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RESPONSES OF COASTAL DUNE FORESTS TO GROUNDWATER CHANGES: INSIGHTS FROM MEDITERRANEAN AND TROPICAL ECOSYSTEMS

RESPOSTAS DE FLORESTAS DUNARES COSTEIRAS MEDITERRÂNICAS E TROPICAIS À VARIAÇÃO DO NÍVEL FREÁTICO

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Respostas de florestas dunares costeiras mediterrânicas e tropicais à variação do nível freático

Thesis presented to the Institute of Biology of the University of Campinas in partial fulfillment of the requirements to obtain the degree of Doctor in Ecology.

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Resumo

Em florestas costeiras a água subterrânea é importante para o equilíbrio hídrico do ecossistema. O crescente uso antropogênico e a previsão de períodos de seca aumentarão as taxas de rebaixamento do lençol freático, possivelmente impactando estes ecossistemas nas regiões mediterrânicas e tropicais. As variações espaço-temporais do nível freático podem influenciar processos fisiológicos fundamentais, restringindo o desempenho das plantas e a sua sobrevivência. Neste contexto, o objetivo geral deste estudo foi avaliar as respostas ecofisiológicas de florestas dunares costeiras à variação do lençol freático em condições climáticas contrastantes.

Em duas florestas dunares costeiras mediterrânicas (úmida e semi-árida) e uma tropical (*restinga*) foi modelado o nível freático, quantificada a contribuição de diferentes fontes de água para as plantas através de uma abordagem isotópica, e utilizada uma combinação de índices de reflectância foliar e de parâmetros isotópicos para caracterizar fisiologicamente a vegetação lenhosa dominante. A sua variação foi avaliada sob condições de disponibilidade hídrica contrastantes e ao longo de gradientes espaciais de profundidade do lençol freático.

No *capítulo 2*, mostramos que na região mediterrânea de clima semi-árido os tipos funcionais de plantas coexistentes se segregam ao longo de um amplo espectro de uso de água, quando em condições de seca. Mudanças sazonais relevantes no uso da água em direção a camadas mais profundas do solo ocorreram nos dois climas. No entanto, sob um regime de precipitação tropical, tendo em conta as fontes de água usadas, não encontramos diferenciação entre espécies.

No *capítulo 3*, mostramos que sob um clima mediterrânico e pressão antropogênica, o aumento da profundidade do lençol freático provoca ajustes na tomada de água em direção a camadas mais profundas do solo na estação seca, mas não em todos os tipos de plantas. Em condições semi-áridas, observou-se maior uso de água subterrânea e sua limitação afetou negativamente características relacionadas com capacidade fotossintética e estado hídrico das plantas, independentemente das fontes de água usadas. As espécies mostraram distintos intervalos operacionais, mas uma sensibilidade fisiológica comum à seca hidrológica.

No *capítulo 4*, mostramos que na *restinga*, em condições menos úmidas, a variação topográfica do nível freático influencia a profundidade de tomada de água, mas não influencia as condições fisiológicas da vegetação. A densidade, biomassa e diversidade não influenciaram o desempenho fisiológico das plantas. Comparativamente, a disponibilidade de luz foi um fator importante na variação das características fotossintéticas, particularmente no particionamento de espécies de canópia e sub-bosque.

Interações entre condições climáticas, hidrologia e capacidade de resposta da vegetação determinaram os padrões ecofisiológicos das plantas. Descobrimos que embora o ajuste abaixo do solo à variação do nível do lençol freático seja global, sua contribuição para o estado fisiológico das espécies lenhosas não o é. O impacto das variações do nível freático mostrou-se maior com o aumento da aridez. Em condições semi-áridas, o efeito combinado da escassez de água nas camadas superficiais do solo e de água subterrânea promove o estresse fisiológico e hídrico na comunidade lenhosa. Assim, plantas sob este contexto hidrológico são mais suscetíveis de sofrer com o aumento da profundidade do lençol freático.

Palavras-chave: ecohidrologia; ecofisiologia vegetal; uso de fontes de água; ecossistemas dunares mediterrânicos; floresta de restinga

Abstract

Ecosystems can be profoundly influenced by groundwater. Predicted droughts and anthropogenic groundwater use will increase the rates of water table lowering in both mediterranean and tropical regions. Spatio-temporal variations in water table depth can have a great impact on physiological fundamental processes, constraining plant performance and, ultimately, survival. In coastal dune forests, groundwater can be particularly important for ecosystem water balance and a relevant water source for vegetation. Our understanding of the impacts of groundwater changes on these ecosystems globally is still poor. Thus, the overall aim of this study was to investigate the ecophysiological responses of coastal dune forests to groundwater changes across contrasting climatic conditions.

We modeled water table depth, quantified the contribution of different water sources to plant water through Bayesian isotope mixing models, and used a combination of leaf spectral reflectance and isotope data to characterize ecophysiology of the dominant woody vegetation. Their variation was evaluated under contrasting temporal water availability conditions and along spatial gradients of groundwater table depth, in two mediterranean (humid and semi-arid) and one tropical coastal dune forest (*restinga*).

In *chapter 2* we found that in semi-arid mediterranean coexisting plant functional types segregate along a wide spectrum of contrasting water-source use under extremely dry conditions, evidencing great soil water partitioning. Furthermore, we confirmed that the water-source-use behavior accompany broader strategies of drought resistance. Relevant seasonal water-use shifts towards deeper soil layers occurred across different climates, but in the tropical site we did not found differentiation among the woody species.

In *chapter 3* we found that under a mediterranean climate and antropogenic pressure, increasing depth to groundwater trigger water uptake adjustments towards deeper soil layers only in the dry season, but not in all plant functional types. Moreover, we observed a greater use of groundwater in semi-arid conditions. Under this climatic context, groundwater lowering negatively affects traits related to both photosynthetic capacity and plant water status, regardless plants' water-sources-use strategy. Species showed distinct operating physiological ranges but a common physiological sensitivity to hydrological drought.

In *chapter 4* we found that in *restinga*, under less-wet conditions, topographic variation of water table depth influenced plants' water-uptake depth, but not their physiological conditions. Under the studied conditions, stand density, biomass and diversity had a negligible influence on plants physiological performance. Comparatively, light availability is a major driver of photosynthetic traits' variations, particularly partitioning over and understory species.

We have highlighted the ecophysiological responses of coastal dune woody vegetation to groundwater lowering in different climatic contexts. In these ecosystems, interactions between climatic conditions, hydrology and vegetation response capacity determined plant ecophysiological patterns. We found that while groundwater influence on water-uptake depth is globally prevalent, its contribution to overall physiological status of woody species is not. The physiological impact of increasing depth to groundwater showed to be greater with increasing aridity. Under semi-arid conditions, the combined effect of soil- and ground-water scarcity, promote physiological and water stress in the woody community. Thus, plants under this hydrological context are more susceptible to suffer from water table depletion.

Key-words: ecohydrology; plant ecophysiology; water sources use; humid and semi-arid Mediterranean; *restinga* forest

Sumário

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Chapter 2

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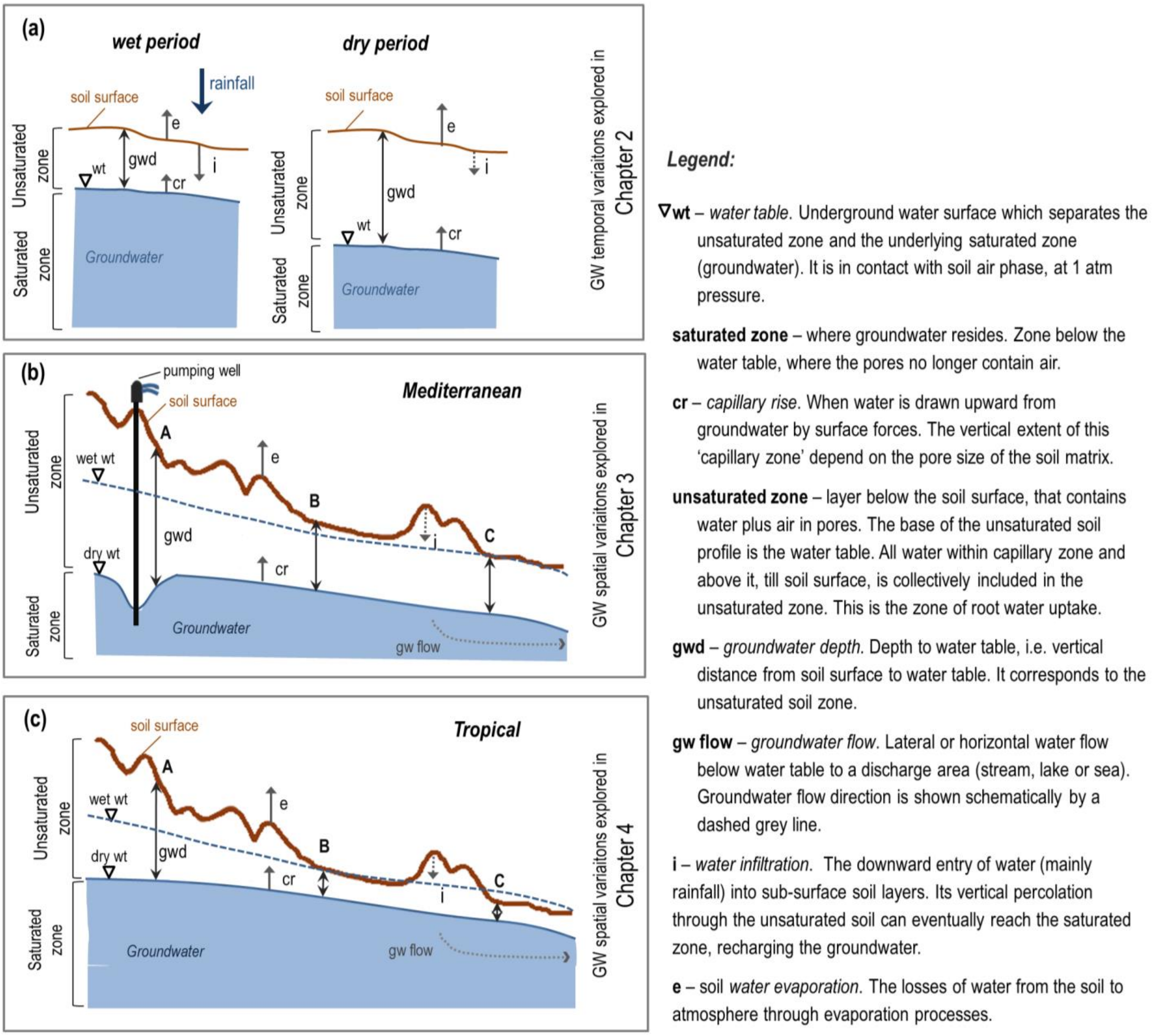
Chapter 1

1 General Introduction

Plants need water to survive, and thus, the distribution, composition and structure of plant communities are directly influenced by spatiotemporal patterns in water availability. The fluctuations of groundwater levels may have different spatiotemporal dynamics than soil moisture but have a comparable importance for ecosystem water budgets and plant growth (Breshears & Barnes 1999; Asbjornsen et al. 2011; Villalobos-Vega et al. 2014). Groundwater can profoundly shape and sustain ecosystems, and its influence is globally prevalent.

As groundwater can serve as an important water resource for woody vegetation, its limitation will directly affect groundwater-dependent plants (Dawson & Pate 1996; Naumburg et al. 2005; Miller et al. 2010; Sommer & Froend 2014; Garcia-Forner et al. 2016; Palacio et al. 2017; Barbeta & Peñuelas 2017). Thus, the intensifying groundwater changes raise concerns particularly in environments with a long dry season, such as mediterranean climate regions (Zencich et al. 2002; Naumburg et al. 2005; Zunzunegui et al. 2011), but also in tropical regions (Saha et al. 2009; Rossatto et al. 2012; Colón-Rivera et al. 2014). Broadly, increasing variability in precipitation can greatly influence groundwater recharge and, under scenarios of future climate change, it is expected an increase in groundwater lowering rates (Williams et al. 2007; Taylor et al. 2012; Kirtman et al. 2013). However, groundwater depth (i.e. distance from surface soil to water-table) forms an environmental gradient not fully captured by the climate (Fan et al. 2013). Additionally, groundwater depth will be affected by edaphic characteristics and the changing patterns of anthropogenic groundwater use, as increasing withdrawal with reduced recharge can significantly decrease groundwater levels (Kopp et al. 2013; Kløve et al. 2014) (Fig. 1).

In seasonally dry environments where groundwater accessibility and soil water scarcity fluctuate sharply, plants may exhibit unique responses to the hydrologic cycle (Naumburg et al. 2005; Newman et al. 2006; Jackson et al. 2009). Also, in more humid environments, where wetlands or saturated soils are predominant, the controls on ecosystem functions are often water table fluctuations (e.g. flooding dynamics) and hydroperiod (Rodriguez-Iturbe et al. 2007). Substantial change in water tables, whether by flooding or drying, can thus drive changes in vegetation composition patterns (see supporting Table S1 for detailed examples). Understanding stand and species contrasting sensitivities to groundwater perturbations, and the ecophysiological processes involved, across hydroclimatic regimes are important challenges.



Legend:

∇wt – *water table.* Underground water surface which separates the unsaturated zone and the underlying saturated zone (groundwater). It is in contact with soil air phase, at 1 atm pressure.

saturated zone – where groundwater resides. Zone below the water table, where the pores no longer contain air.

cr – *capillary rise.* When water is drawn upward from groundwater by surface forces. The vertical extent of this 'capillary zone' depend on the pore size of the soil matrix.

unsaturated zone – layer below the soil surface, that contains water plus air in pores. The base of the unsaturated soil profile is the water table. All water within capillary zone and above it, till soil surface, is collectively included in the unsaturated zone. This is the zone of root water uptake.

gwd – *groundwater depth.* Depth to water table, i.e. vertical distance from soil surface to water table. It corresponds to the unsaturated soil zone.

gw flow – *groundwater flow.* Lateral or horizontal water flow below water table to a discharge area (stream, lake or sea). Groundwater flow direction is shown schematically by a dashed grey line.

i – *water infiltration.* The downward entry of water (mainly rainfall) into sub-surface soil layers. Its vertical percolation through the unsaturated soil can eventually reach the saturated zone, recharging the groundwater.

e – soil *water evaporation.* The losses of water from the soil to atmosphere through evaporation processes.

Figure 1. Cross-sections showing variations in groundwater depth. Definitions of concepts used in this study are presented in the inner legend.
(a) At wetter periods, when rainfall occurs, infiltration of rainwater is an important process of water input in the unsaturated zone of the soil profile. In sandy soils the infiltration rates are higher compared to other soil textures, and water drainage can easily recharge groundwater. Groundwater depth is generally lower compared to dry periods. The gap of dry soil (soil layer between groundwater capillary rise and infiltration

zone) is expected to be narrower than during dry periods. At dry periods, water table levels decline and groundwater depth increases. When precipitation is not available, top wetting is minimal and infiltration processes are negligible. At this dry periods soil water evaporation is high, commonly exceeding infiltration, and moisture tends to move upwards in the sandy soil profile. Accordingly, water upward movement (bottom-up vertical flow), through capillary rise of the groundwater, can be an important process in these dry periods. The soil dry gap is expected to be higher compared to wetter periods. This *temporal* dynamic of groundwater can occur in seasonally dry coastal dune ecosystems in different climatic regions (e.g. tropical and mediterranean), potentially affecting vegetation (explored in Chapter 2). The amount of rainfall and groundwater depth will present different magnitudes (e.g. mean groundwater depth is typically few meters at tropical sites and groundwater depth can reach more than 10 m in mediterranean sites), but the seasonal processes involved are similar.
(b) The groundwater depth varies greatly from place to place, not only due to precipitation regime, but also due to topography and anthropogenic water use. Groundwater pumping alters groundwater recharge rates and can further exacerbate the dry season water table drawdown under a mediterranean climate. This factor and the natural topography imply a *spatial* gradient of groundwater depth under the same climatic pressure (e.g. points A-C). This can have an effect on water-use and physiological performance of coastal dune vegetation (explored in Chapter 3).
(c) Under a wetter climatic regime (as in the tropical region), the groundwater depth will vary in narrower ranges, but topography still allows the existence of a groundwater depth (spatial) gradient (e.g. A-C). The different access to groundwater can have below and above ground implications in vegetation of tropical coastal dune forests (this is explored in Chapter 4).

The impacts of groundwater changes on vegetation will depend not only on temporal and local factors, but also on plant species. For example, water table fluctuations can exert controls on vegetation depending on plant species' tolerance to water shortage conditions (Naumburg et al. 2005). A decline in the water table can lead to increased plant moisture stress, reduced growth and increased mortality if belowground adjustments are not made (Oliveira et al. 2005; Nepstad et al. 1994, 2007; Gessler et al. 2007; West et al. 2012) (Fig. 2). This is true even in typically wet ecosystems, if the groundwater changes are atypical or too rapid. Conversely, excessively high levels of soil water or frequent flooding can lead to reduced transpiration and constrain species performance if plants are not tolerant to low levels of oxygen (Santiago et al. 2004; Jackson & Colmer 2005; Krauss et al. 2007). Interactions between climate, hydrology and vegetation response capacity, will thus define plant community performance and assemblage (Horton et al. 2001; Naumburg et al. 2005; Sommer & Froend 2011; Asbjornsen et al. 2011; Kløve et al. 2014; Silvertown et al. 2015) (Fig. 2). However, similarly to drought strategies, there are costs and limits to the benefits that acclimation processes to groundwater changes can deliver in a given environment (Horton et al. 2001; Zunzunegui et al. 2005; Valladares et al. 2007; McDowell et al. 2008; Hartmann et al. 2018; McDowell et al. 2018).

One of the main processes involved in responses to increasing groundwater depth is belowground adjustments (Naumburg et al. 2005; Palacio et al. 2017; Fan et al. 2017). Studies in temporarily dry ecosystems of different climatic regions have shown that woody species tend to respond to seasonal low water availability by shifting water uptake from shallow water to deep water (Jackson et al. 1999; Hasselquist et al. 2010; Ellsworth & Sternberg 2015; Grossiord et al. 2017). Furthermore, spatial variation in groundwater depth (controlled by local factors, such as topography and human water extraction) was also pointed out as a factor shaping the use of water from deep soil layers (Zencich et al. 2002; Rossatto et al. 2012). Thus, both temporal and spatial changes of groundwater depth can have important implications for plant belowground adjustments and in turn affect plant's growth (Zunzunegui et al. 2002; Nepstad et al. 2007; Barbeta et al. 2015). In fact, ecohydrological factors are one of the most general drivers of niche differentiation for plants and water-uptake depth is an important factor involved in the segregation of hydrological niches, even in ecosystems where water is not limiting (Araya et al. 2011; Silvertown et al. 2015).

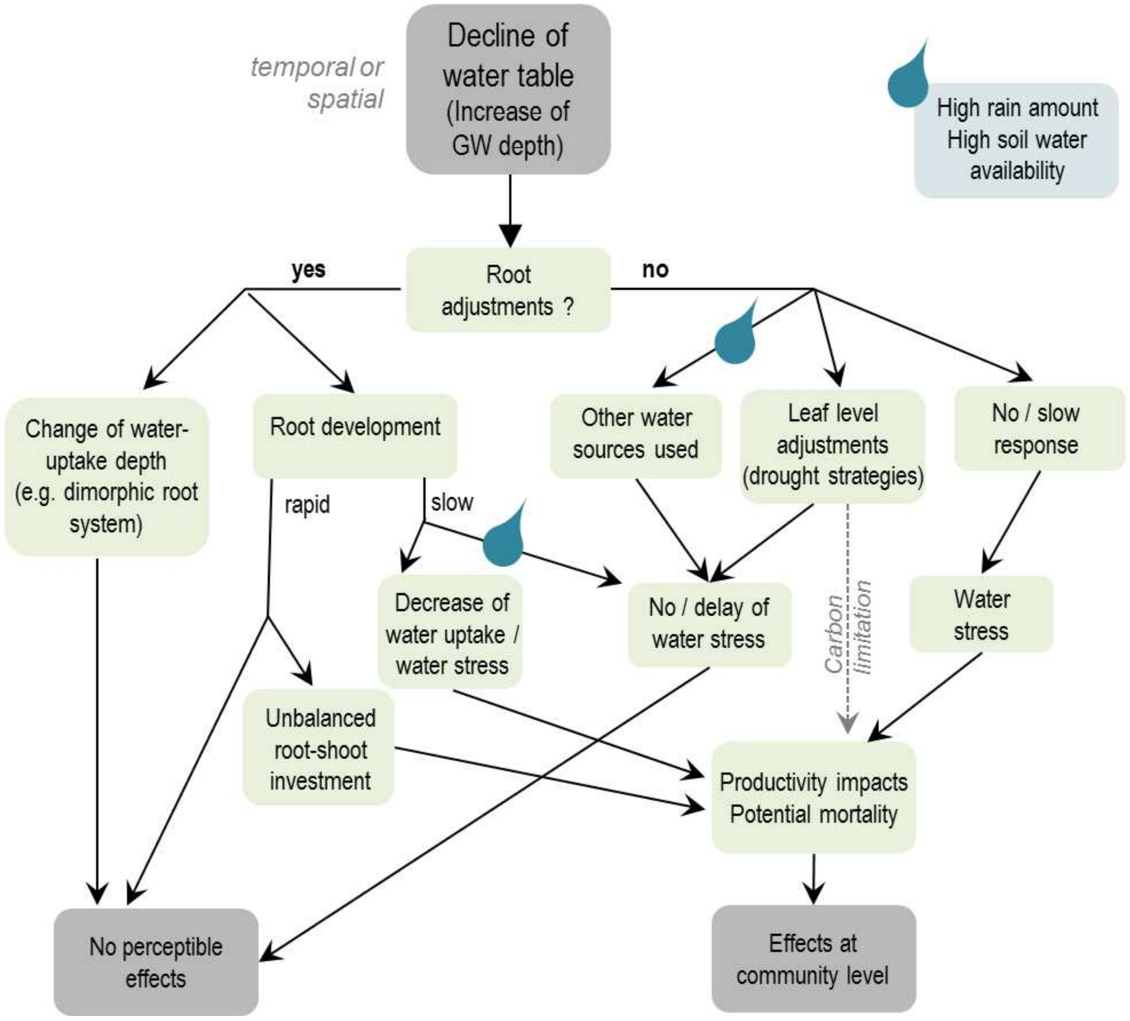


Figure 2. Simplified conceptual model of some possible effects of a dropping water table on a community that was in equilibrium and using groundwater as a significant source of water (adapted from Naumburg et al. 2005). Belowground adjustments (such as root water-uptake depth) and/or aboveground regulation (such as stomatal regulation) to site-specific variations in water-table are expected as a way to buffer or alleviate plant water stress. Under low water availability specific physiological performance and distribution along gradients of groundwater depth will, to a great extent, be determined by plants plasticity in water acquisition, and also tolerance to water shortage. Ecophysiological adjustments thus allow some species to sustain (or increase) resource-use efficiency rates, subsequently maintaining their fitness under varying groundwater conditions. Still, there are some species that are not able to make enough adjustments or that decrease their physiological performance to limiting values, and ultimately face die-back within the community.

Hence, study parameters related to plant water acquisition, such as water uptake depth, are important to better understand water-sources-use and plants' dependency on groundwater. Determining where plants mostly acquire water in contrasting hydrological conditions, for instance through isotopic approaches, may elucidate not only strategies of water-resources acquisition but also soil-water partitioning among species (Filella & Peñuelas 2003; Silvertown 2004; Peñuelas et al. 2011; Silvertown et al. 2015; Valladares et al. 2015).

