth) (Table 1). There was a positive linear relationship between average δD_{xyl} and $\Delta\Psi_{PD}$ ($R^2 = 0.38$; $F = 8.24$; $p < 0.05$; $y = 0.006\times + 1.0495$). When we removed two species (*Loudetiopsis* sp. and *Vellozia* sp.), evaluated as outliers by theoretical quantiles and Cook's distance inspection (Aguinis et al. 2013), average δD_{xyl} explained 69% of $\Delta\Psi_{PD}$ ($R^2 = 0.69$; $F = 24.88$; $p < 0.0001$; $y = 0.012\times + 1.205$).

A strong positive relationship was observed between averaged δD_{xyl} and coefficient of variation of stomatal conductance (Fig. 4b; $R^2 = 0.78$; $p < 0.0001$; $F = 46.26$; $y = 0.576\times + 73.76$), meaning that shallow-rooted plants have a wider variation in seasonal stomatal conductance than deep-rooted plants (Supplementary material S5). The Ψ_{TLP} of the grasses and the sedge diverged from the woody and rosette species, with substantial variation between individuals, ranging from −1.3 to −2.54 MPa. The *sobole* rooted species C. *pubescens* and rosette *Vellozia* sp. had the lowest Ψ_{TLP} -3.02 ± 0.15 and −3.09 ± 0.39 MPa, respectively. The lignotuber species had intermediate Ψ_{TLP}, 2.04 MPa for *L. warmingianum* and −1.75 MPa for *Mikania* sp. The three deepest-rooted plants *E. seidelli, M. clausenii* and *L. aurea* had less negative Ψ_{TLP} ranging from −1.4 to −1.8 MPa. The Ψ_{TLP} was not related to δD_{xyl} ($R^2 = 0.01$; $F = 0.241$; $p = 0.63$; $y = -0.003\times-2.347$) when we considered grasses and woody species together (Fig. 4c). However, when we considered only woody species, the δD_{xyl} explained 40% of variation in Ψ_{TLP} (Fig. 4c; $R^2 = 0.40$; $F = 6.12$; $p < 0.03$; $y = -0.023\times-3.928$). Assuming that lignotubers may decouple rooting depth from aboveground hydraulic functioning because of their hydraulic trait differences (Küppers et al. 1987),

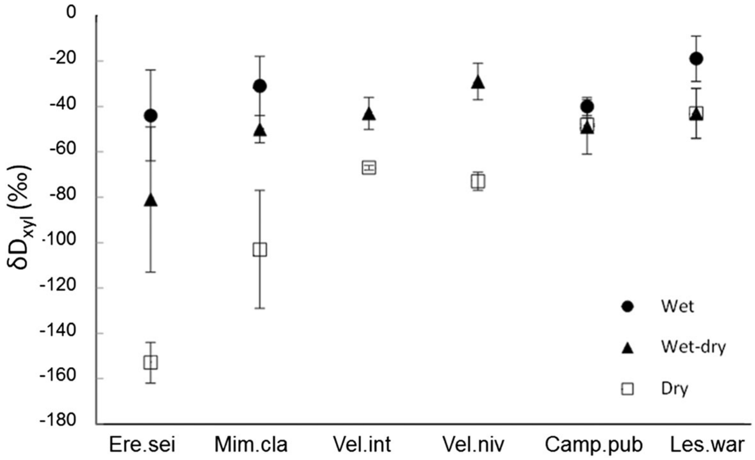

Fig. 3 Seasonal changes in hydrogen isotopic composition (δD_{xyl}) on six species with contrasting root architecture collected during the wet season (circles - November 2011), the transition between wet to dry seasons (triangles - June 2011) and dry season (squares - August 2012) in Serra da Canastra National Park, Brazil. Species are sorted from left to right from deepest to shallowest roots. The species are represented as: Ere.sei, *Eremanthus seidelii* – Mim.cla, *Mimosa clausenii* – Vel.int, *Vellozia intermedia* – Vel.niv, *Vellozia nivea* – Camp.pub, *Campomanesia pubescens* – Les.war, *Lessingianthus warmingianum*. Water samples from Vel.int and Vel.niv were not extracted for the wet season period because we had problems during the cryogenic distillation process

we found that the δD_{xyl} explained 87% of variation in Ψ_{TLP}, when we considered only woody species without lignotubers ($R^2 = 0.87$; $F = 50.81$; $p < 0.0001$; $y = -0.038\times-5.230$).

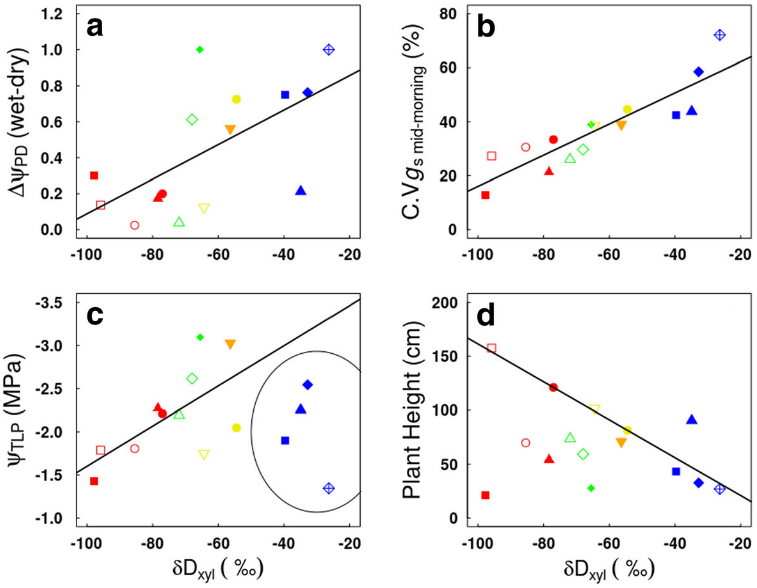

Fig. 4 Relationship between rooting depth inferred by the deuterium isotopic composition (δD_{xyl}) and **a** variation on pre-dawn water potential between wet and dry season ($\Delta\Psi_{PD}$ wet-dry); **b** Coefficient of variation of stomatal conductance (CV $g_{s\ \text{mid-morning}}$); **c** Water potential at turgor loss point (Ψ_{TLP}). **d** Plant height (cm) for a *campos rupestres* plant community at Serra da Canastra National Park. The colors are the most representative roots types found in this survey (Fig. 2; Table 1). The symbols represents the species as described on Table 1. The root type is classified as dimorphic root (*red*), Velloziaceae fasciculate (*green*), lignotuber/xylopodium (*yellow*), *sobole* stem tuber (*orange*) and fasciculate grasses (*blue*). The continuous lines (**a**, **b** and **c**) represent the linear model between each response variable and root depth. Grasses (blue dots inside of semi-circle, Fig. **c**) were not included in the regression analysis (see results). The line on Fig. **d** is the limit relationship from a quantile regression with τ = 0.75

Species were separated in two groups by the Euclidian distance criterion: deep-rooted (continuous line, Fig. 5) and shallow-rooted species (dotted line, Fig. 5), indicating that rooting depth is a key trait defining plant water use strategies. Within the deep-rooted group, *E. seidelli* and *M. clausenii* and the two Velloziaceae species (open diamond and open triangle, Fig. 5) formed two separate groups.