Moreover, differential water use may depend on plant traits such as rooting depth, leaf habit and growth form (Nippert & Knapp 2007; Moreno-Gutiérrez et al. 2012; Rosado et al. 2016; Barbeta & Peñuelas 2017; Jackson et al. 1999; Hasselquist et al. 2010; Esquivias et al. 2014; Ellsworth & Sternberg 2015; Grossiord et al. 2017). Conversely, a differential reliance on water-sources and specific water-use strategies might lead to distinct ecophysiological responses to fluctuations in groundwater availability. As responses of woody species to extreme climatic events (e.g. droughts) can be strongly related to their functional traits (Lloret et al. 2016), their responses to hydrological changes might be related as well. In other words, the responses to groundwater depth may differ between plant functional types. For example, some plants may show important shifts in water-uptake depth and others may show variations in carbon acquisition. The function, performance and, ultimately, survival of woody species within a community will depend significantly on water availability, their ability to use the available water and their overall water strategies (McDowell et al. 2008; West et al. 2012; Skelton et al. 2015; Pivovaroff et al. 2016).

In coastal dune ecosystems, where there is little water retention during dry periods, deep soil water sources, as groundwater, can be a particularly important water source for vegetation (Zencich et al. 2002; Alessio et al. 2004). In sandy soils, deep infiltration, soil and groundwater recharge, rapid depletion and groundwater capillary rise are important processes in soil moisture variation (Wilcox 2010; Fan et al. 2017). Common characteristics can be shared by forests of seasonally dry coastal stabilized dunes across climatic regions: sandy poor soils, presence of both trees and shrub species, a relative shallow water table and noticeable variations on groundwater level (harboring temporarily flooded habitats as temporary ponds or dune slacks) (Zunzunegui et al. 1998; Díaz Barradas et al. 1999; Zunzunegui et al. 2005; Assis et al. 2011; Máguas et al. 2011; Joly et al. 2012; Marques et al. 2015). However, hydrological differences may occur due to their different precipitation regime. In contrast to humid lands, relatively high proportions of evaporation with respect to precipitation characterize the water balance of dryland ecosystems, such that losses to subsurface flow and soil moisture storage are typically lower than in more humid sites (Wilcox et al. 2006). Nevertheless, even at different ranges, both temporal and spatial variations in groundwater can greatly alter water balance of coastal dune forests in different climatic regions (Fig. 1). Thus, these are particular relevant ecosystems to study in the context of groundwater changes. Furthermore, knowledge about whether and to what extent groundwater affects the woody vegetation in these ecosystems globally is lacking. Although through different mechanisms, similar ecohydrological implications for vegetation functioning can occur. For example, in tropical sites where generally high water availability sustain high levels of diversity and biomass, small changes in the water-resources may indirectly have a great influence on plants water status through competition pressure. On the other hand, in water-limited ecosystems a more direct effect of groundwater limitation on plants performance is expected. Nevertheless, in both cases interactions between vegetation and the hydrologic cycle can contribute to alterations in plants' physiological conditions, but the underlying causes can differ. There is a need for deeper mechanistic understanding between what drives plant overall performance (water, carbon and nutrients uptake), and how it might be linked to hydrological temporal and spatial patterns across different climatic regions.

Community-level assessments of water and carbon acquisition patterns are becoming more frequent (West et al. 2012; Skelton et al. 2015; Lloret et al. 2016; Pivovaroff et al. 2016; Martínez-Vilalta & Garcia-Forner 2017), but we still do not know enough about their relationships with particular hydrological gradients. Additionally, using a set of ecophysiological responses would eventual help to better define a more integrated trait syndrome that would characterize general water use, hydrological drought resistance strategies and vulnerability of plants to under-going groundwater changes. Through the evaluation of physiological and water-sources-use adjustments of plants, we can potentially identify mechanisms underlying the hydrological impacts that threaten the persistence of ecosystems in their current structures and compositions. The quantification of the relative contribution of groundwater to plant water status and to their overall physiology, under contrasting water availability conditions and along spatial gradients of water-table depth, can contribute to disentangle to what extent this water factor influences and constrains overall physiology of vegetation.

Thus, the overall *aim* of this study was to investigate the ecophysiological impacts of groundwater changes in coastal dune forests. The study would fulfill the gaps of knowledge about woody species' water-source-use and physiological responses to groundwater depth under contrasting climatic conditions (Fig. 1). We specifically explored:

(i) The water-sources-use of the woody community under contrasting temporal water availability conditions (Chapter 2) in two different hydroclimatic sites: mediterranean (Chapter 2.1) and tropical (Chapter 2.2). Moreover, the extent of differentiation in the main sources of water used among woody species was also evaluated.

(ii) Ecophysiological responses of different plant functional types along a groundwater depth gradient, in two mediterranean coastal dune forests under anthropogenic groundwater drawdown. We explored the impacts of groundwater lowering on below- and above-ground adjustments of woody species in two seasonally dry ecosystems differing in aridity (Chapter 3).

(iii) The relative importance of groundwater depth in shaping the physiological performance of woody vegetation in a tropical dune forest under a less-wet period. We evaluated whether and how plants' physiological condition and water-uptake depth change along a groundwater depth gradient (Chapter 4).

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Table S1. Some studies developed in different habitats considering groundwater (GW) changes, vegetation composition responses and the processes involved. – Continue in next page

Habitat	Local	Communities	Species number	Assessment time	Cause of GW changes	Vegetation pattern driven by GW	Processes involved	Reference
Peatland – forest complex	British Columbia	Tsuga–Rhytidiadelphus; Sphagnum ; Pinus–Sphagnum woodland	99	1 season in 2 years	Climatic	Highest level of GW table helped distinguish the upland Tsuga–Rhytidiadelphus forest from all other communities.	Species capacity (root system) to deal with GW related factors: pH and dissolved oxygen	Asada et al. 2003
River bank	China	riparian vegetation	14	2 years	Climatic	Plant species became less diverse, the structure of plant communities became simpler, and the diversity and abundance indices of the plant species decreased with declining ground-water level	Salt- and drought-tolerance; regenerating capability; reproduction strategies; root system	Chen et al. 2006; Kong et al., 2009
Peatland	Netherlands	Grassland; aquatic and bank vegetation	122 (32 terrestial; 90 aquatic)	3 years	Anthropogenic	Increasing terrestrial species richness with rising GW and decreasing fluctuations	Bank vegetation responded to K + concentration, and winter and summer GW levels; aquatic vegetation to pH, NH4 and water temperature	Best et al. 1995
Wetland forest	Iberian Peninsula	Alnus and Salix forests	2	1 season in 2 years	Climatic	Increased site saturation favored a shift in species dominance from Alnus to Salix and resulted in a higher degree of multi-stemmed tree architecture; Overall site productivity decreased with prolonged saturation.	Increased sprouting; anaerobiosis in the rooting zone; Competition (Alnus seems to be a good competitor in well-aerated soils); nutrient limitation.	Rodríguez-González et al., 2010
Coastal wet dune slacks	Netherlands	herbaceous (angiosperms) plant communities	5	3 years	Anthropogenic	In permanent waterlogged conditions the biomass of wetland species was highest. Although fluctuating water levels had led to lower total biomass production, germination and seedling growth show a positive effect:	Ecological preference; recruitment requirements also pose a limitation to the distribution	Bakker et al., 2007
Wet meadow and river foreland	Poland	wet meadows and frequently submerged(/flooded river foreland) grasslands	19	6 weeks	Experimental	River forelands were classified as tolerant to complete submergence (~100% of survival after long submerged period), whereas species from wet meadows showed relatively intolerant, intermediate or tolerant responses. Meadow species showed lower production of biomass of leaves, stems, fine roots and taproots, compared with floodplain species.	Species traits that enable them to cope with oxygen limitation in the root zone and with unfavorable biogeochemical reduction processes. However, deeper flooding seems to require solutions at the whole plant level, which they do not have (eg, shoot elongation).	Banach et al., 2009

(cont. Table S1) Habitat	Local	Communities	Species number	Assesment time	Cause of GW changes	Vegetation pattern driven by GW	Processes involved	Reference
River bank and fluvial plain	China	Xero, meso and hydrophytic trees, bushes and herbs (6 community types)	29	3 years	Climatic	Community diversity and richness declined (bimodal pattern) with increasing GW depth and salinity; but eveness reach a lowest value in intermedium GW depth and salinity.	Deep roots as an advantage of perennial herbs over all other groups; Intolerance of GDV species to GW depth (GWD), salinity (SAL) and total dissolved solids (TDS), HCO3 , Ca2+, pH, and SO4 2. Tree species are capable of dealing with large values of GWD and low values of SAL and TDS, and shrubs species are more adapted to low GWD and high SAL and TDS. The perfect combination of GWD and SAL for GDV species diversity is 2m and 1,8 gl-1.	Zhu et al., 2012
Peatland (bog and forest)	Canada	Bog and forest communities	5 (dominant)	1 season assesment but long-term responses (86 years)	Anthropogenic	Bulk density increased with water table decline and more strongly fluctuating GW; Enhanced tree growth observed with drainage.	Hydraulic gradients and flow patterns have changed due to increased evapotranspiration and interception and positive feedbacks occur throughout the years.	Talbot et al., 2010; Kopp et al., 2013
Forest	Germany	Scots pine forest	-	Simulation (based in 20 years GW Mean)	Climatic	With increasing GW depth there is a decrease in species diversity and productivity, but higher landscape patchiness	Drought tolerance: shift in the natural species composition towards more drought tolerant species.	Lasch et al., 2002
Dune ponds	Spain	dune ponds shrub and herbaceous communities	10	8 years	Anthropogenic and Climatic	With decreasing GW and during the dry period hygrophytic species showed regression, with high mortality of some species, while the xerophytic species advanced to lower areas.	Hydraulic adaptations and drought intolerance; scrub species with fast response growth	Zunzunegui et al., 1998
Fire-protected savannas	Brazil	cerrado communities (herbaceous, shrubs and trees)	55	16 months	Climatic	Low minimum distance between the soil surface and water table depth (reached during the wet season) and the relatively large fluctuations in GW limit tree density and diversity at low elevations.	Species responsive capacity: savanna trees cannot cope with extended waterlogging during the wet season and with low soil water availability during the dry season*	Villalobos-Veja et al., 2014; *but see Silva, 2015
Coastal sandy plain	Australia	Hydro, Meso and xerophytic communities	30	35 years	Climatic and Anthropogenic	The change in composition was primarily manifested as a shift towards non-woody, shallow-rooted species not dependent on specific hydrological conditions. The ranges of GW depths at which individual states occur suggest that as GW depth increases, the degree of habitat specificity decreases	Drought tolerance and root system; Shifting communities from hydrophytic to xerophytic dependent on adaptation traits. Specific GW level defines non-linear shifts in communities.	Sommer and Froend 2011; Sommer and Froend 2014

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Chapter 2

Seasonal water-sources-use of woody species in coastal dune forests

2.1 Water source partitioning among plant functional types in a semi-arid dune ecosystem

Antunes C., Díaz Barradas M.C., Zunzunegui M., Vieira S., Máguas C. (2018) Water source partitioning among plant functional types in a semi-arid dune ecosystem. *Journal of Vegetation Science*. 0, 1-13. http://dx.doi.org/10.1111/jvs.12647 .

2.2 Water sources used by woody species under contrasting water availability in a tropical coastal dune forest

2.1 Water source partitioning among plant functional types in a semi-arid dune ecosystem

Antunes C., Díaz Barradas M.C., Zunzunegui M., Vieira S., Máguas C. (2018) Water source partitioning among plant functional types in a semi-arid dune ecosystem. *Journal of Vegetation Science*. 0, 1-13. http://dx.doi.org/10.1111/jvs.12647

2.1.1 Abstract

Question(s): The aim of this work was to characterize the main water sources used by the woody plant community of a semi-arid coastal dune ecosystem. We ask: Do the seasonal water sources used by plants differ between functional types? Does the seasonal drought promote shifts towards deeper soil layers in all plants? Does greater use of deep soil water enables the maintenance of a more favorable plant water status? Do water-sources-use strategies mirror overall drought strategies?

Location: Semi-arid coastal dune system in Doñana Biological Reserve, southwest Spain.

Methods: We analyzed the oxygen isotope composition of xylem water of fourteen coexisting woody species and compared it to water sources (soil water at different depths, rain and groundwater), both in spring and dry summer. Bayesian isotope mixing models were used to estimate the proportion of each water source used by plants. We tested the influence of different (categorical) functional traits on plants' water-source-use. We evaluated the relationships between the use of deep soil water and ecophysiological parameters related to water economy (leaf δ^{13}C and reflectance water-index).

Results: A greater similarity between species was found in periods of higher water availability (spring). Contrastingly, during the dry period the functional traits considered showed a significant effect on the water sources used and a high inter-specific differentiation was observed. Accordingly, species clustered in five water-use functional groups, exploring water from shallower to deeper soil layers. Greater use of deep soil layers in the dry season was linked to a better seasonal maintenance of plant water status.

Conclusions: Coexisting plant functional types segregated along a spectrum of water-source-use under extremely dry conditions, evidencing great soil water partitioning. Relevant seasonal water-use shifts towards deeper soil layers were observed, but not in all functional types. Furthermore, we confirmed that the water-source-use behavior accompanied broader strategies of drought resistance.

Key-words: soil water partitioning; water sources segregation; coexistence; groundwater use; Mediterranean; functional groups; sandy coastal ecosystem; dry conditions; stable isotope mixing model; water-use shifts; drought

2.1.2 Introduction

The function, performance and, ultimately, survival of plant species living in dry ecosystems depend significantly on water availability as well as on the ability to use the available water (Austin et al. 2004; Grossiord et al. 2017; Palacio et al. 2017). In semi-arid systems, interspecific (belowground) competition for water defines plant communities (Fowler 1986; Eissenstat & Caldwell 1988; Casper & Jackson 1997; Schenk & Jackson 2002). Nevertheless, different species can coexist by using different ranges and proportions of resources (Silvertown et al. 1999; Filella & Peñuelas 2003; Silvertown et al. 2015; Valladares et al. 2015; Palacio et al. 2017). Hence, temporal and spatial (vertical) water partition is expected to represent an important process in these seasonal dry ecosystems. This is especially relevant in sandy soils with low water retention (Veneklaas & Poot 2003; Alessio et al. 2004). When exposed to an extreme decrease in soil water availability and in the absence of rain, dune woody plants will need to rely on another water source and/or readjust root system and water uptake to new water levels. In fact, under drought, water adjustments can and must be made, otherwise the impacts will be detected in plant performance and eventually in plant dieback (Nepstad et al. 2007; Padilla & Pugmaire 2007; Allen et al. 2010; Barbeta et al. 2015).

There is evidence of water sources use segregation between growth forms in several environments (Silvertown et al. 1999, 2015; Nippert & Knapp 2007; Barbeta & Peñuelas 2017). In fact, an important differentiation in Mediterranean communities is established between tree species with deep roots, with more constant water and nutrient resources, and shrubby species with shallow roots, which use episodic rainwater and associated nutrient uptake (Moreno-Gutiérrez et al. 2012). Water-use under drought will determine the degree to which a plant experiences a given hydric variation, with deep-rooted species being possibly buffered from drought to a greater extent than shallow-rooted species (Nepstad et al. 1994, 2007; Oliveira et al. 2005; West et al. 2012). Besides growth form, other key functional traits can play a role in water-sources-use patterns (Leng et al. 2013; Barbeta & Peñuelas 2017). Leaf phenology is one of them. Studies in Mediterranean scrub, desert savannas, and tropical dry forests have shown that evergreen species shift water uptake from shallow water to deep water in the dry season (Jackson et al. 1999; Hasselquist et al. 2010; Esquivias et al. 2014; Ellsworth & Sternberg 2015; Grossiord et al. 2017). As an adaptation for water absorption and for protection against water deficit, leaf shape can also affect water-use patterns of plants (Wright et al. 2006; Antunes et al. 2018). Additionally, an important functional syndrome related with water-use is plant water requirement. Species can explore different niches according to their relative requirement of soil moisture; hygrophytes will grow in a moist habitat and xerophytes are adapted to survive in an environment with little water (Ackerly 2004). This classification has been pointed out as key in differentiating semi-arid Mediterranean coastal dune plants' physiological patterns (Zunzunegui et al. 2005, 2009, 2011).

Although the basis for niche segregation by rooting depth in plant communities have been studied (Silvertown et al. 1999; Filella & Peñuelas 2003; Araya et al. 2011; Peñuelas et al. 2011; Silvertown et al. 2015; Palacio et al. 2017) there are water-source-use differences between plants that still have been overlooked. Attempts to understand mechanisms underlying plant responses to drought have led to the emergence of distinct hydraulic and water strategies among coexisting species (McDowell et al. 2008; West et al. 2012; Skelton et al. 2015; Pivovaroff et al. 2016). However, when exploring plant water strategies, specific quantification of water sources used by plants is not usually used. This is an important piece of the puzzle since it can be linked to the ability to explore different water sources (and in some cases buffering the drought effect). Furthermore, it can clarify soil water partitioning (i.e. differentiation of water-uptake depth) among species. Because partitioning of water based solely on differences in rooting depth does not explain patterns of water uptake for many species (Jackson et al. 1999; Oliveira et al. 2005; Nippert & Knapp 2007; Ellsworth & Sternberg 2015), quantifying the relative contribution of different water sources to the composition of the xylem water is utmost relevant. One valuable approach to do so is through Bayesian stable isotope mixing models (Parnell et al. 2013; Stock et al. 2016; Palacio et al. 2017).

The aim of this work was to characterize the woody community seasonal water source use and enlighten the relevance of segregation in the sources of water used by woody species in a semi-arid coastal dune ecosystem. Through an isotopic approach, and considering fourteen species of widely contrasting functional traits, we explored: (i) which are the main water sources used by plants under contrasting water availability conditions, (ii) the influence of functional traits on patterns of water sources use, (iii) the seasonal water-source-use variation and possible shifts towards deeper soil layers in drier conditions, (iv) the effect of water-sources used on plant water status, and (v) the link between water-use functional groups and plants' drought strategies.

2.1.3 Methods

Study site and plant species

The study was conducted at a semi-arid Mediterranean coastal dune ecosystem at Biological Reserve of Doñana, southwest of Spain (Aridity index=0.47) (UNEP 1992; http://www.cgiar-csi.org/data/global-aridity-and-pet-database). It showed a typical western Mediterranean climate, with a total annual rainfall (August 2012 – August 2013) of 566 mm (weather station at RBD: 36°59'19"N; 6°26'35"W) (see Appendix S1). During the dry season, the well-drained sand, underlying the sandy plant community in Doñana scrub, exacerbates soil drying and water limitation. Soil water content, measured at 10 cm depth, was reduced by 88% from spring to summer, reaching very low values in the dry season (see Appendix S1).

The sand mantle is mostly covered by Mediterranean scrub vegetation with local patches of the original Juniperus and Quercus woodlands (Muñoz-Reinoso & García Novo 2005). To represent the dominant vegetation of the studied sandy ecosystem, we selected fourteen woody plant species, with contrasting functional traits. The categorical functional traits considered were: growth form (GF), water requirements (WR), leaf longevity (LL) and leaf form (LF) (Table 1). The following classes within each trait were considered: GF – shrub or tree; WR – xerophytic, mesophytic or hygrophytic; LL – summer semi-deciduous, evergreen, aphyllous; LF – narrow, broadleaf, needle, scale, spike/aphyllous. The sampled species and their functional classification (based on Díaz Barradas et al. 1999; Zunzunegui et al. 2005, 2011; Castroviejo 2012) are described in Table 1.

Table 1. Species' name, family, code, number of sampled individuals in each sampling date (*n*) and traits considered: growth form (GF), water requirements (WR), leaf longevity (LL) and leaf form (LF). Classes considered were: GF – shrub (shrub) or tree (tree); WR – xerophytic (xero), mesophytic (meso) or hygrophytic (hygr); LL – summer semi-deciduous (semd), evergreen (ever), aphyllous (aphy); LF – narrow (narr), broadleaf (brle), needle (need), scale (scal), spike/aphyllous (aphy). Species classification based on Díaz Barradas et al. (1999), Muñoz-Reinoso & García Novo (2005), Zunzunegui et al. (2005, 2011) and Castroviejo (2012).

Species	Family	code	GF	WR	LL	LF	*n*
Cistus libanotis	Cistaceae	Cl	shru	xero	semd	narr	3
Cistus salviifolius	Cistaceae	Cs	shru	hygr	semd	brle	3
Corema album	Ericacea	Ca	shru	xero	ever	narr	8
Erica scoparia	Ericacea	Es	shru	hygr	ever	narr	33
Halimium calycinum	Cistaceae	Hc	shru	xero	semd	narr	14
Halimium halimifolium	Cistaceae	Hh	shru	meso	semd	brle	42
Juniperus phoenicea	Cupressaceae	Jp	tree	xero	ever	scal	24
Lavandula stoechas	Lamiaceae	Ls	shru	xero	semd	brle	6
Phillyrea angustifolia	Oleaceae	Pa	shru	hygr	ever	brle	4
Pinus pinea	Pinaceae	Pp	tree	xero	ever	need	23
Quercus suber	Fagaceae	Qs	tree	hygr	ever	brle	5
Rosmarinus officinalis	Lamiaceae	Ro	shru	xero	semd	narr	9
Stauracanthus genistoides	Fabaceae	Sg	shru	xero	aphy	aphy	3
Ulex australis	Fabaceae	Ua	shru	xero	aphy	aphy	3

To better characterize the woody community at landscape level, and encompass eventual local heterogeneity, we consider a study area covering the prevalent dune formations (of ~7 km2). Eighteen sampling plots (20 m x 20 m), separated by at least 200 m, were distributed throughout the study area. In each plot, we sampled 3 to 4 species, belonging to contrasting functional types. We assured that, within each plot, species of: both GF classes, at least two WR classes (except in one plot), at least two LL classes, and at least two LF classes were sampled (see Appendix S2).

When possible, we sampled three individuals per species within plot (replicates), resulting in a total of 180 (adult) individuals sampled in each sampling date (Table 1, Appendix S2).

Isotope composition of xylem water and water sources ($\delta^{18}O$)

To access the water being used by plants, lignified woody stems of each plant individual selected were collected in two contrasting water conditions: spring (humid) and summer (dry) (Appendix S1).

To determine available water sources for the roots, water samples were collected from soil, rain and groundwater. Rainwater was sampled on one pluviometer equipped with liquid paraffin to prevent evaporation (at 36°59'19"N, 6°26'35"W). We considered the rainwater collected over the 2 weeks prior to stem sampling. Groundwater samples were collected in piezometers or wells reaching the water table on the two periods of stem sampling. Mean depth to water table was 2.8 and 3.9 m in spring and summer sampling dates, respectively. Soil samples were collected at 3 depths: 10 cm, 30 cm and 50 cm in each sampling plot with 3 replicates per depth, on the same days of stem sampling. Having in mind the extreme conditions of Mediterranean dune sites, especially in summer, soil and twig samples were immediately stored in airtight vials, sealed with a cap and parafilm and placed in a cool ice chest until they could be stored. All samples were kept in the fridge until isotopic analysis.

Water from xylem and soil samples was extracted using a custom-made cryogenic vacuum distillation system housed at SIIAF (Ehleringer & Dawson 1992; West et al. 2007). All water samples were kept at 4°C until isotopic analysis. When the isotopic fraction of the available water sources (soil water, rain, and groundwater) is known, an analysis of the oxygen isotopic composition of xylem water provides information about the water sources being used by the plant at the time of study (Ehleringer & Dawson 1992; Dawson 1993; Dawson & Pate 1996). We used stem water $\delta^{18}O$ values (rather than δD) as a measure of plant water sources because xerophytic plants might be able to discriminate against deuterium during soil water uptake by roots (Ellsworth & Williams 2007). Oxygen stable isotope ratio analyses were performed by headspace equilibration, on an Isoprime (Micromass, UK) SIRMS, coupled on continuous flow mode to a Multiflow (Micromass, UK) auto-sampler and sample equilibration system. Analytical uncertainty is <0.1‰.

Soil water at 10, 30 and 50 cm depth and groundwater are from now on named SW10, SW30, SW50 and GW respectively.

Quantification of water sources used by plants

The relative contribution of different water sources to the composition of the xylem water was estimated by Bayesian stable isotope mixing models using the graphical user interface and model framework MixSIAR for R (Parnell et al. 2013; Stock et al. 2016). In our study, the model used as input the individual isotope values of xylem water $\delta^{18}O$ of each plant ('mixture' raw data), all sources described in water sources (mean plus standard deviation) and a matrix for potential fractionation (set to 0). We calculated the water use proportion in the two seasons and in each plot separately, using an individual-based model to obtain separate estimates for each of the plants. We ran models using the individual plant ('ID' within the 'plot') values of xylem water $\delta^{18}O$, the sampling plot water sources mean (plus standard deviation) and the 'process only (n=1)' error structure option. We set the Markov Chain Monte Carlo to 'long' or 'very long' model run, until the diagnostics were satisfying. Spring precipitation was (prior) aggregated with SW10 because their isotopic composition was similar. Thus, spring mixing model ran with only 4 sources (Precipitation+SW10, SW30, SW50 and GW). Summer mixing model ran with the 4 sources sampled (SW10, SW30, SW50 and GW), since it didn't rain in the two weeks prior to stem sampling (see Appendix S3). Details on extremely enriched xylem water samples are given in Appendix S4.

Physiological parameters

The following ecophysiologcal traits were measured in all plants (n=180) (Table 1) in both spring and summer sampling dates (April and September respectively; Appendix S1).

Leaf carbon isotope composition ($\delta^{13}C$)

Leaf $\delta^{13}C$ is a good proxy of leaf-level intrinsic water use efficiency, which is given by the ratio between leaf net photosynthetic rate and stomatal conductance (Farquhar et al. 1989; Dawson et al. 2002). Mature leaves (4-10, depending on leaf size) were collected from each plant. The bulk leaf samples were dried at 60ºC for at least 48 h, and milled to fine powder in a ball mill (Retsch MM 2000, Germany) for isotopic analysis. Leaf $\delta^{13}C$ was determined by continuous flow isotope ratio mass spectrometry (CF-IRMS) on a Sercon Hydra 20-22 (Sercon, UK) stable isotope ratio mass spectrometer, coupled to a EuroEA (EuroVector, Italy) elemental analyser. Uncertainty of the isotope ratio analysis, calculated using values from 6 to 9 replicates of secondary isotopic reference material interspersed among samples in every batch analysis, was $\leqslant$ 0.1‰.

Reflectance water index

We used a nondestructive optical method, based on the reflectance of light by an intact leaf. Spectral reflectance was measured using a UniSpec Spectral Analysis System (PP Systems, Haverhill, Massachusetts, USA) and carried out in 6 full expanded leaves per plant. Plant water index (WI) was calculated per leaf as WI = R900/R970, where R900 and R970 are the reflectance at wavelengths of 900 nm and 970 nm (Peñuelas et al. 1997). A mean value of the 6 measurements per plant was considered. This index is highly related with plant water content, so it can be used as a proxy for water status in the plant (Claudio et al. 2006).

Additionally, the plant water content (PWC) was calculated as:
PWC(%) = -620+684*WI (following Peñuelas et al. (1997)).

Statistical analysis

To explore the influence of different traits on the patterns of plants' water-source use, firstly we performed Principal Component Analyses (PCA) based on all individuals information (n=180), using (i) the relative contribution of the water sources to xylem water per season, and (ii) the seasonal differences of the relative contribution of the water sources (spring to summer variation, i.e. summer minus spring values) (see Appendix S5). Positive values represent an increase of the proportions of water-source use from spring to summer, and negative values a decrease from spring to summer. Secondly, we tested if these water-source-use patterns were dependent on growth form (GF), water requirements (WR), leaf longevity (LL) or leaf form (LF). For that, based on the individual scores of PCAs' first axis (PC1), we performed an analysis of variance (factorial ANOVA) to test the main effects of the four categorical functional traits (GF, WR, LL and LF) on: (i) the water used by plants in spring (PC1spring~GF+WR+LL+LF), (ii) the water used by plants in summer (PC1summer~GF+WR+LL+LF), and (iii) the water-sources-use seasonal variation (summer minus spring) (PC1diff~GF+WR+LL+LF). Lastly, we explored the differences of water-sources used between plant functional groups. To do so, we used *a priori* functional classification for each plant as the concatenation of the four trait variables (GF&WR&LL&LS). We performed Tukey HSD post-hoc multiple comparisons of means to test differences in water-sources-use between the *a priori* functional groups (PC1~FG4traits; with PC1spring, PC1summer and PC1diff as dependent variables). Then, we used the grouping resulting from the post-hoc information to define the new (*a posteriori*) functional groups of water-source-use.

We further tested the relationships between the proportions of use of deep soil water (i.e. the contribution of water from deeper than 0.5 m soil layers to xylem water, i.e. % of GW used by plants) and the physiological parameters (leaf $\delta^{13}C$ and WI in spring and summer, and seasonal variation of leaf $\delta^{13}C$ and PWC) through Pearson correlations (for the community and per a posteriori functional group). We also explored the differences in the physiological parameters (leaf $\delta^{13}C$ and WI) and in the seasonal variation of leaf $\delta^{13}C$ and PWC among the functional groups by performing an analysis of variance (ANOVA) and post-hoc Tukey HSD. Preliminary analyses were performed to ensure that there was no violation of statistical assumptions.

All statistical analyses were performed in R 3.2.3 (R Core Team, 2015).

2.1.4 Results

A greater similarity between species' water-sources-use was found in periods of higher water availability (spring). In this season, we only found a significant effect of growth form on plants' water source usage (F = 173.63, p<0.0001, Table 2).

Table 2. Results of the variance analysis (ANOVA) conducted to test the effect of growth form (GF), water requirement trait (WR), leaf longevity (LL) and leaf form (LF) on spring water source use (Spring WU), summer water source use (Summer WU) and seasonal variation of water source use (Seasonal variation WU). Bold represents significant effects (p<0.01).

		Spring WU			Summer WU			Seasonal WU variation		
Effect	df	*SS*	*F*	*p*	*SS*	*F*	*p*	*SS*	*F*	*p*
GF	1	215.55	173.63	**<2e-16**	66.68	44.50	**3.4e-10**	26.03	15.17	**1.4e-4**
WR	2	2.85	1.15	0.32	76.85	25.65	**1.8e-10**	38.37	11.18	**2.7e-5**
LL	2	6.96	2.80	0.06	27.68	9.24	**1.5e-4**	16.81	4.90	**0.009**
LF	3	8.01	2.15	0.10	29.57	6.58	**3.1e-4**	21.40	4.16	**0.007**

The composition of xylem water of shrubs was better explained by precipitation mostly present at top-soil (SW10), while water used by trees was mostly from soil layers between 30 and 50 cm (Fig. 1a). At this period, some individuals of the xerophytic tree *J. phoenicea* were using a high percentage of groundwater (>60%) (Fig. 1a). During the dry season (summer), we observed a high range of water sources used by plants, from shallower to deeper soil layers, depending on plant functional traits. Growth form, water requirements, leaf longevity, and leaf form showed a significant effect on the sources of water used in summer (Table 2). After testing differences in water source use, the following new functional groups were defined: xerophytic shrubs with narrow leaves (NXs), semi-deciduous mesophytic shrubs (sMs), aphyllous/spike or semi-deciduous broad-leaf shrubs (Os), evergreen hygrophytes (eH) and evergreen xerophytic trees (eXt) (see Appendix S6). These functional types acquired water from different soil depths, with their reliance on deeper soil water increasing in the order of: NXs < Os < sMs < eH < eXt (Fig. 1c).

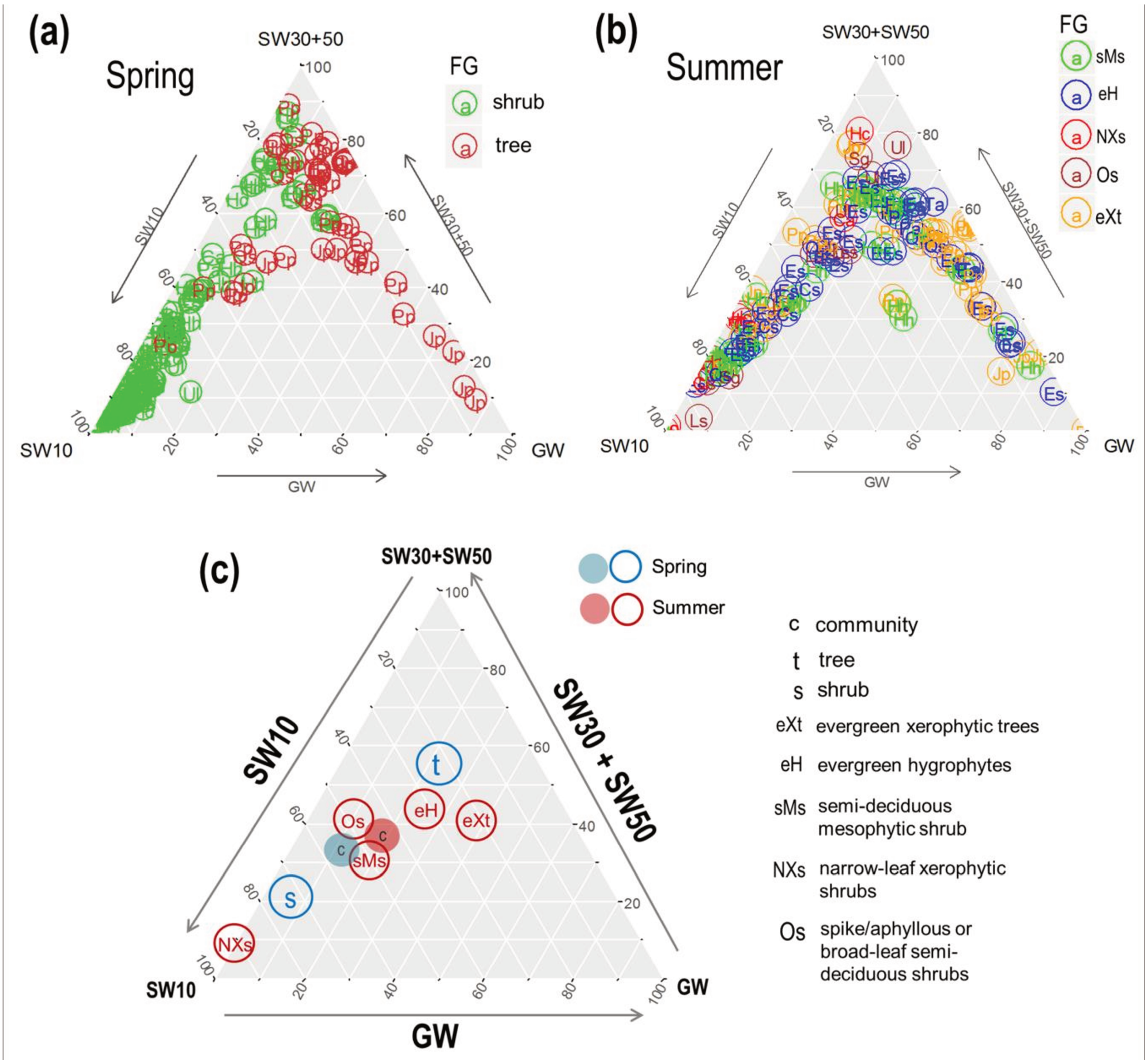

Figure 1. Proportion of water sources used by plants in spring and summer periods. Four water sources considered: soil water at 10, 30 and 50 cm (SW10, SW30, SW50 respectively), and groundwater (GW). In the spring period, SW10 is also representing rain water. SW30 was aggregated with SW50 (posteriorly to mixing model calculation), and represented as SW30+SW50. Species grouping and functional classification was done considering the significant effects of functional traits on water sources use and the comparison between prior classified plant groups (see Appendix S6 for further details). Functional groups listed in the inner legend. **(a, b)** Spring and summer proportions of water sources used (%) by individual plants of each functional group posteriorly identified. Inner labels represent species name (see species codes in Table 1). **(c)** Mean proportion of water sources used (%) by the functional groups posteriorly defined (unfilled circle) and by overall woody community (filled circle) in each sampling period (spring: blue, summer: red).

Accordingly, the highest use of shallow soil layers was observed in xerophytic shrubs with narrow leaves. Evergreen xerophytic trees (eXt) were the functional group that showed a higher use of deep soil layers (Fig. 1c). *Erica scoparia*, an eH shrub species, showed a wide range of water-source-use under dry conditions (Fig 1b).

Although we found no seasonal differences in overall community water source usage, plants presented different seasonal variation in the use of water sources depending on particular functional traits (Fig. 2, Appendix S6). Similarly to summer, species' seasonal adjustments in water-sources were influenced by growth form, water requirements, leaf longevity and leaf form (Table 2). Accordingly, the following functional groups were identified: NXs, Os, sMs, eXt, eHt and eHs (Fig. 2, Appendix S6). Each of these functional groups showed a distinct pattern of seasonal (spring to summer) water-source-use variation (Fig. 2). The highest spring-to-summer variation towards groundwater table was observed in evergreen hygrophytic shrubs and trees (eHt by substituting SW50 and eHs by substituting SW10). Contrastingly, NXs showed the lowest seasonal variation towards deeper soil layers (Fig. 2), and, unlike other shrubs, a very high use of shallow soil layers in the dry period (Fig. 1).

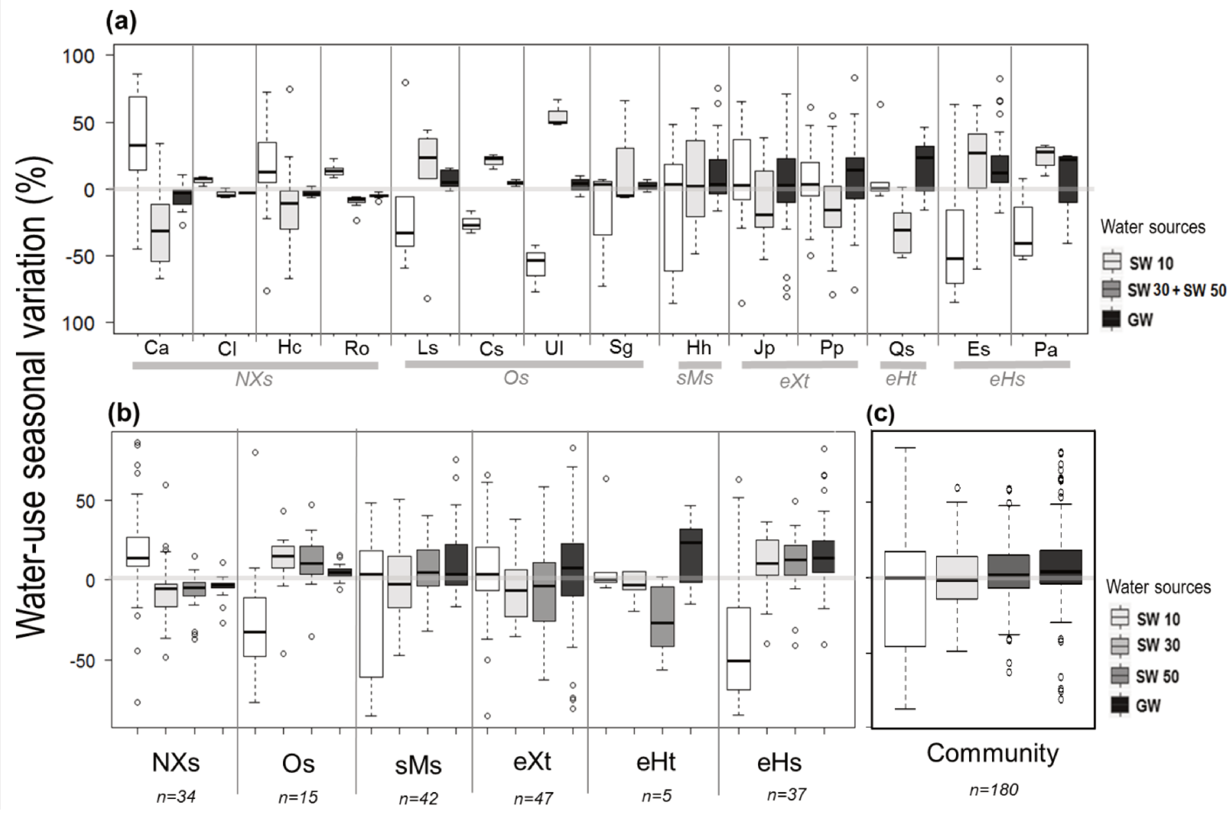

Figure 2. Water-source-use seasonal variation (spring-to-summer, %) considering: **(a)** species, **(b)** functional groups and **(c)** overall woody community. Positive values represent higher values in summer compared to spring, and negative values lower values in summer compared to spring. Water sources considered were: soil water at 10, 30 and 50 cm (SW10, SW30, SW50 respectively) and groundwater (GW) (white to dark grey, as described in inner legend). **(a)** Species name represented with a code as described in Table 1. For figure simplification, SW30 was aggregated with SW50, and represented as SW30+SW50 in this panel. Grey horizontal thick line below species code indicates the species included in the grouping of (b). **(b)** Functional groups' definition was done considering the significant effects of functional traits on seasonal variation of water source use and the comparison between prior classified plant groups (see Appendix S6): xerophytic shrubs with narrow leaves (*NXs*), semi-deciduous broad-leaf or aphyllous/spiky shrubs (*Os*), semi-deciduous mesophytic shrubs (*sMs*), evergreen xerophytic trees (*eXt*), evergreen hygrophytic tree (*eHt*) and evergreen hygrophytic shrubs (*eHs*). Number of plants considered is represented for each functional group (*n*).

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In spring, functional types that used a low proportion of water from deep soil layers (i.e. greater use of precipitation/shallow soil layers) showed lower values of leaf δ^{13}C (low water use efficiency) and higher values of plant water status (WI) (Fig. 3a, and see Appendix S7). In this period eXt plants showed the highest values of mean δ^{13}C (higher stomatal control) and lower values of WI (Fig. 3a). Contrastingly, in summer, the high reliance on water from deep soil layers of eXt and eH implied a higher mean WI (Fig. 3b). However, no significant correlations were observed for δ^{13}C patterns (Fig 3b, Appendix S7). Under dry conditions, although a high intra-group variability in physiological parameters was observed, it was not related with the proportion of use of deep soil water (see Appendix S7). Still, there was a better mean seasonal maintenance of water index values (positive spring to summer variation), accompanied by low mean seasonal enrichment of leaf δ^{13}C, in the eXt and eHt functional groups (Fig. 3c). Thus, plant functional types that generally used a higher proportion of deep soil water during the dry sampling showed a better maintenance of their water content (Fig. 3c, Appendix S7).

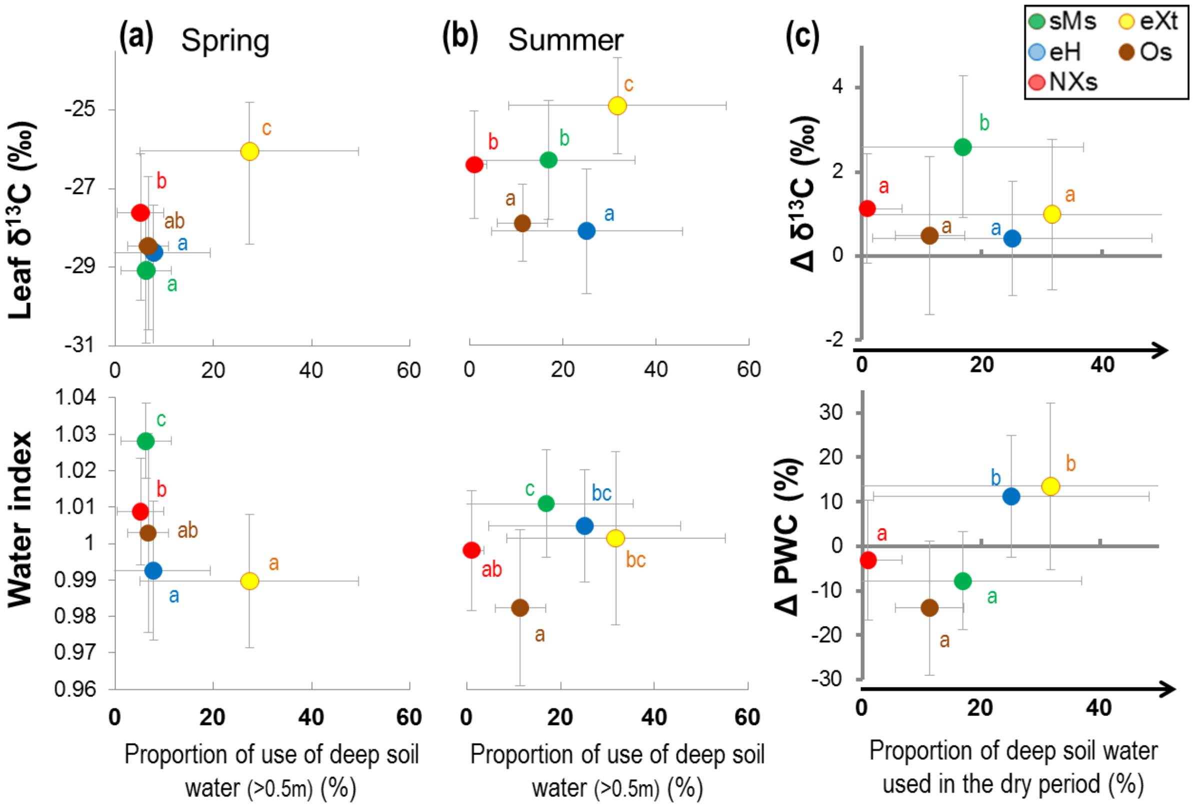

Figure 3. Plant physiological parameters (leaf δ^{13}C and reflectance water index) and the proportional use of water from deep soil layers (depper than 0.5 m) in **(a)** spring and **(b)** summer. **(c)** Spring to summer seasonal variation (summer minus spring values, Δ) of the physiological parameters leaf δ^{13}C (Δ δ^{13}C, ‰) and plant water content (Δ PWC, %), and the proportion of use of deep soil water in the dry period. Each functional type is represented by a different color as indicated in the inner legend: xerophytic shrubs with narrow leaves (NXs, n=34), semi-deciduous broad-leaf or aphyllous/spiky shrubs (Os, n=15), semi-deciduous mesophytic shrubs (sMs, n=42), evergreen xerophytic trees (eXt, n=47), evergreen hygrophytes (eH, n=42). Dots represent mean values per functional type and bars represent their standard deviation. Significant differences between functional types are denoted with different letters (*p<0.05).

2.1.5 Discussion

Our results showed evidence of great soil water partitioning under water limited conditions. Although overall woody community seasonally maintained the mean use of water sources, relevant water-uptake depth adjustments were made by different functional groups. Thus, coexisting plant functional types in this semi-arid Mediterranean ecosystem segregate along a wide range of contrasting water source usage under extremely dry conditions.