Discussion

We found substantial evidence to support our hypothesis of vertical root partitioning of soil volume based on root architecture and the strong vertical gradient of the δD and $\delta^{18}O$ in the bulk of soil and xylem water during dry season. Additionally, the results support our second hypothesis that rooting depth is strongly related to water stress avoidance mechanisms, such as stomatal regulation and leaf Ψ_{TLP}. Letten et al. (2015) and Silvertown et al. (2015) highlighted the need to identify differences in physiological processes and the diversification of hydraulically related traits that might allow HNS in spatial and temporal scales. Our results demonstrate the functional coordination between belowground water acquisition traits (rooting depth) and aboveground water relations (stomatal control and water potential at leaf turgor loss point) that allow survival, growth and partition of water use in this seasonally dry environment. Taken together, our results provide evidence on the existence of a spectrum of hydraulically related traits that are evolutionary viable in this environment and that might be implicated in the coexistence of a high number of plant species in the *campos rupestres* communities (Araya et al. 2011; Alcantara et al. 2015; Castro et al. 2016; Letten et al. 2015; Silvertown et al. 2015).

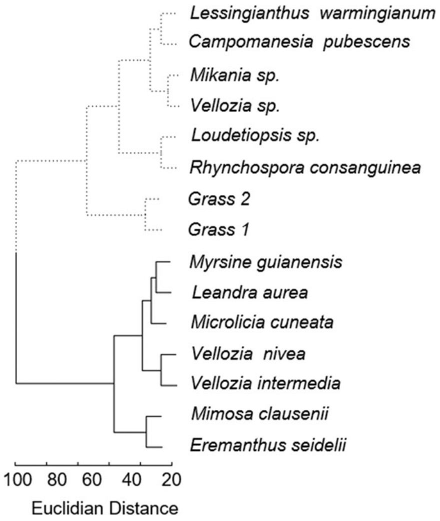

Fig. 5 Hierarchical clustering dendrogram based on Euclidian distance showing the similarities between 15 *campos rupestres* species from Serra da Canastra National Park, Brazil based in five traits related to water-use strategies: δD_{xyl}, $\delta^{18}O_{xyl}$, $\Delta\Psi_{PD}$, CV $g_{\text{mid-morning}}$, Ψ_{TLP}. The continuous line is represented by deepest-rooted species and dashed line by shallowest–rooted plants

Diversity of root morphologies and depth

Several rooting types accessed water in a variety of depths in *campos rupestres* and they were well correlated with δD_{xyl} and δD or $\delta^{18}O$ in the bulk soil (Fig. 1; Fig. S2). This high diversity of underground architectures is expected in water-limited environments (Schenk and Jackson 2002). The ability of plants to grow through the rock fissures enables roots to segregate along quartzite rock outcrops accessing deeper soil layers and spreading horizontally (Poot and Lambers 2003; Schwinning 2010). For example, two woody plants *L. aurea* and *C. pubescens*, were able to adjust their root size and architecture to grow into rock fissures allowing roots to forage for water stored in the rocky matrix (see Oliveira et al. 2016).

One third of the species in our survey showed dimorphic root systems. The δD_{xyl} of dimorphic rooted plants showed 2.6 times less enrichment in D during the dry season (average $\delta D_{xyl} = -86‰$; see Fig. 1 and Fig. 4) in contrast with the fasciculate root type ($\delta D_{xyl} = -33‰$). We do not have δD and $\delta^{18}O$ data for bulk soil water during the rainy season, making the interpretation of changes in δD_{xyl} composition between seasons in dimorphic roots difficult (Fig. 3). However the higher net seasonal changes in δD_{xyl} composition for dimorphic rooted plants (Fig. 3) indicates that this root architecture allows plants to use water from many sources in the soil as observed in other communities (Dawson et al. 2002; Oliveira et al. 2005b; Rossatto et al. 2013).