Water sources partitioning under drought

In periods of high rainwater availability at shallow soil layers (spring), a higher similarity of water-sources-use between species was found. Contrastingly, in the dry season, we observed segregation in the sources of water used by woody species in a semi-arid dune ecosystem (Fig. 1). Even though water-sources-use differentiation among functional types, a mechanism to reduce competition for limited resources and contribute to patterns of coexistence in woody community, has been long supposed in this ecosystem, empirical verification has been lacking. Based on differences of summer water uptake depth among species, we were able to identify five distinct water-source-use strategies, from a strong reliance on soil water from the upper layer to water uptake predominately from deeper soil layers (Table 3). Thus, our results supported a soil-water partitioning among several functional types in response to changes in soil water content (Jackson et al. 1999; Filella & Peñuelas 2003; Nippert & Knapp 2007; Palacio et al. 2017).

When water availability was high, shrubby species (regardless being evergreen or semi-deciduous, hygrophytic or xerophytic) explored shallow soil layers where precipitation is available, and trees explored slightly deeper soil layers, where moisture is still high in spring. Although, under this wetter conditions, individuals of *J. phoenicea* seems to make a greater use of deeper soil layers [consistent with previous water-use studies that pointed to deeper rooting system of *Juniperus* sp. (West et al. 2007, 2008; Grossiord et al. 2017)], most species were mainly dependent on water from shallow (<50 cm) soil layers. These results point to the importance of water from upper soil layers in spring and a benefit of rainfall pulses for several species (as also seen in Williams & Ehleringer 2000, Chesson et al. 2004 and Grossiord et al. 2017). When water is not limiting, several species can exploit the same water resource, and an increased overlap in soil water exploitation among coexisting species can exist without great implications to plants performance.

Table 3. Functional classification of the studied species based on water-source-use (WU) patterns. Species code as in Table 1. *A priori* functional groups (FG) defined as a concatenation of the four trait categories considered in this study (leaf form, leaf longevity, water requirement and growth form). For classification name, the first letter of each trait' classes (Table 1) was used. *A posteriori* FGs were defined considering the comparison of water sources use patterns between *a priori* FGs, for spring, summer and spring-to-summer WU variation. An overview of the main water source used and of the water uptake shifts made in dry periods by each *a posteriori* FG is also provided: the mean water sources used by the different FGs ranged from shallow to deep soil layers as indicated by dashed arrow; spring to summer water-uptake depth shifts are shown as up- or downward arrows representing the direction of water uptake changes (e.g. downward arrows indicate seasonal changes to deeper soil layers). See Fig. 2 for functional groups' quantitative water-source-use in spring and summer and Fig. 3 for quantitative water-source-use seasonal variation.

Species	***a piori* FG**	***a posteriori* FG**				**Main water sources used**	**Mean seasonal WU shifts**
		Spring WU	**Summer WU**	**Seasonal variation WU**	**Description**		
Cl	NsXs	shrub	NXs	NXs	narrow-leaved xerophytic shrubs	shallow	↑
Hc	NsXs						
Ro	NsXs						
Ca	NeXs						
Cs	BsHs		Os	Os	aphyllous/spike or semi-deciduous broad-leaf shrubs		↓
Ls	BsXs						
Sg	AaXs						
Ua	AaXs						
Hh	BsMs		sMs	sMs	semi-deciduous mesophytic shrub		↔
Es	NeHs		eH	eHs	evergreen hygrophytic shrubs		↓↓↓
Pa	BeHs						
Qs	BeHt	tree		eHt	evergreen hygrophytic tree		↓↓
Pp	NEeXt		eXt	eXt	evergreen xerophytic trees	deep	↓↓
Jp	SeXt						

Species differences in water uptake depth developed during the dry summer, a seasonal shift also observed previously in other dry ecosystems (West et al. 2007; Saha et al. 2009; Grossiord et al. 2017; Palacio et al. 2017). During the drier period, when precipitation is very scarce or absent, despite similar growth form, the xerophytic shrubs show reliance on water in surface soil layers, while evergreen hygrophytic shrubs explored deeper soil layers (exhibiting greater shifts of water sources used in response to reduced water availability) (Fig. 2). Thus, growth form was not the only trait influencing plants' water-source use under summer dry conditions. Water requirements, leaf longevity and leaf form (particularly in xerophytic shrubs) were shown to be important traits for water sources explored. We inferred a common strategy among xerophytic shrubs with narrow leaves, possibly competing for water in surface soil layers even during periodic drought. In agreement with other studies (Álvarez-Cansino et al. 2010; Moreno-Gutiérrez et al. 2012), we showed that this functional group tended not to use water from deep layers of soil nor groundwater as a main water source throughout the seasons, showing a particularly distinct signature of xylem water. This 18O-enriched values can be further linked to a distinct stem hydraulic process occurring in this plants, as an evaporative enrichment of xylem water can take place when sap flow is limited (Martín-Gómez et al. 2017). By contrast, evergreen hygrophytic shrubs and xerophytic tree species explored deeper soil layers, possibly avoiding competition at surface layers when this water resource is limiting and exploring a more available water source. Although a considerable intra-specific variability in seasonal shifts was observed, both studied evergreen xerophytic trees generally responded to (seasonal) precipitation reduction in a similar way, which is not in agreement with other *Juniperus-Pinus* studies (Grossiord et al. 2017; but see Williams & Ehleringer 2000). Furthermore, the individual short-term changes in water-uptake depth of hygrophytic shrubs and xerophytic tree species point to the existence of an active dimorphic root system. A dimorphic root system is considered a major advantage in water limited habitats, allowing plants to explore water from different soil layers, to take advantage of any rain event and of water from a deep soil profile (Ehleringer & Dawson 1992; Dawson & Pate 1996; Rossatto et al. 2012; Barbeta et al. 2015). Although it has been shown that once shallow soils become dry, trees may switch water access and subsequently garner water from deep sources, we showed that also certain shrub species (e.g. *E. scoparia*) can do this water-sources-use shift in this ecosystem.

Linking water sources use to drought strategies

The summer shifts in water source use towards deeper soil layers can be seen as a way to explore a more stable water supply, thus supporting evergreen trees and hygrophytes water demand and seasonal maintenance of their water status (Fig. 3). We confirmed that, not only important differences in water sources use exist, but that they are linked to general strategies to cope with water stress in this ecosystem. The observed water use trends agreed well with the previously observed seasonal changes in water potential and leaf physiology (Zunzunegui et al. 2005, 2011). This indicates an important relationship between the depth of water extraction and leaf physiological traits in this semi-arid Mediterranean ecosystem. Xerophytic shrubs with narrow leaves (mostly semi-deciduous species, but including the evergreen *C. album*), that showed a distinct water-source-use in our study, were previously considered as poikilohydric-type, with a pronounced decrease in water potential and a severe drought stress (Zunzunegui et al. 2005, 2011). The absence of a summer shift to deeper soil layers denote a low capacity of this functional group to change to more available water sources, which forces them to a great morpho-physiological specialization targeted to greater photosynthetic activity and water status in the peak of growing season (spring) (Fig. 3). This is an adaptive trait for small-sized shallow rooted plant species heavily dependent on fluctuating shallow soil water pool that is subjected to rapid evaporation (Schwinning & Ehleringer 2001; Alessio et al. 2004; Saha et al. 2009; Moreno-Gutiérrez et al. 2012; Sardans & Peñuelas 2013). The summer semi-deciduous mesophytic shrub *H. halimifolium* was previously assigned to the center of an avoidance-tolerance gradient, pointed out as a species of wide plasticity and with different roots' depth depending on the topographic position (Zunzunegui et al. 2002, 2009, 2011; Wegener et al. 2015). This corroborates with our observation of high intra-specific variability of summer water sources usage (accompanying a low mean seasonal variation of water-sources-use) and the observed high adjustment in water use efficiency during the dry period. Consistent with the previously defined conservative water-use strategy of evergreen sclerophyll species in hygrophytic sites within this ecosystem (Zunzunegui et al. 2011), we observed a general summer maintenance of water status of hygrophytes. However, a particularly high intra-specific variability within the summer season was observed in this functional type. Nevertheless, the higher proportion of water obtained from deeper soil layers during the dry season observed for the studied evergreen hygrophytes and tree species seems to allow (at least partly) the avoidance of severe water stress, and further support the presence of turgid leaves throughout the year (Merino et al. 1995; Zunzunegui et al. 2011). Thus, we demonstrated that, similarly to other studies, the depth of soil water uptake is related to broader water-use strategies, and associated with plant habit, leaf phenology and growing period (West et al. 2008; Moreno-Gutiérrez et al. 2012; Schwendenmann et al. 2015; Palacio et al. 2017). Further, we have shown that the same fundamental tradeoff between growth capacity and low-resource tolerance that separates life forms (Chapin et al. 1993) is found within what is commonly considered to be one plant functional type. The degree of separation or aggregation between species based on water-sources-use strategies was related to a differential response to environmental water variation and their water-use strategies. Additionally, species may exhibit multiple strategies, purportedly to reduce competition and facilitate resource acquisition (Schenk 2006; Valladares & Sánchez-Gómez 2006; Angert et al. 2009; Moreno-Gutiérrez et al. 2012; Pivovaroff et al. 2016). Thus, the functional groups identified in our study end up exhibiting a trade among traits, each contributing to alleviate a part of the plant stress. Their water strategies result, therefore, from a combination of leaf level and water uptake depth adjustments under drought.

2.1.6 Conclusion

Unlike spring, a high inter-specific differentiation in water source use was observed during the dry period. In the studied ecosystem, rain, available at shallow soil layers, was an important water source for several plant functional types in spring periods, but under extremely dry conditions, coexisting plant functional types segregated along a wide spectrum of water sources usage. Woody species clustered in five water-sources-use functional groups, exploring soil water from shallower to deeper soil layers. This segregation in water-sources-use was observed not only in different growth forms, but also among species with different water requirements, leaf longevity and leaf form characteristics. The highest use of water from shallow soil layers was observed in narrow-leaved xerophytic shrubs, while evergreen xerophytic trees and evergreen hygrophytic species showed a higher use of deep soil layers. This contrasting (and potentially complementary) water source use should foster plant species coexistence through temporal and spatial segregation of the resource that is most limiting to vegetation (i.e. soil moisture) in this ecosystem. Accordingly, relevant spring-to-summer shifts in the water sources used towards deeper soil layers were observed, but not in all functional types. These water uptake depth adjustments were associated with a more favorable seasonal maintenance of plant water status. Furthermore, our observations confirmed that the water-sources-use behavior accompanied broader strategies of drought resistance.

It is noteworthy to mention that the high intra-specific variability within summer season showed by particular functional groups indicates that other fine-scale (hydrological/ environmental) factors can be playing an important role on their water-sources-use patterns (Araya et al. 2011; Voltas et al. 2015). Thus, further work is needed to better understand functional groups' and overall community water use and strategies along ecohydrological gradients in these semi-arid ecosystems.

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2.1.9 Supporting Information

Appendix S1. Overview of the study site: location of the study area and climatic conditions.

Appendix S2. List of studied species and sample sizes by plot.

Appendix S3. Isotopic composition (δ18O) of xylem water and water sources at spring and summer conditions.

Appendix S4. Detailed description of 18O enriched xylem water.

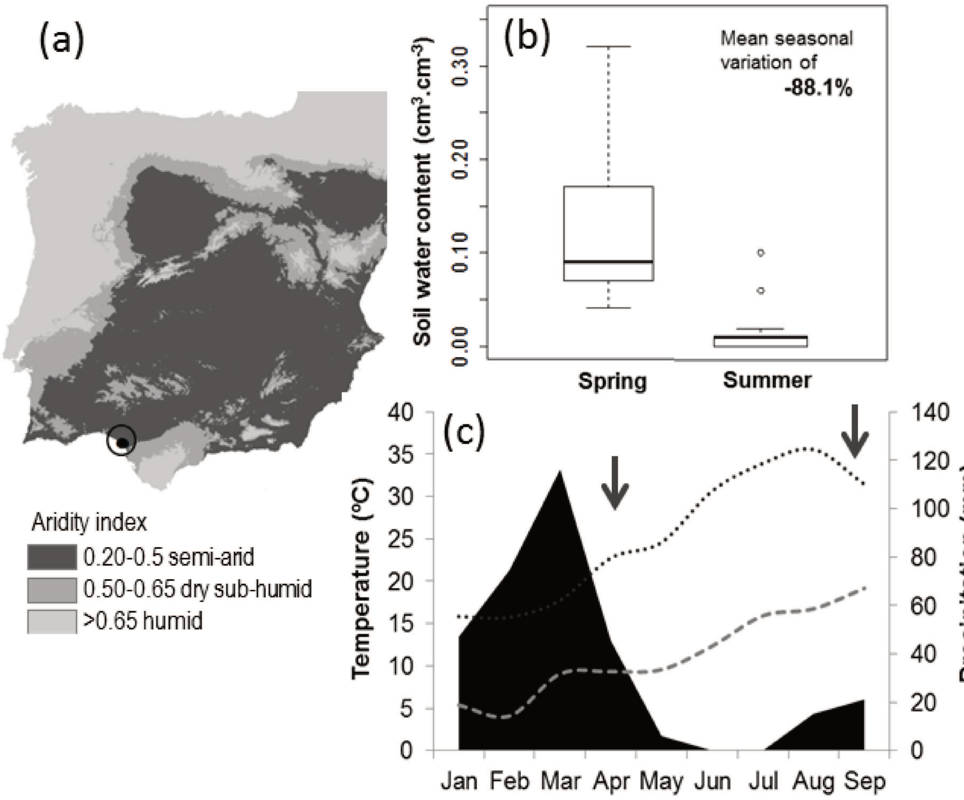
Appendix S5. Principal Component Analyses of water-sources-use.

Appendix S6. Functional groups classification based on water sources used.

Appendix S7. Relationships between physiological parameters and the proportional use of water from deep soil layers.

Supporting information

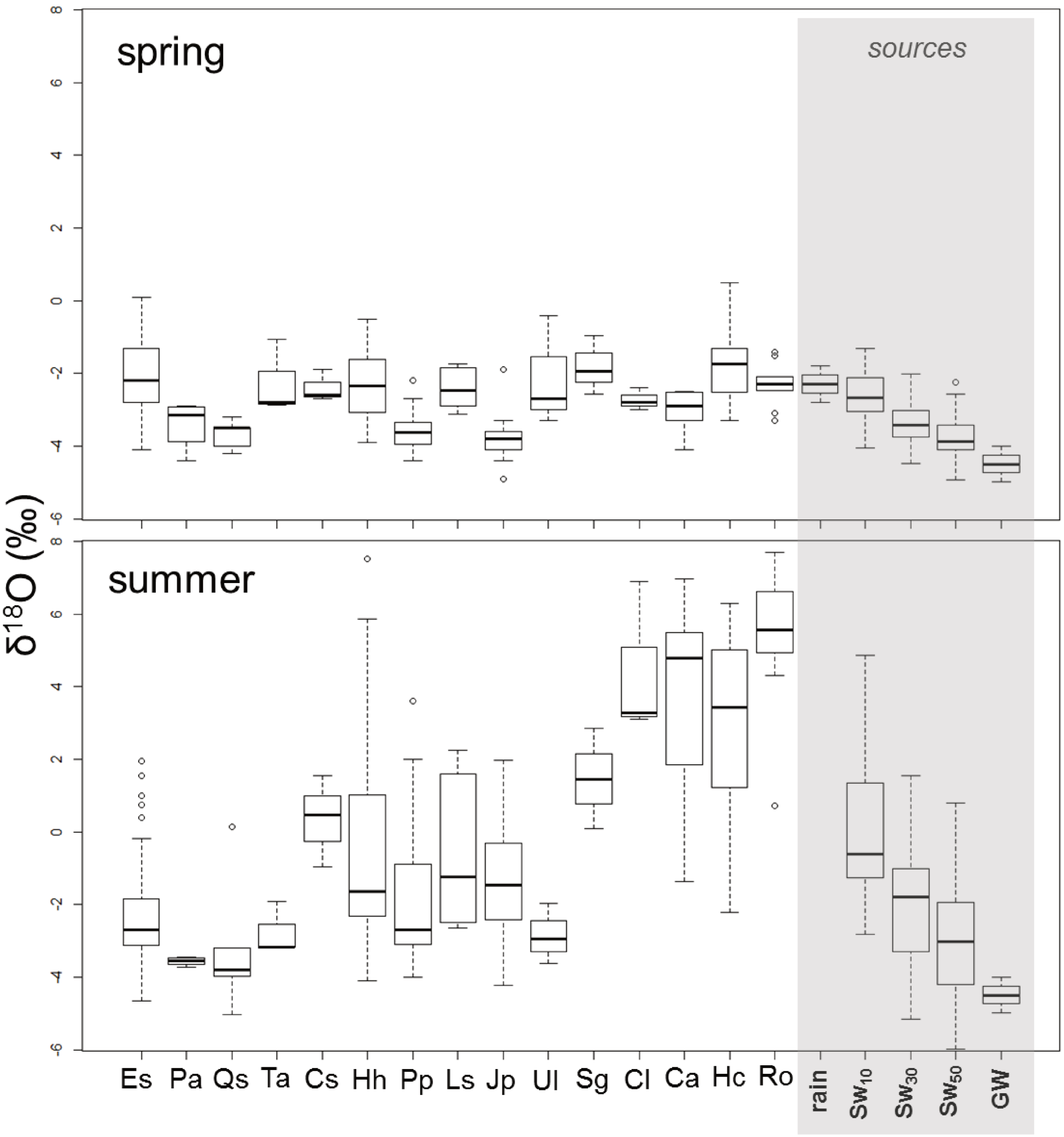
Appendix S1. Overview of the study site: (a) location of the study area at southwest Spain – Doñana Biological Reserve, showing the aridity index classification (UNEP 1992), (b) soil water content at 10 cm depth and (c) climatic conditions: total precipitation (right y-axis, black) and minimum and maximum temperatures (right y-axis, grey dashed lines) from January till September 2013. Down arrows indicate sampling dates (spring and summer sampling). Spring and summer volumetric soil water content was measured at 18 sampling points in the two contrasting climatic conditions.



Appendix S2. List of the studied species and sample sizes by plot. Number of plants sampled per plot (A-R), considering the different functional traits: growth form (GF), water requirements (WR), leaf longevity (LL) and leaf shape (LS). Classes considered were: GF – shrub (s) or tree (t); WR – xerophytic (xero), mesophytic (meso) or hygrophytic (hygr); LL – summer semi-deciduous (semd), evergreen (ever), aphyllous (aphy); LS – narrow (narr), broadleaf (brle), needle (need), scale (scal), spike/aphyllous (aphy). Total n (*n*) and species sampled per plot is also presented in the table. Check species names in Table 1 of the main manuscript.

	GF		**WR**			**LS**					**LL**							
plot	**s**	**t**	**hygr**	**meso**	**xero**	**aphy**	**brle**	**narr**	**need**	**scal**	**aphy**	**ever**	**semd**	***n***	**Species**			
A	9	1	4	3	3	0	7	3	0	0	0	4	6	*10*	Es	Hh	Ls	Qs
B	9	3	3	0	9	0	3	6	0	3	0	6	6	*12*	Es	Jp	Ls	Ro
C	9	1	7	0	3	3	4	3	0	0	3	7	0	*10*	Es	Pa	Qs	Ul
D	9	1	6	3	1	0	6	3	1	0	0	4	6	*10*	Cs	Es	Hh	Pp
E	9	2	5	3	3	0	5	6	0	0	0	5	6	*11*	Es	Hh	Qs	Ro
F	9	3	0	3	9	0	3	6	3	0	0	3	9	*12*	Ca	Hc	Hh	Pp
G	6	1	3	3	1	0	3	3	1	0	0	4	3	*7*	Es	Hh	Pp	
H	5	3	0	3	5	0	3	2	3	0	0	3	5	*8*	Hc	Hh	Pp	
I	8	3	0	3	8	0	3	5	3	0	0	3	8	*11*	Ca	Hc	Hh	Pp
J	6	4	0	3	7	0	3	3	1	3	0	4	6	*10*	Hc	Hh	Jp	Pp
K	6	4	0	0	10	0	0	6	1	3	0	4	6	*10*	Hc	Jp	Pp	Ro
L	6	4	0	3	7	0	3	3	1	3	0	4	6	*10*	Cl	Hh	Jp	Pp
M	7	1	5	3	0	0	5	3	0	0	0	5	3	*8*	Es	Pa	Qs	Hh
N	6	3	0	3	6	3	3	0	3	0	3	3	3	*9*	Hh	Pp	Sg	
O	6	5	3	0	8	0	0	6	2	3	0	8	3	*11*	Ca	Es	Jp	Pp
P	6	4	3	3	4	0	3	3	1	3	0	7	3	*10*	Es	Hh	Jp	Pp
Q	6	5	3	3	5	0	3	3	2	3	0	8	3	*11*	Es	Hh	Jp	Pp
R	6	4	3	3	4	0	3	3	1	3	0	7	3	*10*	Es	Hh	Jp	Pp
Total	128	52	45	42	93	6	60	67	23	24	6	89	85	180				

Appendix S3. Isotopic composition ($\delta^{18}O$) of xylem water and water sources at spring and summer conditions. Species names as codes in Table 1 of the main manuscript. Sources considered were: (spring) precipitation (rain), soil water at 10 cm (SW_{10}), 30 cm (SW_{30}), 50 cm (SW_{50}) and groundwater (GW).



Appendix S4. Detailed description of ^{18}O-enriched xylem water.

We acknowledge that some samples of xylem water presented extremely high δ^{18}O values (particularly narrow-leaved xerophytic shrub species in summer, see Appendix 3). In this cases, xylem water was isotopically enriched compared to soil water, and could be reflecting another water source not sampled in this study or indicating further ^{18}O fractionation processes (thus, not directly reflecting a water source). However, we didn't exclude these samples but rather attributed a 100% top-soil use. The reasons for this decision were:

(a) Some samples of top-soil (SW_{10}) showed comparable ^{18}O-enriched values in summer (i.e. high δ^{18}O, >3 ‰).

(b) The xylem water with δ^{18}O out of the sources' range can be reflecting shallow water sources as from 0-10 cm soil layers or dew. Water from surface soil layers (0-10 cm deep) is expected to suffer a strong isotopic enrichment (high evaporation) in dry periods, and thus show high δ^{18}O values. Also, there is a high frequency of summer dew in Mediterranean coastal habitats (which can show ^{18}O-enriched values), depositing, and consequently available to plants, at <10cm soil layers (Ingraham & Matthews 1995; Dawson 1998; Alessio et al. 2004). These inputs of dew or fog are common in Doñana ecosystem (García Novo & Marín Cabrera 2005). Although we did not sample these possible water sources, both cases (0-10 cm soil layers and dew) are water sources available in very shallow soil layers, so it is plausible to assume the samples' main source as the top-soil (SW_{10}).

(c) Summer rain collected at the site on the previous month of sampling (August), although not considered by us as a source in the mixing model of September, was also analysed (δ^{18}O = -2.06 ‰). Thus, the isotopic signature of this water source is far from the values showed by the plants with extremely enriched xylem water. However, a high residence time (stagnation) of water and fast evaporative enrichment of xylem water through bark/stem transpiration can occur under limited sap flow conditions (and low leaf transpiration) (Martín-Gómez et al. 2017). Under such conditions, the limited stem flow increases water turnover time, reducing the input of fresh, unenriched xylem water, and allowing for accumulative evaporative enrichment. Assuming that this was the case, the (most recent) water-source used (i.e. rain pulses) is still shallow and reasonable to assume the samples' main source as the top-soil (SW_{10}).

(d) It has been described evaporative enrichment in suberized stems of deciduous plants during leafless periods, when a long-lasting water stagnation in the xylem and a partial desiccation occur (Williams & Ehleringer 2000; Bertrand et al. 2012; Ellsworth & Sternberg 2015). Although we didn't study deciduous species, summer semi-deciduous species could show isotopically enriched stem water as a result of evaporation due to leaf shedding. However, the evergreen *Corema album* also showed ^{18}O-enriched xylem water, pointing to the probable influence of other factors besides leafless. It has been also suggested that an additional cause for xylem enrichment and xylem–soil decoupled observations may be the mixture of xylem water with enriched water from the leaf, either through backward diffusion (Dawson & Ehleringer 1993; Brandes et al. 2006; Ellsworth & Williams 2007; Bertrand et al. 2012; Treydte et al. 2014), or indirectly by means of water exchange between xylem and phloem tissues (Cernusak et al. 2005; Brandes et al. 2006; Nardini et al. 2011). However, due to the smaller amount of water expected in the phloem of these plants under dry conditions, xylem–phloem exchange is likely to have greater effect on phloem values than on the xylem (Martín-Gómez et al. 2017). The observed isotopic uncoupling between xylem and soil water, can further suggest a possible effect of intra-specific competition for a limited water resource in these plants, as a large reduction in leaf transpiration rates are expected (which in turn can result in enrichment in stems) (del Castillo et al. 2016). These are plausible situations, but much likely to occur when the plants have a tight stomatal regulation, a limited sap flow and are relying in a more ^{18}O-enriched, scarce and fluctuating water source as SW_{10} (Moreno-Gutiérrez et al. 2012; Gaines et al. 2016; Martín-Gómez et al. 2017). The amount of variation we observed in $\delta^{18}O$ compositions of xylem and water-sources of top-soil layers itself suggests that plants were using water from a highly variable water source as top-soil, pointing to shallow soil water rather than deep soil water or groundwater as the main source of water supporting transpiration.

It is worthy to mention that we conducted all the sampling and lab analysis with extreme care, following standard methodologies, and always having in mind the complexity and extreme conditions of Mediterranean dune systems. To prevent false readings on samples that might have evaporated inside the collection tubes, due to transport from the field, collection tubes with the twigs or soil samples are placed inside the cryogenic vacuum distillation sample vessels (after quick uncapping in the lab cool environment), to allow for the complete sample water to be extracted (even the water that eventually evaporated from xylem and present in the inner walls of the tubes). Thus, because our custom-made cryogenic vacuum distillation system allows placing the tube inside the distillation sample vessels, immediately storing the samples in airtight vials, quickly sealing with a cap and parafilm and placing in cool conditions (kept at 4°C until isotopic analysis) is considered adequate for isotopic analysis without losses of depleted water and further evaporative enrichment of xylem water. Thus, we don’t consider that evaporative enrichment due to colleting and storage played a role in the out-of-range values of some xylem water samples found in summer. Furthermore, the xylem and sources sampling and water analysis were performed equally among the various plant individuals (of various species), and soil water accurately characterized by plot in each site (3 depths x 3 replicates x 18 plots per site) following the same protocol. Thus, if some enrichment occurred due to sampling, storage or extraction, it was most probably equal for every water sample, maintaining the validity of the comparison (between xylem and sources, and between species). Additionally, to reinforce the validity of the data, we repeated the same sampling and analysis the following year (2014) and the results were consistent (data not shown).

Regardless the arguments, and for the sake of comparison with other species (sampled in the same way at the same time), these are distinct patterns of water-use that shouldn't be ignored.

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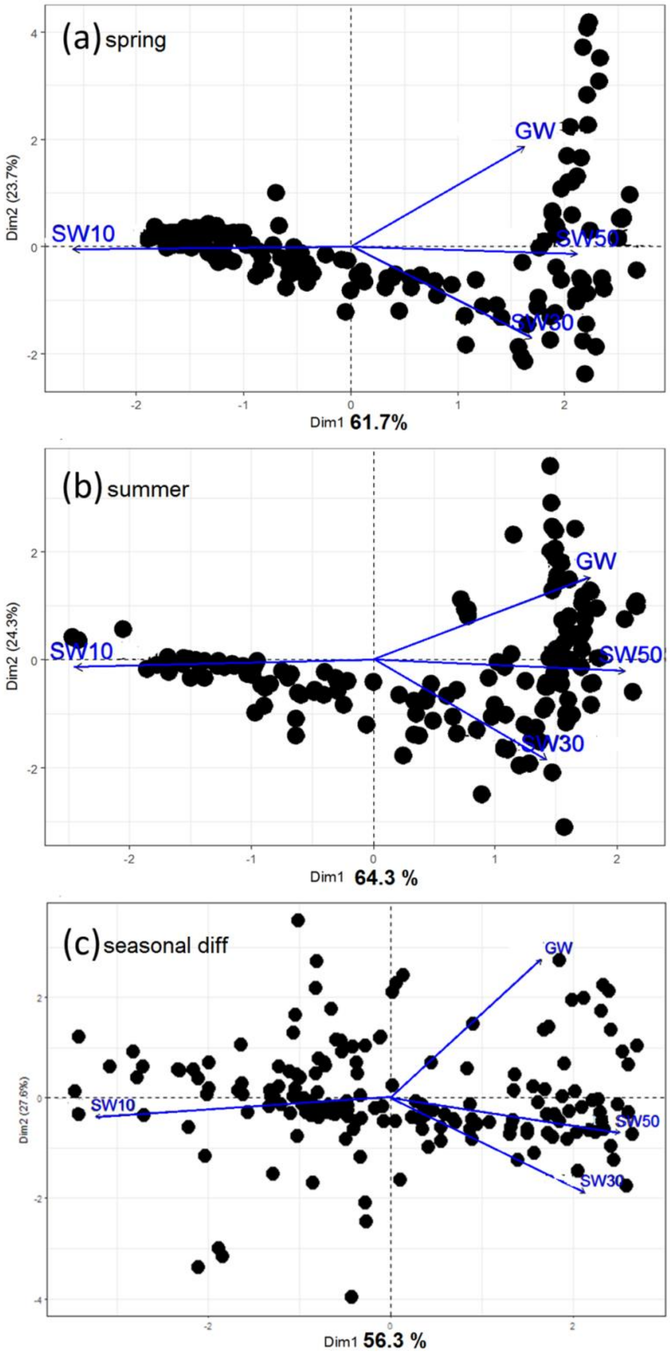
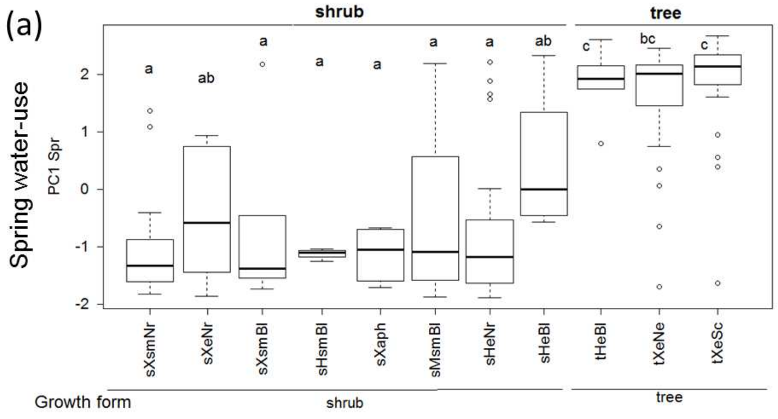
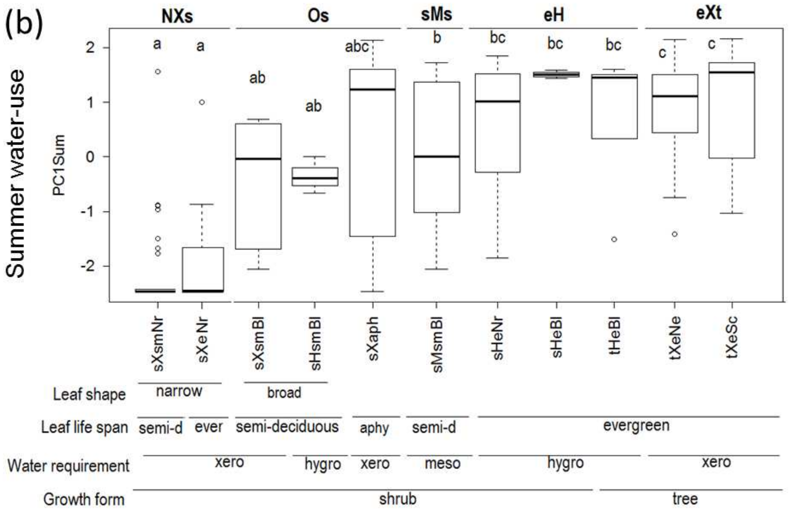
Appendix S5. Principal Component Analyses (PCA) of water-sources-use. PCA considering: (a) spring water-use, (b) summer water-use and (c) seasonal variation of water-use (calculated as diff= summer – spring). Sources considered were: soil water at 10 cm (SW10), 30 cm (SW30), 50 cm (SW50) and groundwater (GW). Spring precipitation was aggregated with SW10. n=183.



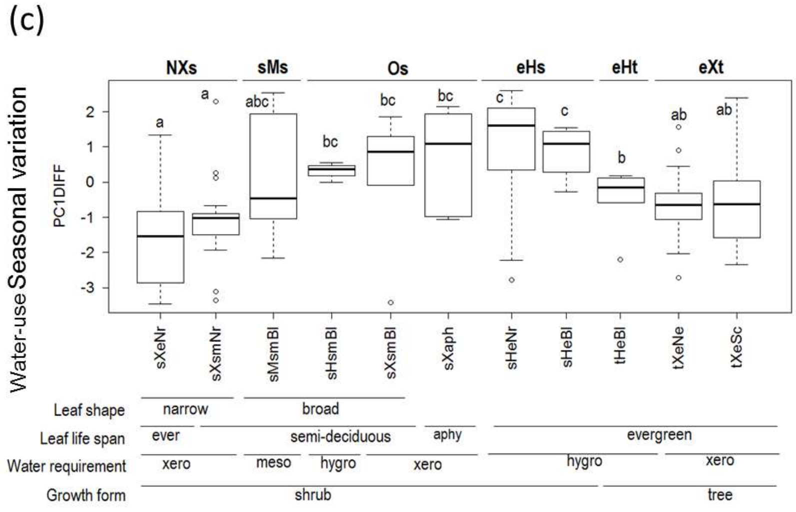
Appendix S6. Functional groups' classification, based on the comparison between prior classified groups (combined traits: GF||WR||LL||LS), and considering the water-sources used (a) in spring, (b) in summer and (c) the seasonal variation (spring to summer) of water sources use. Scores of PC1 (see Appendix S5) were considered for the analysis (PC1Spr, PC1Sum and PC1Diff, accordingly). New functional groups defined for (b) and (c) appear on top of the graphs. Species that are included in the new defined functional groups are listed in left tables. See species' name in Table 1 of the main manuscript.



shrub	tree
Cl	Jp
Cs	Pp
Ca	Qs
Es	
Hc	
Hh	
Ls	
Pa	
Ro	
Sg	
Ua	

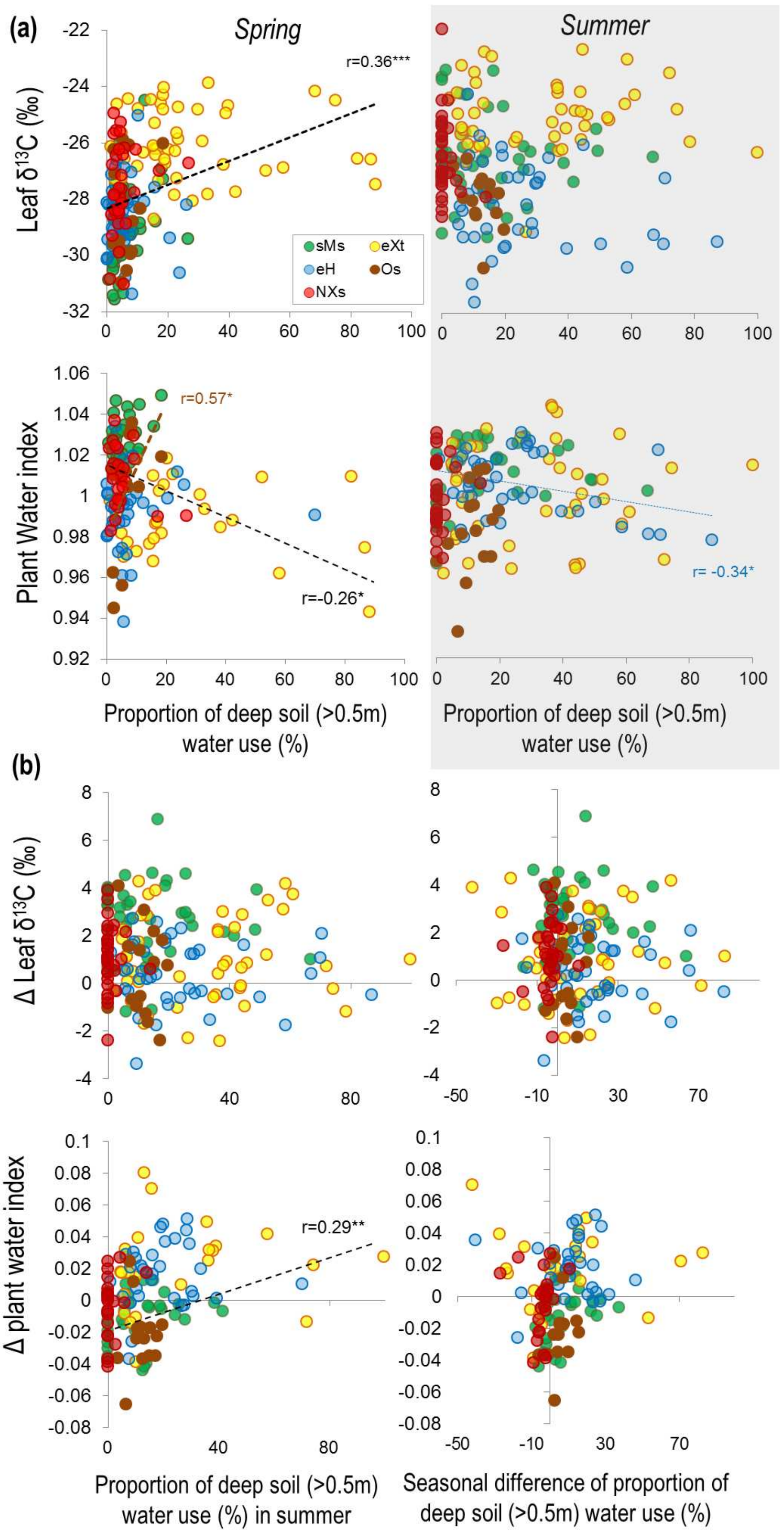


NXs	Os	sMs	eH	eXt
Cl	Cs	Hh	Es	Jp
Ca	Ls		Pa	Pp
Hc	Sg		Qs	
Ro	Ul			



NXs	Os	sMs	eHs	eHt	eXt
Cl	Cs	Hh	Es	Qs	Jp
Ca	Ls		Pa		Pp
Hc	Sg				
Ro	Ul				

Appendix S7. Relationships between physiological parameters and the proportion of use of deep soil.



Relationships between physiological parameters (leaf δ^{13}C and reflectance water index) and the proportional use of water from deep soil layers (>50 cm). (a) spring and summer, (b) seasonal variation (summer minus spring values). Each functional type is represented by a different color as indicated in the inner legend in (a). Significant Pearson correlations are denoted with a dashed line (black for the overall community) and the respective r and p-value (*p<0.05; **p<0.01; ***p<0.001). Total n=180 (see *n* per functional type in Fig. 2 of the main manuscript).

2.2 Water sources used by woody species under contrasting water availability in a tropical coastal dune forest

Antunes C., Silva C., Joly C.A., Máguas C., Vieira S.

2.2.1 Abstract

In seasonally flooded tropical forests, plants must cope with periodic groundwater variations. This is particularly relevant in sandy soils, where soil-water availability changes can be very rapid. The aim of this study was to investigate the extent of water-sources-use differentiation among dominant woody species in a tropical coastal dune forest.

The study was conducted in a restinga forest, São Paulo, Brazil. We sampled 15 woody species. Individuals' growth form, diameter at breast height (DBH) and crown illumination index (CII) were characterized. Through Bayesian isotope mixing models we estimated the proportion of water sources used by plants in two contrasting hydric conditions. We tested whether water-sources-use was (i) different between contrasting water availability conditions; (ii) dependent on growth form, DBH or CII; or (iii) influenced by resource competition.

We found a temporal variation in water-sources-use, but no vertical soil-water partitioning among the woody species. In wetter periods, plants used mainly precipitation, as high soil-water availability and shallow water table limited water uptake to top-soil layers. Contrastingly, during dry periods, with the absence of rain and a deeper water table, plants generally relied on deeper (50 cm) unsaturated soil layers. Interestingly, changes in water-sources-use were neither evoked in more dense or diverse plots, nor induced by plant size. Our study supports the hypothesis that restinga plants have dynamic shifts in water-uptake depth due to seasonal water availability changes, particularly groundwater. It enhances our understanding of woody species coexistence and the effects that water variations can have on water-resource use in restinga forests.

Key-words: *restinga* forest; water-sources-use; over and understory species; coastal dune ecosystem; wet and dry conditions; stable isotope mixing model; groundwater availability

2.2.2 Introduction

In the wet lowland tropics, biodiversity, productivity and carbon stocks tend to reach great values (Churkina & Running, 1998; Malhi et al., 2004; Phillips et al., 2010). As plants use the same basic resources (light, CO_2, water, nutrients and space for growth), co-occurring plants would likely compete. However, if the resources are not limiting or species sufficiently partition the abiotic and biotic environments, then different species can coexist (Peñuelas, Terradas, & Lloret, 2011; Silvertown, 2004). In other words, by using different ranges and proportions of resources, i.e. by resource and niche partitioning, co-occurring species can stably exist.

In tropical ecosystems, light is considered to be the most limiting resource for tree growth and survival and a major axis of differentiation for tropical tree species (Boisvenue & Running, 2006; Fyllas et al., 2017; Markesteijn, Poorter, & Bongers, 2007; Poorter, 2001, 2002; Wright, 2002). Thus, traditionally, studies in the tropics have mainly focused on light availability as the key factor determining species coexistence. However, more recently, attention has shifted towards the role of water availability as a factor in tropical species coexistence and resource-use differentiation (Oliveira, Eller, Bittencourt, & Mulligan, 2014; Rosado, Joly, Burgess, Oliveira, & Aidar, 2016). Tropical tree species have to cope with periodic water shortage at some point, and even in the wet tropics short dry spells can affect survival in the field (Engelbrecht et al., 2006; Markesteijn, Poorter, Bongers, Paz, & Sack, 2011; McDowell et al., 2018; Nepstad, Tohver, Ray, Moutinho, & Cardinot, 2007; Phillips et al., 2010; Wright et al., 2010). This is particularly relevant in sandy soils, where water availability changes can be very rapid, water retention is low and typically little water is available within the top soil during dry periods. Thus, plant species relying on that water-source will have to re-adjust their water-use for withstanding the periods without precipitation. Because the tropics may experience significant climate change, including more frequent and more extreme moisture deficits, in this century (e.g. Williams, Jackson, & Kutzbach, 2007), the question of how the world's richest ecosystems respond to moisture deficits (or 'drought') is a central concern. Particularly, how tropical sandy forests that experience seasonal water availability variations adjust their water-source-use merit further explanation.

Species existence along gradients of water availability will, to a great extent, be determined by the ability of species to acquire water and tolerate water shortage. For example, water stress of individual tropical plant species alleviates if they are able to adjust their root systems to site-specific conditions, such as variations in water table (Fan et al., 2017; Oliveira & Joly, 2010; Zea-Cabrera, Iwasa, Levin, & Rodríguez-Iturbe, 2006). Temporal plasticity in below-ground resource use could be thus a major driver of soil water-use differentiation among plants and a competitive advantage in acquiring soil resources (Guderle et al., 2018; Kulmatiski, Adler, Stark, & Tredennick, 2017). In addition, plant size can play an important role in dictating plant water-use (Brienen et al., 2017; Frederick C Meinzer, 2003). While tree growth is associated with water availability in seasonal tropical forests, relationships between stem diameter and maximum sap flow have been reported for co-occurring tropical forest tree species (Frederick C Meinzer et al., 2013; Santiago et al., 2004). In fact, tree size and climatic water deficit can control root to shoot ratio in individual trees globally (Ledo et al., 2018). With greater aridity (i.e. scarcity of moisture) trees tend to invest comparatively more resources to acquire soil water as it becomes a more limiting resource for growth and survival. However, water uptake patterns are not solely controlled by root length (and its density distribution) but also by spatial/vertical and temporal shifts in water acquisition (Guderle et al., 2018; Kulmatiski et al., 2017). Moreover, in tropical ecosystems, light-demanding species tend to have different growth rates and root biomass allocation compared with shade-tolerant species(Markesteijn & Poorter, 2009; Poorter, 2001; Poorter & Bongers, 2006). Light demanding species can show higher transpiration rates and greater leaf-level water demand, therefore necessitating an increased water supply to the leaf and increased stem- and leaf-specific hydraulic conductivity (Chave et al., 2009; Markesteijn, Poorter, Bongers, Paz, & Sack, 2011; Sack, 2004; Tyree, Velez, & Dalling, 1998). However, studies comparing coexisting light-demanding and shade-tolerant species within a sandy tropical forest community considering their water-sources usage are still lacking.

Restinga forests are coastal ecosystems, occupying nearly 80% of the Brazilian coastal fringe, with high biological diversity and a particular floristic composition (Assis et al., 2011; Magnago, Martins, Schaefer, & Neri, 2012; Marques, Silva, & Liebsch, 2015). They occur under a tropical climate and in sandy substrate, characterized by seasonal or perennial waterlogging, low water retention and poor nutrients and organic matter (Oliveira & Joly, 2010; Joly et al., 2012). The main limiting abiotic factors of this ecosystem are considered drought, flooding, salinity and low nutrients (Scarano, 2002). Dry spells on the *restinga*s are common, which may decrease the water availability even during the wet season due to the low water retention of sandy soils (Cavalin & Mattos, 2007). Additionally, road constructions along the coast line in the 60s also had an impact on the flooding dynamics of this ecosystem. *Restinga* forest species possess traits to deal with low water and nutrient availability (Gessler et al., 2007; Rosado, Mattos, & Stermberg, 2013; Rosado, Joly, Burgess, Oliveira, & Aidar, 2016; Rosado & Mattos, 2010), but also to flooding (Oliveira, 2011). Additionally to an interspecific variation of physiological traits, variations in water-use among species can occur. However, how over and understory woody species use water in *restinga* forests remains poorly understood. A differential reliance on water-sources might lead to distinct species responses to fluctuations in water availability in this ecosystem. Determining, through isotopic approaches, where plants mostly acquire water in contrasting hydrological conditions may elucidate not only strategies of water-resources acquisition but also soil-water partitioning among woody species in this ecologically relevant ecosystem.

In this study, we investigated the extent of water-source use differentiation among 15 ecologically dominant *restinga* forest woody species. Our objectives were to determine whether patterns of water-sources-use were different between contrasting water availability conditions and dependent on functional traits such as growth form, plant size and access to light. We further explored the possible influence of competition (density, diversity and biomass) on plants water-sources-use. The characterization of soil-water-use strategies will enhance our understanding of woody species coexistence and the effects that water variations can have on water-resource use in this ecosystem.