Grasses with fasciculate roots were hydrologically partitioned from the other study species, with a major proportion of water uptake in the shallowest soils. Our cluster analysis separated the fasciculate root type in all analyses (Figs. 4 and 5), showing the clear functional divergence between grasses and others plants. This is consistent with a recent meta-analysis showing that woody and non-woody plants are functionally contrasting (Díaz et al. 2016). About 80% of worldwide grasses present root systems in the first 30 cm of soil depth (Jackson et al. 1996) and the niche diversification within the grass functional type is explained based on temporal recruitment of niche rather than resource-mediated mechanisms (Schwinning and Kelly 2013). To cope with seasonal water restrictions in the upper soil layers grasses use non-structural carbohydrate reserves as osmotically active molecules to keep physiological activity and tissue turgor under drought conditions (de Souza et al. 2005). Furthermore, some grasses also show C4 metabolism, further facilitating survival in open, hot and dry environments and reduce foliar area during the dry season (de Souza et al. 2005; Garcia et al. 2009; Sarmiento and Monasterio 1983). Leaf senescence during the dry season might explain the lack of correlation between rooting depth of and leaf TLP, suggesting that grasses invest in drought-avoidance instead of tolerance.

We also demonstrated that shallow soil space is partitioned by other woody shallow-rooted species able to coexist with grasses. These species exhibited greater diversity in root architectures, for instance, lignotuber/xylopodium, stem-tuber underground system (*sobole*, underground tree) being furcate along the horizontal surface (Apezzato-da-Glória 2003), and the rosette *Velloziaceae* species with typical monocotyledonous fasciculate roots, which are thicker and deeper than grasses (Fig. S2). In contrast with grasses, these shallow-rooted woody species are evergreen and, therefore, must invest in drought-tolerance traits since they are subject to a high variation on water availability in the upper soil layers (Meinzer et al. 2016).

Soil depth imposes a limit to the investment in plant height in our survey. While shallow soils (not more than 100 cm) only support shorter plants, both short and tall trees explored the deep soil layers. For instance, the tallest treelet *E. seidelli* (about 200 cm) that had access to water stored deeper in the soil and exhibited similar isotopic water source to the shrub *M. clausenii* (about 20 cm height). Plant height or bud height disposition and architecture are usually used for a priori plant categorization in life-forms (growth form), and then used as predictors of the depth of soil water uptake by plants in savannas (Rossatto et al. 2013). Blondel (2003) stressed that many studies rely on intuition to partition a priori species into groups, making it hard to define categories without quantitative methods. Our results show that predicting root depth using growth form defined by plant height can be misleading in *campos rupestres*.

Linking rooting depth with above-ground water relations

Small differences in root architecture can minimize plant competition for water during prolonged droughts (Araya et al. 2011; Silvertown et al. 1999). We demonstrated that rooting depth is a good predictor of leaf water use strategies in a perennial *campos rupestres* community. The acquisition of deep-water sources allowed plants to buffer the effects of seasonal water deficits to the extent that the deep-rooted plants showed less variation in their $\Psi_{PD,}$ a proxy of plant water stress (Fig. 4) (Ivanov et al. 2012; Meinzer et al. 2013; Nepstad et al. 1994; Oliveira et al. 2005a). On the other hand, the shallow-rooted species are subject to a high variation in water availability in the upper soil layers as seen by the larger variation in their Ψ_{PD} (Fig. 4). In those plants, we identify two main drought responses / strategies. The short-term response is related to avoidance strategy (hours to months), where we identified a continuum of stomatal control responses dependent of root depth. The medium term response (months to years) is related to drought resistance expressed in structural reinforcement and osmoregulation and represented by the continuum of variation in TLP (Meinzer et al. 2016; Mitchell et al. 2016). The integration of these short and medium term responses allow partitioning of the shallow soil layers.

The stomatal closure during the dry season represents an important mechanism of short-term regulation of water loss (Fig. 5). Pioneering studies in Cerrado had already suggested that some species have contrasting rates of stomatal conductance between seasons to regulate water loss (Ferri 1944). The water loss control must be unrelated to leaf or branch senescence to avoid the costs of producing new tissues imposed by trade-offs on nutrient economy in the nutrient-impoverished ecosystems, as *campos rupestres* (Abrahão et al. 2014; Oliveira et al. 2015). This study showed that below and aboveground hydraulically related traits divergence allowed specialization along the soil space by showing the presence of hydrological niche axes: at one end, drought avoiders, shallow-rooted plants with higher variation in stomatal conductance between seasons, and at the other end the drought tolerant deeper-rooted plants with lower seasonal variation in g_s.