2.2.3 Methods

Study site

The study was conducted at Serra do Mar State Park, in the *restinga* forest that occurs at Praia da Fazenda, Picinguaba, municipality of Ubatuba, São Paulo, Brazil. Its soil is classified as a sandy Quartzenic Neosol (90.2% of sand), acidic (pH 3.6), chemically poor and with high dilution of nutrients (Assis et al., 2011; Joly et al., 2012; Scarano, 2002). Its climate is considered Tropical/Subtropical Humid (Af/Cfa) (Joly et al., 2012). In the sampling year (2013), total annual rainfall was 2753 mm (Fig. S1 – Supporting information). This sandy coastal ecosystem is subjected to seasonal or perennial waterlogging (Oliveira & Joly, 2010; Oliveira, 2011) and, although a typical dry season doesn’t occur, it suffers a seasonal variation of water availability (including variations on water-table depth) due to variations in precipitation along the year (Fig. S1).

Our study was conducted at a permanent plot previously installed (1ha, 23° 21’ 22” S; 44° 51’ 03” O) (Joly et al., 2012). The area has a stem density of ~1625 ind.ha^{-1} (DBH $\geq$ 4.8cm), 84 species of 32 families, and is dominated by Myrtaceae, Arecaceae e Euphorbiaceae species (Assis et al., 2011). Within this area, eighteen sampling (sub-) plots (10 m x 10 m) were randomly selected, only assuring that they were not contiguous.

Study plant species

In each plot, the most common woody species were selected. We selected at least two species per plot, and species occurring at least in three plots. We sampled 15 woody plant species, (13 from over and 2 from understory) (Table 1). When possible, we sampled three individuals per species in each plot. The total number of individuals per plot is shown in Table S1. The individuals selected were characterized by their growth form, diameter at breast height (DBH) and crown illumination index (Keeling & Phillips, 2007; Joly et al., 2012; Vieira et al., 2008) (Table 1). Understory shrubs with perimeters <15 cm were all attributed the ‘DBH’ value of 4.7 cm.

Table 1. Woody species sampled and their: growth form classification, mean diameter breast height (DBH) and crown illumination index range (CII). 'n' refers to number of individuals sampled in each sampling period.

Species	**n**	**Growth form**	**DBH (cm)** (mean±SD)			**CII** (min-max)		
Eugenia schuechiana	7	tree	7.61	±	1.58	3	–	3
Euterpe edulis	4	tree	5.56	±	0.85	1	–	3
Faramea pachyantha	4	tree	13.52	±	5.74	3	–	3.5
Guapira opposita	7	tree	11.83	±	4.52	3	–	3
Guarea macrophylla	4	tree	6.74	±	1.12	3	–	3
Guatteria sp4	9	tree	9.60	±	3.59	3	–	4
Jacaranda puberula	7	tree	17.58	±	7.04	1.5	–	4.5
Marlierea tomentosa	4	tree	10.53	±	2.22	3	–	5
Maytenus littoralis	9	tree	9.16	±	3.88	3	–	4
Myrcia brasiliensis	4	tree	25.02	±	5.53	3	–	4
Myrcia multiflora	13	tree	10.18	±	1.56	3	–	4
Myrcia racemosa	5	tree	10.32	±	3.23	3	–	3
Pera glabrata	5	tree	9.87	±	3.73	3	–	4
Psychotria sp1	48	shrub	<4.78	±	0.00	1	–	1
Psychotria sp2	9	shrub	<4.78	±	0.00	1	–	1
Total (range)	139		(<4.78	–	33.2)	(1	–	5)

Oxygen isotopic composition ($\delta^{18}O$) of water: xylem and sources

To access the water being used by plants, lignified woody stems of each plant individual selected were collected in two hydric conditions: wet and less-wet (Fig. S1). Under wet conditions, precipitation was highly available and water-table close to surface, while in the less-wet condition soil water moisture of shallow soil layers was minor and groundwater table deeper (Fig. S1).

To determine available water sources for the roots, water samples were collected from soil, rain and groundwater (within the study area). Rainwater was sampled on a pluviometer and groundwater collected in 3 piezometers reaching the water table on the two periods of stem sampling. In each sampling plot, 3 replicates of soil samples were collected at 3 depths: 10 cm, 30 cm and 50 cm, on the same days of stem sampling (total of soil samples = 18 plots x 3 points x 3 depths x 2 sampling periods = 324). Whenever groundwater was above 50 cm deep (wet period), we consider only the depths till reaching water-table (plus groundwater) as possible water sources (per plot). Both soil and twig samples were immediately stored in airtight vials, sealed with a cap and parafilm and placed in a cool ice chest until they were stored in the fridge until further laboratory analysis.

Water from xylem and soil samples was extracted using a custom-made cryogenic vacuum distillation system housed at SIIAF (Ehleringer & Dawson, 1992; West, Hultine, Burtch, & Ehleringer, 2007).

When the isotopic fraction of the available water sources (soil water, rain, and groundwater) is known, an analysis of the oxygen isotopic composition of xylem water provides information about the water sources being used by the plant at the time of study (Dawson & Pate, 1996; E. Dawson, 1993; Ehleringer & Dawson, 1992). Oxygen stable isotope ratio analyses were performed by headspace equilibration, on an Isoprime (Micromass, UK) SIRMS, coupled on continuous flow mode to a Multiflow (Micromass, UK) auto-sampler and sample equilibration system. Analytical uncertainty is <0.1‰.

Soil water at 10, 30 and 50 cm depth and groundwater are from now on named SW_{10}, SW_{30}, SW_{50} and GW respectively.

Quantification of water sources used by dominant woody species

The relative contribution of different water sources to the composition of the xylem water was estimated by Bayesian stable isotope mixing models using the graphical user interface and model framework MixSIAR for R (Parnell et al., 2013; Stock, Jackson, Ward, & Venkiteswaran, 2016). In our study, we calculated the water use proportion in each sampling period separately, using a species(plot)-based model to obtain separate estimates for each species within each plot. The models used as input the individual isotope values of xylem water $\delta^{18}O$ of each plant ('mixture' raw data), all sources described in water sources per plot (mean plus standard deviation) and a matrix for potential fractionation (set to 0) (Fig. S3). We considered 'species' within 'plot' a fixed factor and the sources varying by plot. In the cases where groundwater was at ≤50 cm depths (wet period), isotopic signal of SW_{50} was assumed as equal to groundwater and model ran with the appropriate water sources. We set the Markov Chain Monte Carlo to 'long' or 'very long' model run, until the diagnostics were satisfying.

The only exceptions of this approach were the cases where we denoted a clear disparity of DBH between the individuals from the same species within the plot. In these cases (see Table S1), we ran models using an individual-based model. We calculated individual water use proportion in each plot separately, using plant values of xylem water $\delta^{18}O$, the sampling plot water sources mean (plus standard deviation) and the 'process only (n=1)' error structure option.

Statistical analysis

We tested if the water-source-use patterns were dependent on water availability or functional traits, analyzing the effects of Water conditions of the sampling period (SPWC), Growth form (GFORM), Diameter breast height (DBH) and Crown illumination index (CII) on the proportion of each water-source used by the species (SW_{10}, SW_{30}, SW_{50}, GW) (N=166, 83 per sampling period, see Table S1). For that, we used mixed linear, random intercept models in which SPWC (less-wet, wet), GFORM (shrub, tree), DBH (species mean within plot) and CII (species mean within plot) were used as fixed effects, and species within plot ('SpID') as random effects, using the R package 'nlme' (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2013) [model = lme(MeanSourceUsed ~ SPWC + GFORM + DBH + CII, random= ~1|SpID)].

Variation of water-sources used by plants between the contrasting water conditions (water-use variation, %) were calculated as the difference between the water-source proportion used in less-wet period and the water-source proportion used in the wet period (for each species within plot separately). Negative values would represent decrease in the proportion of the water-source used from wet to less-wet conditions, and positive values an increase of its use. We tested the effects of GFORM, DBH and CII on the water-use variation, considering each water source separately, using the analysis of variance (ANOVA) [Anova(lm(WUvariation~GFORM+DBH+CII))]. Preliminary analyses were performed to ensure that there was no violation of statistical assumptions.

We further explored the influence of density, diversity and biomass on plants water-sources-use and on water-source-use variation. For that, for each plot (n=18) we considered (a) the number of individuals with DBH > 4.8 cm (Density), (b) the Shannon's equitability index calculated as E_H = H/ ls(N) (i.e., Evenness), and (c) the total Biomass (kg, calculate following Vieira et al. 2008). As dependent variables, we considered (i, ii) the mean proportion of each water-source used (%) in both wet and less-wet conditions, (iii) the mean variation between sampling water conditions of water-sources-used by plants, and (iv) the standard deviation of the proportion of each water-source used (%) in both wet and less-wet conditions (representing the variability of water-sources-use). We performed Spearman rank correlations between Diversity, Evenness and Biomass (a-c) and all the variables related with water-sources-use (i-iv). When significant ($p<0.05$), a further linear or quadratic regression was applied.

All statistical analyses were performed in R 3.2.3 (R Core Team, 2015).

2.2.4 Results

After testing the effects of the water conditions (sampling period), growth form, plant size and light access on water-use of woody species, we found that only hydric conditions at the sampling periods had a significant effect on plants' water-use (Table 2). Accordingly, the water used by plants was not influenced by growth form, plant size (assessed by diameter at breast height, DBH) or light access (assessed by crown illumination index, CII) (Table 2).

Table 2. Summary of linear mixed model of the water-sources used by plants (n=166). Explanatory variables tested: Sampling water conditions (SPWC; less-wet, wet), Growth form (shrub, tree), Diameter breast height (DBH) and Crown illumination index (CII). Bold represents significant effects ($p<0.05$).

	Effect	Estimate	SE	t-value	p-value
SW_{10}	(Intercept)	0.191	0.033	5.705	**<0.0001**
+Pp	SPWC	0.622	0.022	27.835	**<0.0001**
	Growth Form	0.019	0.051	0.377	0.707
	DBH	-0.0003	0.002	-0.109	0.913
	CII	-0.030	0.022	-1.337	0.185
SW_{30}	(Intercept)	0.206	0.034	6.022	**<0.0001**
	SPWC	-0.085	0.021	-4.081	**0.0001**
	Growth Form	-0.012	0.053	-0.231	0.818
	DBH	-0.003	0.002	-1.192	0.237
	CII	0.043	0.023	1.875	0.064
SW_{50}	(Intercept)	0.549	0.031	17.704	**<0.0001**
	SPWC	-0.478	0.021	-23.109	**<0.0001**
	Growth Form	-0.007	0.048	-0.151	0.880
	DBH	0.003	0.002	1.616	0.110
	CII	-0.023	0.021	-1.112	0.269
GW	(Intercept)	0.056	0.009	6.554	**<0.0001**
	SPWC	-0.051	0.005	-10.569	**<0.0001**
	Growth Form	0.007	0.013	0.548	0.585
	DBH	-0.0003	0.001	-0.549	0.585
	CII	0.004	0.006	0.642	0.523

The composition of xylem water in the wet condition was better explained by precipitation mostly present at top-soil (SW_{10}), while water used by woody species during the less-wet condition was mainly from 50 cm soil layers (Fig. 1, Fig. S2ab). Within the less-wet period, the species showed a similar use of the water-sources, with only *Myrcia racemosa* and *Maytenus littoralis* showing a higher proportion of SW_{30} use (Fig. S3).

All species increased the use of soil water at 50 cm deep from wet to less-wet conditions, and reduced the use of top-soil water (Fig. 1, Fig. S4). None of the functional traits (growth form, DBH, CII) significantly influenced the (temporal) variation of plants' water-use (Table S2).

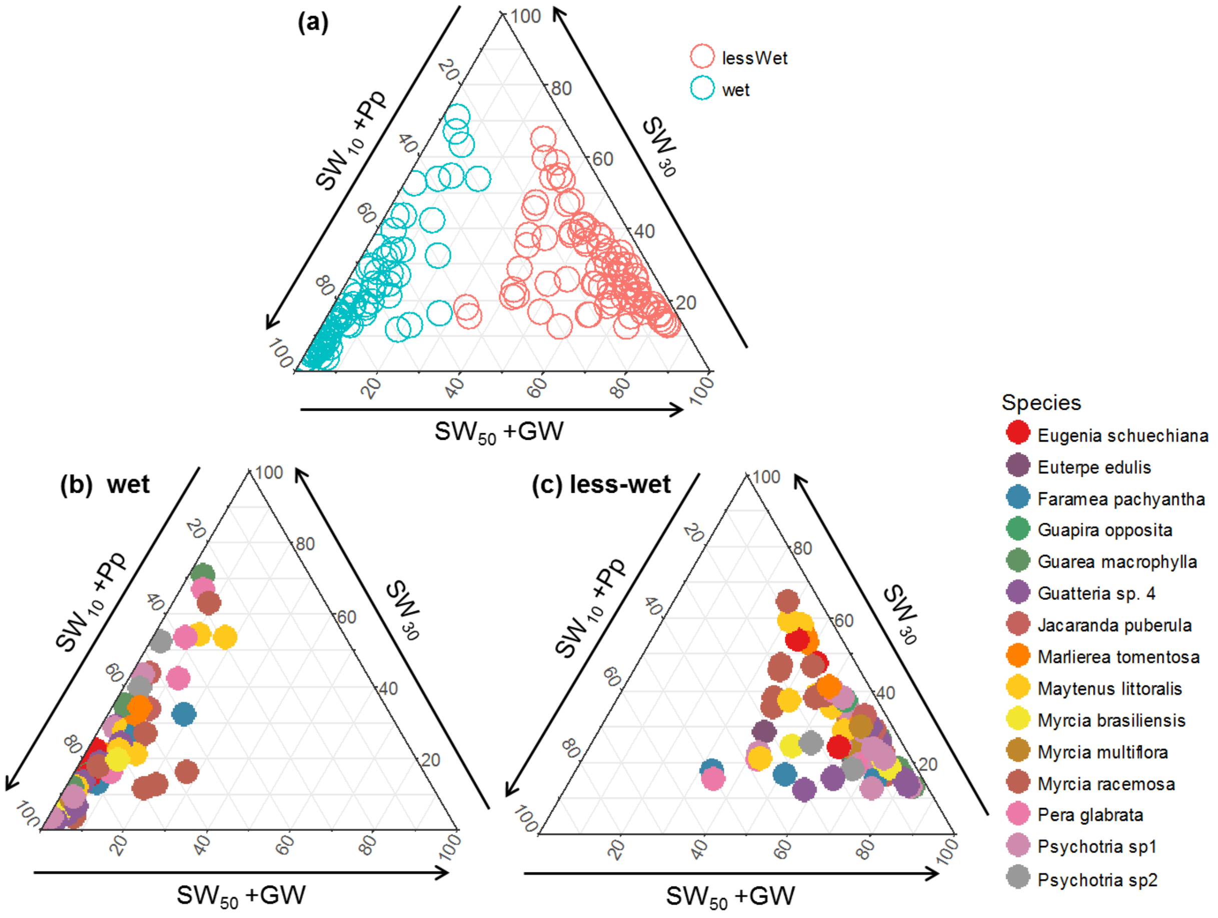


Figure 1 Water sources proportion used by plants in (**a**) both sampling periods (contrasting water conditions), (**b**) the wet period, and (**c**) less-wet period. Water sources considered were: soil water at 10 cm, 30 and 50 cm (SW_{10}, SW_{30}, SW_{50}), precipitation (Pp) and groundwater (GW). For figure simplification, rain-water was aggregated to $SW_{10;}$ and GW to SW_{50} (posteriorly to mixing model calculation). **(a)** Overall woody vegetation water use in wet (red) and less-wet (blue) periods (n=82 in each season). **(b)** Species water use in the wet period. **(c)** Species water use in the less-wet period. Species are represented by different colors as presented in inner legend.

Thus, we found a significant shift towards deeper soil layers under less-wet conditions made by the overall woody vegetation, regardless their size or light access (Fig. 2). From wet to less-wet conditions, the mean increase of SW_{50} use by woody vegetation was 48% and the decreased of SW_{10} use was 61%.

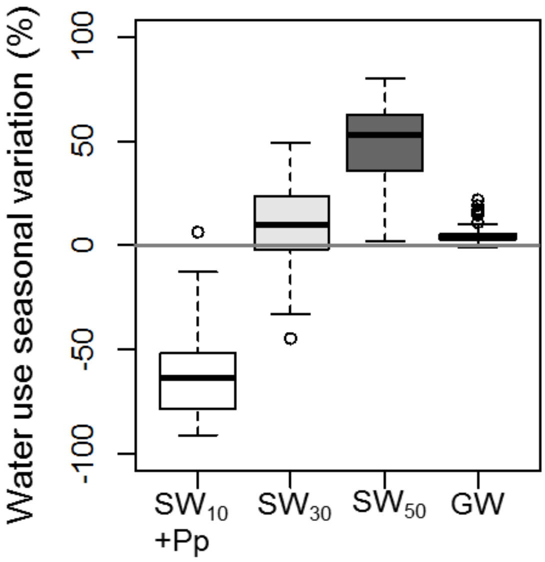


Figure 2 Water-use variation (wet to less-wet conditions, %) considering the overall woody community (n=82). Water sources considered were: soil water at 10 cm, 30 cm and 50 cm (SW_{10}, SW_{30}, SW_{50}), precipitation (Pp) and groundwater (GW). Rain-water was aggregated to $SW_{10.}$ Grey horizontal line represents null seasonal variation (0%).

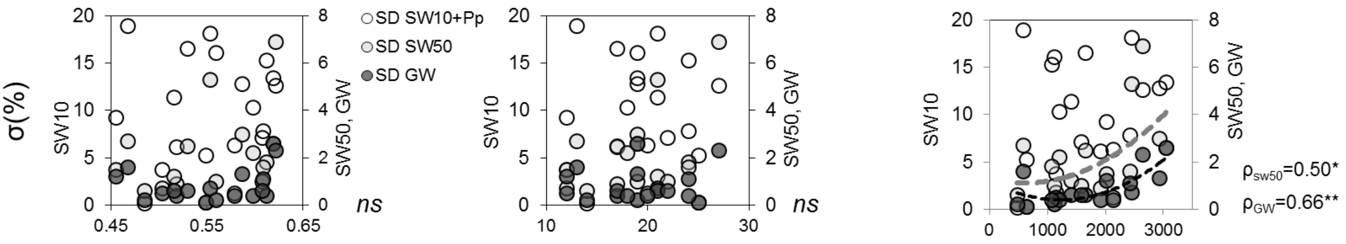
We further explored the influence of potential competition (using plot density, evenness and biomass) on plants water-use in both sampling periods. Neither density nor evenness showed a significant correlation with the mean water-sources used in both wet and less-wet conditions (Table 3). Biomass also didn’t show any significant effect on the proportion of water-sources used in the less-wet season or on its variation between contrasting water conditions (Table 3). Contrastingly, biomass did show a significant effect on the use of soil water from 50 and >50 cm deep (groundwater-use) in the wet period (Table 3). However, a lower contribution of these water-sources occurs in the wet season (Fig. 1). Thus, the main water-source used by plants (SW_{10} in wet periods, and SW_{50} in less-wet conditions) was not influenced by any of the structural characteristics (Table 3).

Table 3. Spearman correlations (coefficient *r*) between diversity and density variables (Evenness, Density and Biomass) and water sources used by plants (mean % of contribution of each water source considered in less-wet and wet sampling periods, and the mean variation of each water source considered). Significant correlations are denoted as *p<0.05, **p<0.01, ***p<0.001. n=18.

		Evenness	Density	Biomass (kg)
wet	SW_{10}+Pp	-0.43	-0.20	-0.36
	SW_{30}	0.44	0.25	0.23
	SW_{50}	0.38	0.15	0.53*
	GW	0.30	0.11	0.73***
less-wet	SW_{10}+Pp	0.24	0.08	0.44
	SW_{30}	0.31	-0.07	0.27
	SW_{50}	-0.19	0.00	-0.30
	GW	0.26	0.13	0.38
variation	ΔSW_{10}+Pp	0.10	-0.09	0.12
	ΔSW_{30}	-0.11	-0.12	-0.09
	ΔSW_{50}	-0.23	-0.01	-0.35
	ΔGW	0.06	-0.03	0.01

Assessing the effects of the potential competition variables on the variability in the water-use (standard deviation of the proportion of water-sources used per plot), we found a positive significant correlation during the less-wet period of both density and evenness (Fig. 3b). Biomass showed, although significant, a weaker correlation (Fig. 3b). Thus, greater mean variability was found in places where density, evenness and biomass were greater, but only in the less-wet period (Fig. 3ab). However, the range of mean variability of water-sources-use assessed in less-wet condition was short: 2 to 13 %.

(a) *wet*



(b) *less-wet*

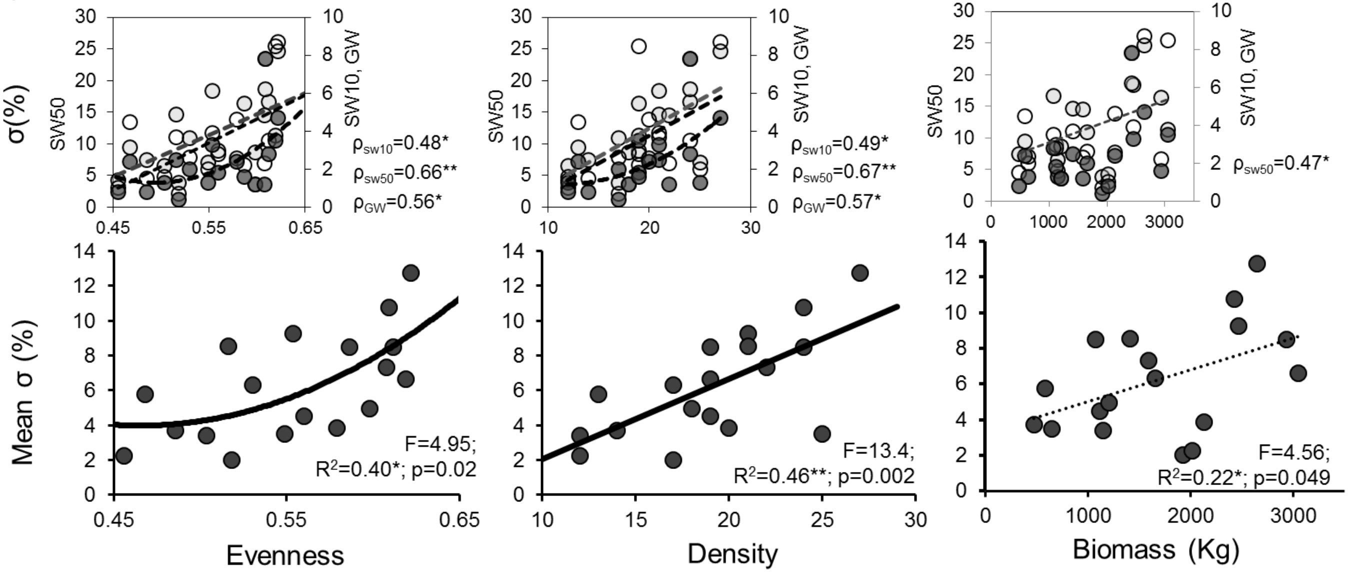


Figure 3. Relationships between abundance variables [Diversity index Evenness, Density and Biomass] and variability of the proportion of water-sources used by plants [σ], in (a) wet and (b) less-wet conditions. (a,b) The water-sources are represented by different colors as indicated in the inner legend. Mean σ (%) is only represented when significant correlations occurred (i.e. less-wet condition). Dashed lines represent the main trend of significant spearman correlation (spearman rho and p-value, $*p<0.05$, $**p<0.01$, $***p<0.001$, indicated in the figure), solid lines the regression fit (either quadratic or linear) when regressors explained more than 25% of the variance, and dotted line the regression fit of very weak significant correlation (further statistical information given in the figure: F- value 'F', R^2 and p-value). *ns* stands for non-significant correlations ($p>0.05$). n=18.

2.2.5 Discussion

We found that the decrease of water availability modified water-sources-use of *restinga* forest's plants, towards less shallow soil layers. From wet to less-wet conditions, woody vegetation shifted their water-uptake from top-soil to 50 cm deep regardless their size, growth form or crown illumination. The common adjustment in water uptake depth implies that woody community has a homogeneous response to water availability and similar strategies of water-resources acquisition. It further points to an absence of a significant soil-water partitioning along a vertical soil profile among woody species (i.e use of water from different depths) within each water condition. In other words, there was a temporal change in the main water-sources used by plants, but there was not a divergence in the water-sources-use among plant functional types.

Differing from other (drier) ecosystems, trees were not using deeper soil layers than understory shrub species (Antunes et al., 2018; Dawson, 1996; Schenk & Jackson, 2002; Scott et al., 2003). Within each water conditions, we did not observed a high degree of partitioning of water resources among species or life-forms, which contrast with other lowland tropical forest studies (Jackson, Cavelier, Goldstein, Meinzer, & Holbrook, 1995). Species sensitive to flooding as *G. opposita* (Oliveira, 2011), did not show a different pattern of water-sources-use compared to the others. Although as been reported that tree size affect water uptake (source and amount) in tropical forest tree species (Bennett, Mcdowell, Allen, & Anderson-Teixeira, 2015; Jackson et al., 1999; Meinzer et al., 1999, 2013), we did not found any control of this factor on plant water-sources-use. However, we acknowledge that although plant size and/or access to light don't affect water-sources used by plants, it can still have important effects on plants physiological (leaf level) performance, particularly plant water status.

Plant communities comprising species with different root water-uptake patterns might be able to better acquire unexploited soil resources compared to communities containing species with similar root distributions (Guderle et al., 2018; Mueller, Tilman, Fornara, & Hobbie, 2013; Schenk, 2006; Silvertown, 2004). Interestingly, in the studied *restinga* forest, such differentiation in rooting patterns among species was not evident and may result in community-specific root water uptake overlap. An efficient water acquisition in this plant community would thus be sustained by enough water supply, other physiological strategies or further water-uptake plasticity (Guderle et al., 2018; Schwendenmann, Pendall, Sanchez-Bragado, Kunert, & Hölscher, 2015; Zea-Cabrera, Iwasa, Levin, & Rodríguez-Iturbe, 2006). Root water uptake profiles shifts may be related to root hydraulic properties (Kulmatiski & Beard, 2013), transpiration of leaves (Jackson, Sperry, & Dawson, 2000; Rosado, Mattos, & Stermberg, 2013; Rosado, Joly, Burgess, Oliveira, & Aidar, 2016), as well as the architecture of root systems and amount of fine roots (de Oliveira Carvalheiro & Nepstad, 1996; Nepstad et al., 1994; Rosado et al., 2011). In fact, the observed water-sources-use changes were accompanied by changes in belowground investment: there was a decrease in the production of fine-roots in the upper soil layer (0-10 cm) from wet to less wet conditions (May to November) (Silva, 2015). As top-soil dries, the use of the water from this layer decreased and implied a decrease in fine-root production on 0-10 cm soil layer (and high turn-over rates). This change in fine-root biomass, similarly to our data, was not dependent on plot total biomass or diversity but rather on the overall water conditions (Silva, 2015). This further underpins temporal/seasonal water availability as a driving force of changes not only on belowground investments (Silva, 2015; Rosado et al., 2011) and on water-use (Rosado et al., 2015) but also on water-sources-use in *restinga* forest woody community.

Unlike other tropical studies (e.g. Rossatto et al. 2012), mean water uptake depth patterns were not linked to vegetation structure. Regardless the density or diversity, there was a maintenance of high proportion of water-use of top-soil (~76%) in the wet period and high proportion of use of soil-water from 50 cm deep (~60%) in the less-wet period. However, where the denser structure predominated, a larger range of different strategies of water uptake occurred. Rather than affecting the main water source used it implied a higher variability. Although short, this variability in patterns of use of water from different depths could help to reduce inter-specific competition and in turn allowing coexistence of a larger number of different species in the same habitat (Silvertown, Dodd, Gowing, & Mountford, 1999). As micro-topographic variations within the study area exists, the positive correlations observed between total biomass and use of water from 50 cm in the wet season can be related to the fact that higher biomass occur where unsaturated soil layers are deeper. Nevertheless, this underpins the weak direct effects that biotic competition had in the patterns of water-sources-use of *restinga* woody species. It further supports the importance of soil water accessibility, particularly groundwater availability to root water uptake (Evaristo & McDonnell, 2017; Kulmatiski, Adler, Stark, & Tredennick, 2017).

Under wet conditions, the proximity of groundwater-table and the overall water availability seems to sustain a low competition for this resource, but also imply a low potential range of water uptake strategies. At coastal lower elevation sites, as *restinga* forests, a more superficial groundwater associated with shallower unsaturated soil profiles could strongly restrict root growth, affecting the range of soil space that can be explored by woody species (Fan, Li, & Miguez-Macho, 2013; Fan, Miguez-Macho, Jobbágy, Jackson, & Otero-Casal, 2017; Joly et al., 2012). Seasonal waterlogging limits roots to the oxygenated soils above the water table, and shallow or aerial roots are common in lowland forests (Fan et al., 2017; Pavlis & Jeník, 2000; Stone & Kalisz, 1991). These restrictive conditions would tend to limit water partitioning among species, reflecting a higher overlap in the use of specific layers of soil, especially in the wet season. In fact, variations in local soil water profile, driven by infiltration above and drainage below, are known causes for rooting depth variations (Evaristo et al., 2017; Fan et al., 2017; Stone and Kalisz 1991). At this sandy soil, roots may easily sense groundwater capillary rise (and capillary fringe) at sites with shallow groundwater table, and in wet periods infiltration commonly meets capillary rise. In periods of lower precipitation, groundwater table lowering occurs and top-soil moisture decreases, and consequently roots will explore deeper unsaturated soil layers (mainly 50 cm deep) where greater moisture is likely to be found. Accordingly, during dry spells (less-wet conditions) it is probable that all the woody species, and as no apparent water competition occurred, were exploring the most favorable layer of soil. More than 50% of the water uptaken by plants was supplied by deeper soil layers (≥ 50 cm deep), suggesting that this increase in deep soil reliance is a fundamental mechanism to cope with top-soil water scarcity. It highlights the relevant role that groundwater depth can have on supplying moisture to upper soil layers and on molding the plants' reliance on specific water-sources.

2.2.6 Conclusion

We found a temporal variation in water-sources-use, but no vertical soil-water partitioning among the woody functional types within each water availability condition. Relevant seasonal adjustments of water uptake depth in neotropical ecosystems have been already observed (Jackson et al., 1999; Markewitz, Devine, Davidson, Brando, & Nepstad, 2010; Meinzer et al., 1999; Oliveira et al., 2005; Rosado, Joly, Burgess, Oliveira, & Aidar, 2016; Rossatto, de Carvalho Ramos Silva, Villalobos-Vega, Sternberg, & Franco, 2012). However, to our knowledge, none have disentangled the water-sources-use of *restinga* forests under contrasting water availability conditions. Our study supports the idea that plants water-sources-use is affected by dynamic shifts in water uptake due to water availability changes. A pre-requisite for such changes is the ability of plant species to rapidly acclimate and explore different soil layers: shallow water-sources in wet and deeper in less-wet conditions. Interestingly, this dynamic water uptake was not enhanced in more diverse plots or evoked by specific plant traits such as plant size. Thus, in spite of differential ability of plants to cope with water shortage and flooding, plants may show convergence of water-use responses to soil water availability. Particularly, groundwater depth seems to be a most constraining factor of water-sources-use by the woody community. In wet season, a shallow water-table limits roots to top-soil layers and in less-wet season, with the absence of rain and deeper water-table, plants generally explore deeper unsaturated soil layers where moisture is higher due to capillary rise of water-table.The dangers of flooding are well known, but this study provides isotopic evidence of the almost exclusive dependence of woody species of *restinga* on 50 cm soil water layers (highly dependent on groundwater bottom-up inputs) in dry periods. This suggests that a more extreme lowering of water table could be a possible threath to the continued development and survival of woody species. It is thus necessary to manage dry season groundwater levels so that they remain accessible to woody *restinga* plants. Further studies of how spatial gradients of groundwater depth affects water-uptake depth in this system are of great interest. Additionally, it would be of most importance to know how these water-source-use patterns are related with physiological status of woody species in this ecosystem.

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2.2.9 Supporting Information

Table S1. Number of samples of woody species sampled per plot in each sampling campaign.

Table S2. Results of the variance analysis, testing the effect of growth form, plant size (DBH) and light access (CII) on the (temporal) variation of the use of different water-sources.

Figure S1 Meteorological data and groundwater-table depth of the study site (*Restinga* forest).

Figure S2. Oxygen isotopic composition ($\delta^{18}O$) of xylem water of the studied 15 species and water sources considered at wet and less-wet conditions.

Figure S3. Water sources contribution to xylem water considering the 15 woody species studied in the less-wet period.

Figure S4. Seasonal variation (in %) of water-sources used by plants (differences between less-wet and wet period).

Supporting information

Table S1. Number of samples of woody species per plot (in each sampling campaign). Species names described in Table 1 and Fig S2. Numbers in bold denote cases of plats consider individually (as referred in Methods section), being all other considered as a mean value of species' water-sources-use.

	plot	Ee	Es	Fp	Gm	Go	Gsp	Jp	Mb	Ml	Mm	Mr	Mt	Pg	Psp1	Psp2	
1	L001	1				1									3		
2	L100							2		2					3		
3	L013							1	1		2				3		
4	L020	3			1												
5	L025	1		2		1		1			1				3		
6	L031				1		1	2						1	3	3	
7	L033									1		1		1	3	3	
8	L038											4	1		3		
9	L046		1			1				1		1	1				
10	L053	2		1					1					**2**	3		
11	L006														3	3	
12	L060		1	1		1						**4**	1		3		
13	L067		1				1	1		2		2		1	3		
14	L074				2		2			1			2		3		
15	L078		1				**3**			**2**					3		
16	L008				3				1		1	1			3		
17	L086						1		1						3		
18	L094						1								3		
Total samples		7	4	4	7	4	9	7	4	9	4	13	5	5	48	9	*139*
Total plots		4	4	3	4	4	6	5	4	6	3	6	4	4	16	3	*76*
Total n after mixing model		4	4	3	4	4	8	5	4	7	3	9	4	5	16	3	*83*

Table S2. Results of the variance analysis (ANOVA) conducted to test the effect of growth form, plant size (DBH) and light access (CII) on the (temporal) variation of the use of different water-sources. Water sources codes as described in methods.

		SW_{10} + Pp			SW_{30}		
Effect	df	SS	F	p	SS	F	p
Growth Form	1	0.033	0.713	0.401	0.049	1.298	0.258
DBH	1	0.087	1.890	0.173	0.002	0.040	0.842
CII	1	0.132	2.868	0.094	0.044	1.185	0.280
residuals	79	3.642			2.967		

		SW_{50}			GW		
Effect	df	SS	F	p	SS	F	p
Growth Form	1	0.010	0.281	0.598	0.000	0.018	0.893
DBH	1	0.093	2.519	0.117	0.002	1.103	0.297
CII	1	0.048	1.297	0.258	0.000	0.046	0.832
residuals	79	2.931			0.151		

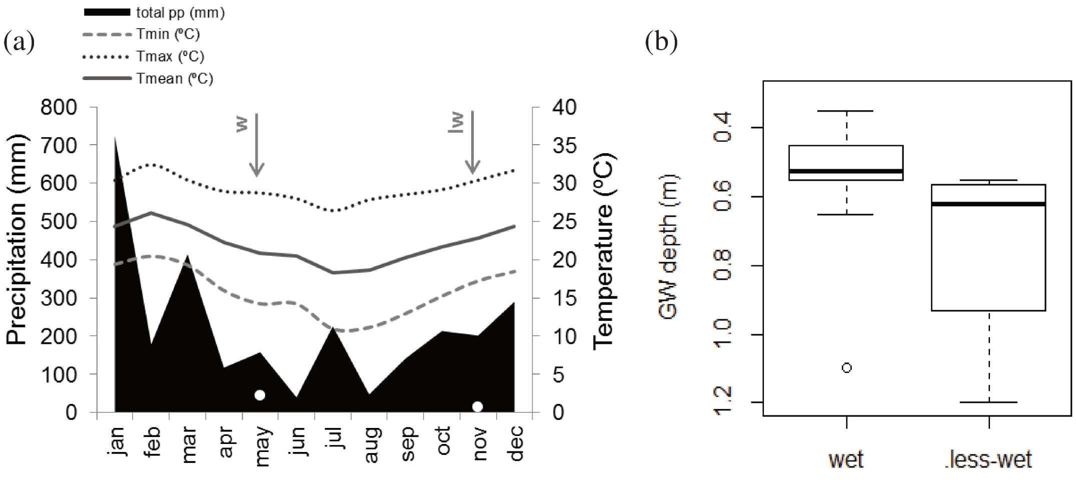

Fig. S1 Meteorological data and groundwater-table depth of the study site *restinga* forest. (a) Monthly total precipitation (total pp, left y-axis, mm), Monthly mean temperature (Tmean, grey line, right y-axis, ºC), Monthly mean of maximum and minimum temperature (Tmin and Tmax, dashed grey line, right y-axis, ºC), from January till December 2013. Data from Ubatuba climatic station (CIIAGRO online). Wet and less-wet sampling campaigns marked as down arrows (w and lw, 25-30/May and 25-30/Nov, respectively). White dots denote weakly precipitation of sampling campaigns. (b) Groundwater-table depth (GWdepth, m) in the wet and less-wet sampling dates (n=14 and n=3, respectively).

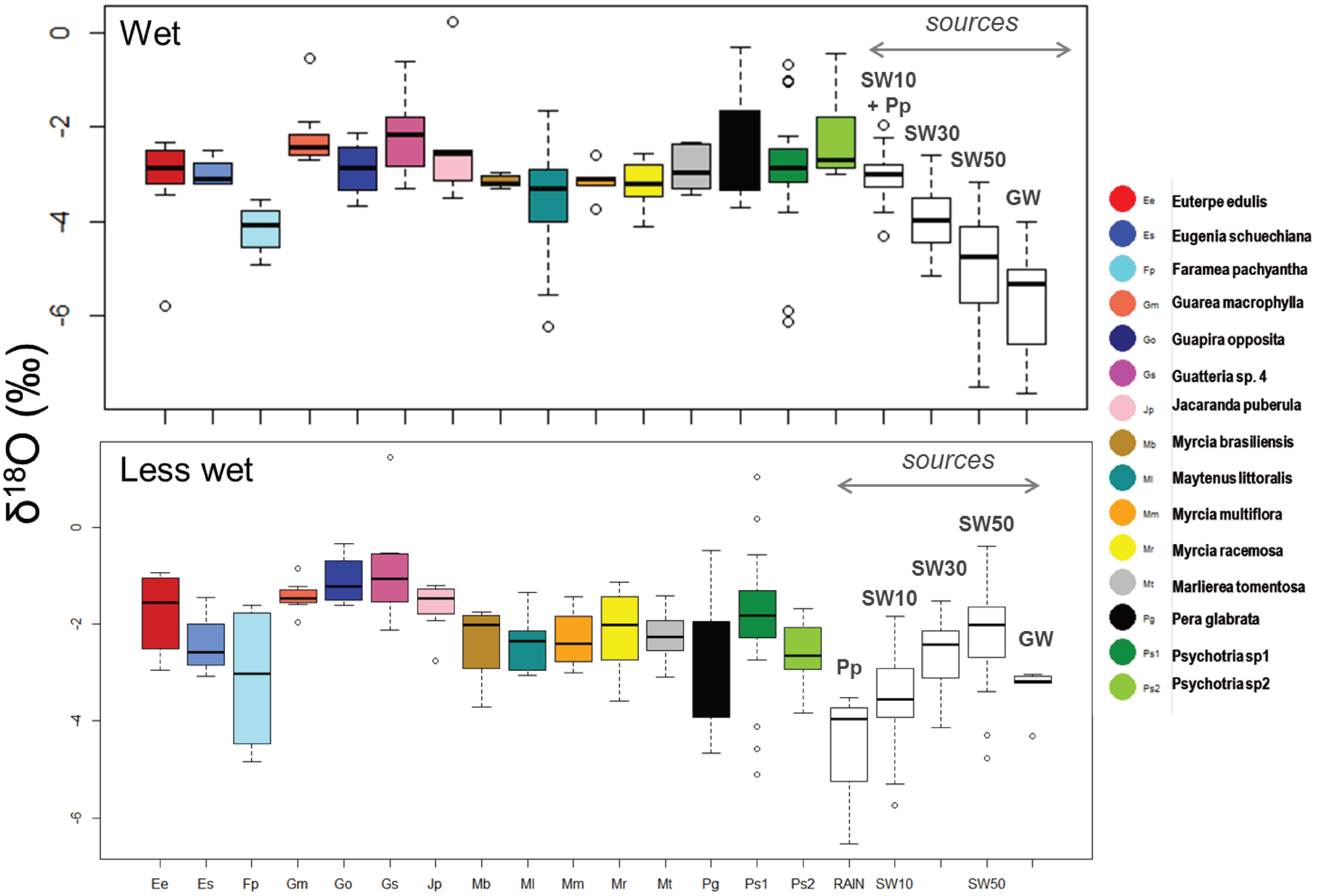

Figure S2. Oxygen isotopic composition ($\delta^{18}O$) of xylem water of the studied species and water sources considered at wet and less-wet conditions. Species names in right inner legend. Sources considered were: (spring) precipitation (Pp), soil water at 10 cm (SW10), 30 cm (SW30), 50 cm (SW50) and groundwater (GW). Precipitation was aggregated with SW_{10} in wet period, since it presented a similar isotopic signal.

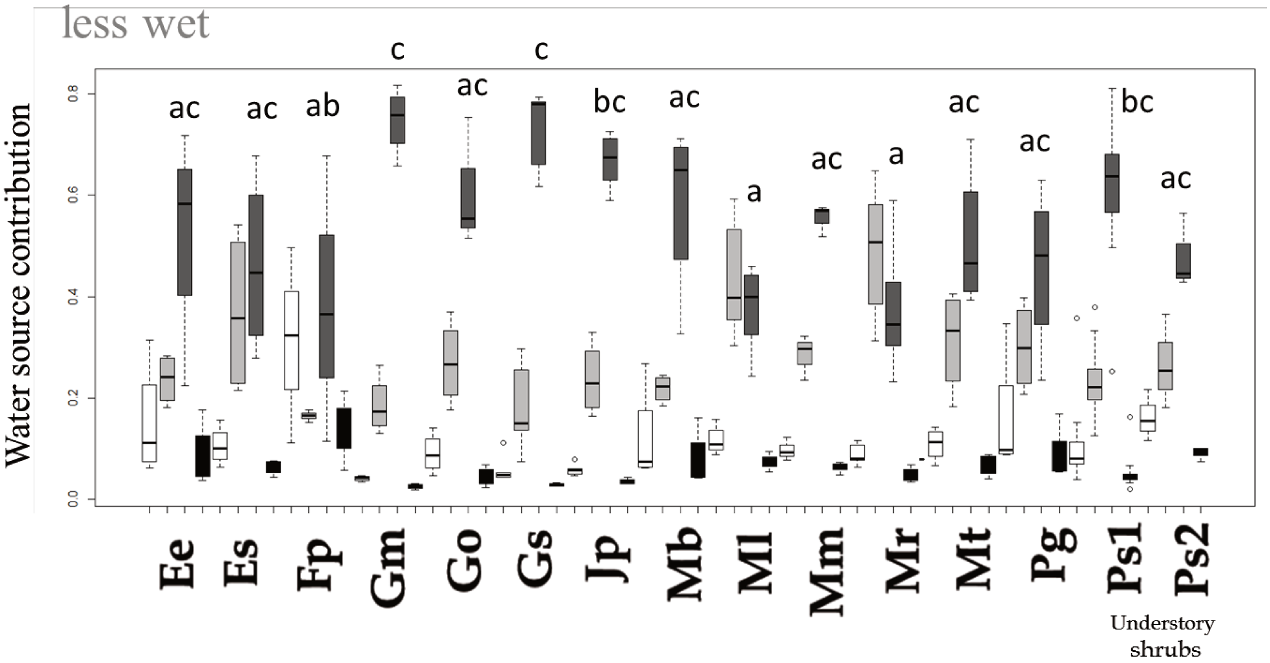

Figure S3. Water sources contribution to xylem water of the woody species studied in the less-wet period. Water sources considered were: soil water at 10, 30 and 50 cm (SW_{10}, SW_{30} and SW_{50}) and groundwater (GW), represented by white, grey, dark grey and black, respectively. Note that precipitation was aggregated with SW_{10}. Significant differences of variation of the main water-source used (SW_{50}) among species are represented by different letters. For species names, see Table 1 or Figure S2 inner legend.

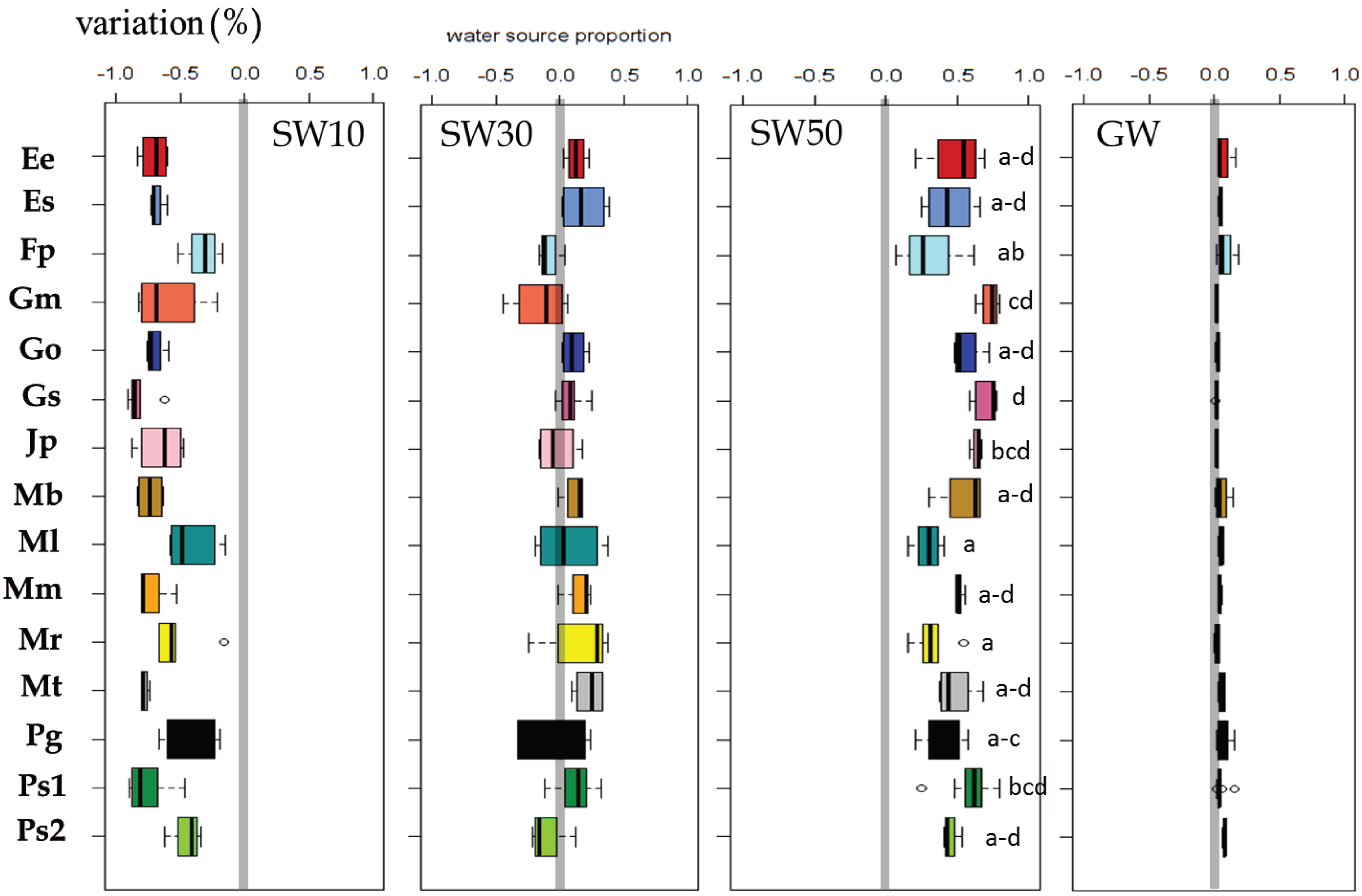

Figure S4. Seasonal variation (in %) of water-sources used by plants (differences between less-wet and wet period). Water sources considered are soil water at 10, 30 and 50 cm (SW_{10}, SW_{30} and SW_{50}) and groundwater (GW). Note that precipitation was (posterior) aggregated with SW_{10}. Grey line represents null seasonal variation (0%). Significant differences of variation of the water-source that suffered the highest seasonal increase (SW_{50}) among species are represented by different letters. For species names, see Table 1 or Figure S2 inner legend.