Campos rupestres plants also exhibit a wide range of variation in drought resistance, as shown by the variation in Ψ_{TLP} between species. Ψ_{TLP} values varied from those found in semi-desert biomes as in shallow-rooted *C. pubescens* and *Vellozia* sp. (−3 MPa) to values close to those observed in tropical wet forests (−1 MPa) (Bartlett et al. 2012). When considering all species, we did not find a correlation between Ψ_{TLP} and rooting depth (Fig. 4). This is expected because several species in this community are drought avoiders and might not invest in costly structural foliar traits that confer resistance (e.g. drought-deciduous grasses). However, when we consider only evergreen woody plants, we found that access to soil water (rooting depth) is a good predictor of Ψ_{TLP} (Fig. 4). More negative Ψ_{TLP} for shallow-rooted plants suggest this to be an important drought tolerance trait for species that are subject to more frequent changes of soil water potential. In contrast, deep-rooted species can avoid water shortage by accessing stored water in deep soil layers and invest their carbon in roots rather than expensive structural reinforcement traits (Bartlett et al. 2012; Mitchell et al. 2016).

We also found contrasting leaf Ψ_{TLP} within woody shallow-rooted species (Fig. 4). Plants with lignotubers showed lower Ψ_{TLP} than *Velloziaceae* group and *C. pubescens* with *sobole* root type. We believe that the lignotuber structure might work as a hydraulic capacitor and buffer the effects of drought and fluctuating water supplies (Lenz et al. 2006; Myers 1995; Oliveira et al. 2014). Even if the main function of lignotubers is still under debate, there is some evidence of carbon source importance to late dry season sprouting (*see* Bond and Midgley 2003). We believe that the soluble sugar content might increase the tissue osmotic potential creating a pressure gradient to hold water molecules and increase water storage. Root relative capacitance tends to be higher in soils with shallower water table (Dietrich et al. 2013). Thus, if lignotubers act as a buffer against water scarcity, we hypothesize that lignotuber plants prioritize investment in well-developed underground structures rather than building robust leaf cell walls (Ding et al. 2014; Lenz et al. 2006). As an alternative hypothesis, if lignotubers have higher tissue capacitance, the δD_{xyl} of this group of plants may not represent the soil depth where these plants are taking up water, but rather represent a mixture of many sources of water (soil plus stored water).

Our results clearly show that we must consider species differences in root architecture when predicting leaf Ψ_{TLP} based on root depth. In fact, there is a higher functional diversity related to leaf Ψ_{TLP} within shallow-rooted plants. The narrower hydrological niche in shallow-rooted plants can cause overlap in water uptake, which may lead to differences on the underlying mechanisms that lead to segregation, e.g. differences in leaf Ψ_{TLP} (Schwinning and Kelly 2013). Despite the peculiarities of each species to cope with water deficits, root depth was a good predictor of Ψ_{TLP} for woody species.

Conclusion

Our results show that rooting depth is a good predictor of seasonal stomatal conductance, variations in predawn leaf water potentials, and drought resistance traits such as leaf turgor loss point for woody plants, evidencing functional integration of below and aboveground hydraulic traits. We demonstrated that leaf hydraulic traits co-vary with rooting depth, forming one axis of trait variation for a broad range of species in a biodiversity hotspot. We believe that this diversity of hydraulic strategies is implicated in the coexistence of an extremely high level of plant biodiversity in the *campos rupestres*. Our results support previous work by Letten et al. (2015), which empirically demonstrated that soil moisture depth emerged as a best predictor of species co-occurrence in a fire-prone, sandstone and low nutrient costal heathland in Australia. We believe our findings clearly illustrate ecological mechanisms driving niche partitioning and its underlying tradeoffs.

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