Chapter 3

Ecophysiological responses of woody vegetation to groundwater depth in mediterranean coastal dune ecosystems

3.1 Contrasting plant water-use responses to groundwater depth in coastal dune ecosystems

Antunes C., Díaz Barradas M.C., Zunzunegui M., Vieira S., Pereira, Â., Anjos A., Correia O., Pereira M.J., Máguas C. (2018) Contrasting plant water-use responses to groundwater depth in coastal dune ecosystems. *Functional Ecology,* 32 (8): 1931-1943. http://dx.doi.org/10.1111/1365-2435.13110 .

3.2 Groundwater drawdown drives ecophysiological adjustments of woody vegetation in a semi-arid coastal ecosystem

Antunes C., Chozas S., West J., Zunzunegui M., Diaz Barradas M.C., Vieira S., Máguas C. (2018) Groundwater drawdown drives ecophysiological adjustments of woody vegetation in a semi-arid coastal ecosystem. *Global Change Biology*. https://doi.org/10.1111/gcb.14403

3.1 Contrasting plant water-use responses to groundwater depth in coastal dune ecosystems

Antunes C., Díaz Barradas M.C., Zunzunegui M., Vieira S., Pereira Â., Anjos A., Correia O., Pereira M.J., Máguas C. (2018) Contrasting plant water-use responses to groundwater depth in coastal dune ecosystems. *Functional Ecology,* 32 (8): 1931-1943. http://dx.doi.org/10.1111/1365-2435.13110

3.1.1 Abstract

Groundwater lowering can produce dramatic changes in the physiological performance and survival of plant species. The impact of decreasing water availability due to climate change and anthropogenic groundwater extraction on coastal dune ecosystems has become of increasing concern, with uncertainties about how vegetation will respond in both the short and long terms.

We aimed to evaluate the water-use responses of different plant functional types to increasing groundwater table depth and how this would affect their physiology in Mediterranean coastal dune systems differing in aridity.

We modeled water table depth, quantified the contribution of different soil layers to plant water through Bayesian isotope mixing models, and used a combination of spectral and isotope data to characterize plant ecophysiology. We found that increasing depth to groundwater triggered water uptake adjustments towards deeper soil layers only in the dry season. These adjustments in water sources use were made by conifer trees (*Pinus pinea, P. pinaster*) and hygrophytic shrubs (*Erica scoparia, Salix repens*) but not by the xerophytic shrub *Corema album*. Moreover, we observed a greater use of groundwater under semi-arid conditions. Accompanying the greater use of water from deep soil layers as a response to increasing groundwater depth, the semi-arid dimorphic-rooted conifer tree *P. pinea* and hygrophytic shrub *E. scoparia* declined their water content (WI), without implications on photosynthetic parameters, such as chlorophyll content (CHL), photochemical index (PRI) and $\delta^{13}C$. Unexpectedly, under semi-arid conditions, the shallow-rooted xerophytic shrub *C. album*, associated with an absence of water-sources-use adjustments, showed a decline in WI, CHL, and PRI with groundwater table lowering.

We provide insight into how different species, belonging to different functional types, are acclimating to groundwater changes in a *region* experiencing climatic drought and a scarcity in groundwater due to anthropogenic exploitation. Greater depth to groundwater combined with limited precipitation can have a significant effect on plants' water-sources use and ecophysiology in semi-arid coastal dune ecosystems.

Key-words: groundwater drawdown; water table depth; humid and semi-arid Mediterranean; water sources; groundwater dependency; plant functional types; physiological adjustments; strategies of water use.

3.1.2 Introduction

Groundwater is an important source of water for plants and humans, especially in environments with a long dry season, such as Mediterranean climate regions (Naumburg, Mata-gonzalez, Hunter, Mclendon, & Martin, 2005; Zencich, Froend, Turner, & Gailitis, 2002). Predicted future droughts under current scenarios of climate change will intensify groundwater limitation in Mediterranean ecosystems, through reduced precipitation and increased evapotranspiration, both of which will diminish recharge and possibly increase groundwater lowering rates (Kirtman et al., 2013; Taylor et al., 2012). Climatic variations influence groundwater patterns in a complex way, with a number of direct and indirect effects, such as those that severely affect coastal sandy ecosystems through salt water intrusion at reduced groundwater inflow conditions (Antonellini & Mollema, 2010). Additionally, groundwater will be affected by the changing patterns of anthropogenic groundwater use, as increasing withdrawal with reduced recharge can significantly decrease groundwater levels (Kløve et al., 2014; Kopp et al., 2013).

Climate and human-induced changes in groundwater/surface water interactions will directly affect groundwater-dependent ecosystems (Cooper, Sanderson, Stannard, & Groeneveld, 2006; Naumburg et al., 2005). In sandy soils, where there is little water retention during dry season, groundwater present at shallow depth could potentially be an important water source for vegetation (Zencich et al., 2002). As such, changes in groundwater can alter the ecosystem water balance and, consequently, lead to shifts in species composition, water-use and physiological performance for terrestrial and riparian vegetation (Sommer & Froend, 2014; Villalobos-Vega et al., 2014). These impacts of groundwater changes will depend not only on local factors, such as groundwater recharge, land-use changes, water holding capacity of the soil layers, precipitation dynamics and human extraction rates, but also on plant species (Asbjornsen et al., 2011). Studies with fully coupled vegetation–hydrology models conducted so far show complex interactions between climate, hydrology and vegetation response capacity, all of which will define community assemblage and performance (Horton, Kolb, & Hart, 2001; Kløve et al., 2014; Naumburg et al., 2005; Silvertown, Araya, & Gowing, 2015; Sommer & Froend, 2011).

Plant responses and acclimation to groundwater limitation imply adjustments at various levels and may vary among functional types (Esquivias, Zunzunegui, Barradas, & Álvarez-Cansino, 2014; Imada, Yamanaka, & Tamai, 2008; Meinzer et al., 2013; Zunzunegui, Diaz Barradas, & García Novo, 1998; Zunzunegui, Díaz Barradas, & García Novo, 2000). Crucial traits in vegetation responses to water source limitation are growth form, rooting depth, water requirement and hydraulic strategy. These traits will determine how and to what degree a plant experiences a given hydric variation (Moreno-Gutiérrez, Dawson, Nicolás, & Querejeta, 2012; Schenk & Jackson, 2002; Silvertown et al., 2015). Thus, differential water-use and physiological responses to groundwater changes are expected to be found when comparing different functional types. Deep-rooted trees can reach water in deeper soil layers and are therefore possibly more buffered from drought than shallow-rooted species (Jackson, Sperry, & Dawson, 2000; Oliveira et al., 2005). Plants that are more dependent on groundwater, such as hygrophytes, when exposed to extreme rates of groundwater lowering, will need to readjust their root system and water uptake to a deeper water table, rely on another water source or be temporarily compensated by soil moisture. Otherwise, plant performance will be compromised and, eventually, plant dieback will occur (Padilla & Pugmaire, 2007; Sperry, Hacke, Oren, & Comstock, 2002). Under strong xeric conditions, declining groundwater levels may change the relative importance of soil water and groundwater as the main water sources, which could lead to physiological adjustments depending on plants' water strategies (Rossatto, de Carvalho Ramos Silva, Villalobos-Vega, Sternberg, & Franco, 2012; Zencich et al., 2002). Still, uncertainties about how the vegetation of sand dunes will respond to groundwater variations in both the short and long terms still exist. Particularly, knowledge gaps remain regarding water uptake depth and physiological adjustments of contrasting plant functional types to a combined effect of low rainwater availability and low groundwater resources exacerbated by human exploitation.

Our aim was to evaluate the water-use responses of different plant functional types to increasing groundwater table depth and how this would affect their physiology in Mediterranean coastal dune systems differing in aridity. We ask 1) how do conifer trees (*Pinus pinaster, P. pinea*), hygrophytic shrubs (*Erica scoparia, Salix repens*), and xerophytic shrub *C. album* vary in water source usage along a gradient of depth to groundwater under different water availability conditions (i.e. at different seasons and aridity conditions), and 2) what the impact of groundwater table depth is on plant physiological performance.

We hypothesize that, under dry conditions, increasing depth to groundwater will lead to (i) a decline in the proportion of groundwater use by hygrophytic shrubs, (ii) the maintenance of a high groundwater use, through water uptake adjustments toward deeper soil layers, by conifer trees, and (iii) an absence of water-use changes by xerophytic shrubs. We also hypothesize that, associated with the water-use adjustments to groundwater table lowering, (iv) hygrophytic shrubs will decrease water content and photosynthetic activity, and (v) deep-rooted conifer trees will be physiologically buffered from the decline of the water table.

3.1.3 Materials and methods

Study sites and plant species

The study was conducted at two coastal dune ecosystems under anthropogenic groundwater lowering and differing in aridity: (i) Mediterranean humid site (*humidMed*) – Osso da Baleia, in the centre of Portugal [aridity index (AI) = 0.91], and (ii) Mediterranean semi-arid site (*semi-aridMed*) – Biological Reserve of Doñana, southwest of Spain (AI=0.47) (UNEP, 1992; http://www.cgiar-csi.org/data/global-aridity-and-pet-database) (Fig. 1). A detailed description of the study sites is presented in Table S1 (Supporting Information). Both sites showed a typical western Mediterranean climate (Fig. S1). The humidMed site showed a higher amount of precipitation (total annual$_{\text{Aug2012-Aug2013}}$ rainfall = 755.4 mm) comparing to the semi-aridMed site (total annual$_{\text{Aug2012-Aug2013}}$ rainfall= 566.1 mm) (Fig. S1).

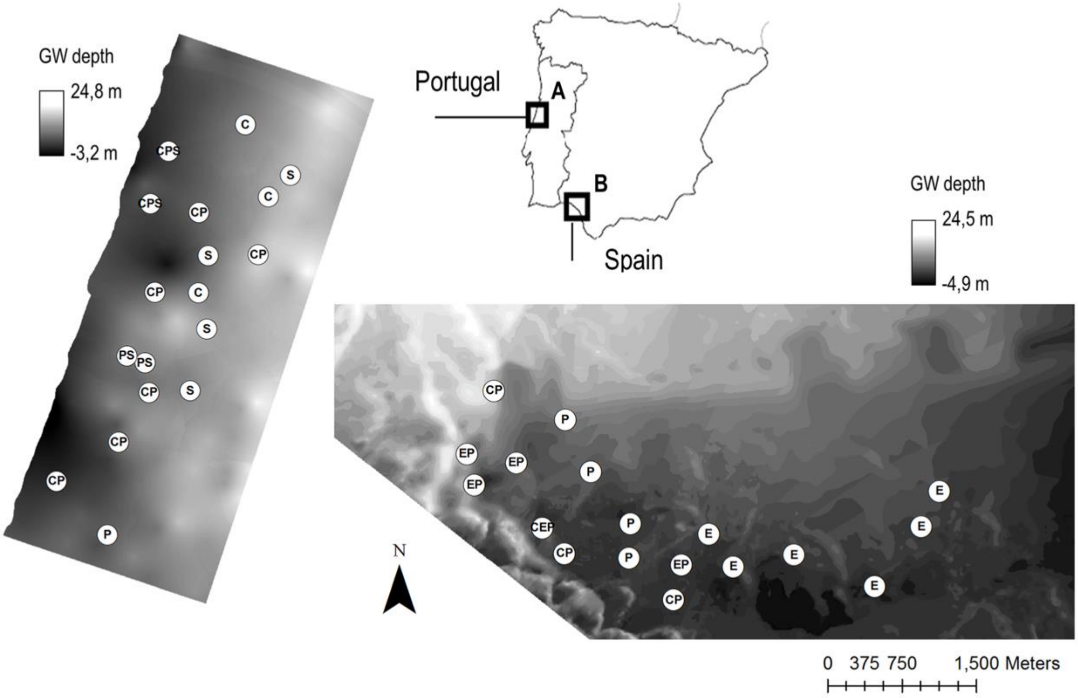

Figure 1. Study sites: (A) humid Mediterranean (Portugal) and (B) semi-arid Mediterranean (Spain), and selected sampling points (white dots). Letters inside dots represent the sampled species: (C) *Corema album*, (P) *Pinus sp*., (E) *Erica scoparia*, (S) *Salix repens*. Maps of groundwater table (GW) depth (i.e. distance from soil surface to water table) produced with summer data (month of August) for each site. Negative values denote areas with surface flow/ inundation, i.e. where water table is above soil surface.

Topography and human groundwater extraction define a spatial gradient of depth to groundwater within each site (Table S1). The ecology of the study sites is especially well suited for studying the water-use patterns of plants in relation to groundwater table depth, because the precipitation input can be considered equal along the spatial gradient of depths to groundwater within each season and site. Within each site, 18 sampling plots (20 m x 20 m) were distributed throughout each study area, using the 5 sampling points from Máguas et al. (2011) in the humidMed site and the 6 sampling points from Serrano & Zunzunegui (2008) in the semi-aridMed site, and by randomly distributing the remaining points by defining a minimum distance of 300 m between them (Fig. 1).

Within each site, we focused on woody plant species with different growth forms and water requirements. Based on common attributes, species that show similar responses to the environment can be grouped in plant functional (response) types (Díaz & Cabido, 2001). Therefore, species sharing common (functional) traits were classified in a similar plant functional type (even when sampled in different climatic sites). Woody plant species sampled and the functional classification considered in this study are described in Table 1 (Canadell et al., 1996; Castroviejo, 2000; Díaz Barradas, Zunzunegui, Tirado, Ain-Lhout, & García Novo, 1999; Máguas et al., 2011).

Table 1 Species' information and functional type classification considered in the study.

Functional type	**Species**	**Growth form**	**Root system**	**Water requirement**	**Habitat**	**GW depth sampling range (summer)**	**Site**
Conifer tree	*Pinus pinaster*	Tree	Deep/ dimorphic	Xerophyte	Dune crests, slopes and border of dune slacks	3.19 – 19.07m	humid
Conifer tree	*Pinus pinea*	Tree	Deep/ dimorphic	Xerophyte	Dune crests and slopes, and border of temporary ponds	1.59 – 12.28m	semi-arid
Hygrophytic shrub	*Erica scoparia*	Shrub	Dimorphic	Hygrophyte	"Monte Negro" scrub type, inner belt of sandy temporary ponds	0.94 – 6.97m	semi-arid
Hygrophytic shrub	*Salix repens*	Shrub	(likely) Dimorphic	Hygrophyte	Dune slacks	3.19 – 19.07m	humid
Xerophytic shrub	*Corema album*	Shrub	Shallow	Xerophyte	Dune crests and slopes	3.19 – 19.07m / 2.79 – 12.28m	humid and semi-arid

Hydrological data

The water table level was monitored in both study areas. In the humidMed site, water table level (height above sea level) was automatically recorded by piezometer divers located in 4 piezometers and 20 pumping wells throughout the dune system, with a sampling rate of 15 minutes, throughout the year. In the semi-aridMed site, water table level was measured every month with a portable probe in PVC piezometers (diameter 6 cm) installed in 7 sampling points.

Monthly groundwater absolute level (height relative to sea level), calculated from the average of daily records from each well and piezometer in the humidMed site, was interpolated within the study area by kriging, with external drift (Goovaerts, 1997) using Geostatistical Analyst Extension of ArcMap (ArcGIS 10.3.1. for desktop). A digital terrain model (DTM) was used for both sites: in the humidMed site, the ASTER Global DEM from NASA (30 m resolution); in the semi-aridMed site, the MDT05-LIDAR from CNIG Spain (5 m resolution). Details of the kriging procedure and DTM correction are available in Supporting Information (Table S2). Using the corrected DTM (representing the actual dune surface altitude) and the previously obtained monthly groundwater absolute level maps (representing the actual water table altitude), monthly maps showing the groundwater table depth for the whole area were created (corresponding to the difference between the corrected DTM and the groundwater absolute level map) (Fig. 1). Mapping and outputs were produced in ArcGis 10.3.1 for desktop. Groundwater table depth (i.e. depth to groundwater) was then calculated per sampling point and is referred to as ‘groundwater depth’ throughout the manuscript (Fig. 1).

Our sampling points end up distributed along a spatial groundwater depth gradient that ranged: (i) in spring, from 0.076 to 11.43 m in the semi-aridMed site, and from 3.14 to 17.90 in the humidMed site; (ii) in summer, from 0.94 to 12.28 m in the semi-aridMed site, and from 3.19 to 19.07 m in the humidMed site.

Shallower groundwater depths were observed in winter months and an overall recharge effect occurred (Fig. S1). A greater seasonal groundwater lowering was observed in the semi-arid site compared with the humid site (Fig. S2).

Physiological parameters

We analyzed plant isotopic data and reflectance indices from the 36 sampling plots, 18 in the semi-aridMed site and 18 in the humidMed site (Fig. 1). In each sampling plot, all the following physiological measurements were carried out in at least one species, and under contrasting water availability conditions: (a) in late spring, end of April – early May 2013, and (b) in summer, early September 2013 (Fig. S1). When possible, 3 plants per species were assessed in each plot. A total of 78 and 68 plant individuals were considered in the humidMed and the semi-aridMed sites, respectively. The number of plants per species and the number of plots where the species were assessed ([*n*]) were the following: 31[11] and 12[4] of *C. album* for humidMed and semi-aridMed sites, respectively*;* 24[8] of *S. repens;* 23[10] of *P. pinaster;* 33[11] of *E. scoparia*; and 23[12] of *P. pinea.*

Reflectance indices

We used a nondestructive optical method, based on the reflectance of light by an intact leaf. Spectral reflectance was measured using a UniSpec Spectral Analysis System (PP Systems, Haverhill, Massachusetts, USA) and carried out in 6 different leaves per plant. Mean value of the 6 measurements per plant was considered. The following reflectance indexes were calculated:

Chlorophyll index (CHL) = *R*750/*R*705, where *R750* and *R705* are the reflectance at wavelengths of 750 nm and 705 nm (Peñuelas, Frederic, & Filella, 1995). This index has been found to be well correlated with leaf chlorophyll content on a number of plant species, and can provide information about photosynthetic potential, primary production and nutrient status (Richardson, Duigan, & Berlyn, 2002).

Photochemical Index (PRI) = (*R531 -R570) / (R531+R570*), where *R531* and *R570* are the reflectance at wavelengths of 531 nm and 570 nm (Peñuelas, Llusia, Pinol, & Filella, 1997). There is an emerging consistency in the relationship between PRI and light use efficiency, which can be used as an index of photosynthetic activities, associated with changes in leaf development or stress in the long term (Wong & Gamon, 2015).

Water Index (WI) = *R*900/*R*970, where *R900* and *R970* are the reflectance at wavelengths of 900 nm and 970 nm (Peñuelas et al., 1997). This index can be used as a proxy for plant water content (Claudio et al., 2006).

Leaf carbon isotope composition

Leaf δ^{13}C is primarily related to intrinsic water use efficiency, the ratio between photosynthesis and stomatal conductance (Farquhar, Ehleringer, & Hubick, 1989). Mature leaves (2-10, depending on leaf size) were collected per plant. The bulk leaf samples (in each season n= 43 *C. album,* 24 *S. repens,* 23 *P. pinaster,* 33 *E. scoparia*, and 23 *P. pinea*) were dried at 60 ºC for at least 48 h and milled to fine powder in a ball mill (Retsch MM 2000, Germany) for carbon isotopic analysis. Leaf δ^{13}C was determined by continuous flow isotope mass spectrometry (CF-IRMS), on a Sercon Hydra 20-22 (Sercon, UK) stable isotope ratio mass spectrometer, coupled to a EuroEA (EuroVector, Italy) elemental analyser for online sample preparation by Dumas-combustion. Uncertainty of the isotope ratio analysis, calculated using values from 6 to 9 replicates of secondary isotopic reference material interspersed among samples in every batch analysis, was ≤ 0.1‰.

Analysis of xylem and sources water $\delta^{18}O$

To determine available water sources for the roots, water samples were collected from soil, rain and groundwater. Rain water was sampled in both sites on pluviometers equipped with liquid paraffin to prevent evaporation. We considered the rain water collected over the 2 weeks prior to plant sampling (n=6 in semi-aridMed site and n=4 in humidMed site). Groundwater samples were collected in piezometers (sampled after bailing to remove stored casing water) or wells reaching the water table (n=3 for both sites), on the two periods of physiological sampling, and were used to characterize this water source, as the groundwater isotopic signal is expected to be quite stable due to low evaporation processes (Newman, Breshears, & Gard, 2010). Soil samples were collected at 3 depths: 10 cm, 30 cm and 50 cm in each sampling plot with 3 replicates per depth, on the same days of physiological sampling. To access the water being used by plants, lignified woody stems of each plant individual selected for physiological measurements were collected (in each season n= 43 *C. album*, 24 *S. repens*, 23 *P. pinaster*, 33 *E. scoparia* and 23 *P. pinea*). Having in mind the extreme conditions of Mediterranean dune sites, especially in summer, soil and twig samples were immediately stored in airtight vials, sealed with a cap and Parafilm, and placed in cool conditions to avoid evaporation. All samples were kept at 4 ºC until water extraction and isotopic analysis. Water from xylem and soil samples was extracted using a custom-made cryogenic vacuum distillation system housed at SIIAF (Ehleringer & Dawson, 1992; West, Hultine, Jackson, & Ehleringer, 2008). The system allows placing collection tubes (with the twigs or soil) inside the cryogenic vacuum distillation sample vessels. Thus, after being uncapped, the refrigerated tubes were rapidly placed in the system vessels, and the circuit sealed. This allows preventing false readings on samples that might have evaporated inside the collection tubes, by letting the complete sampled water to be extracted.

When the isotopic composition of the available water sources (soil water, rain and groundwater) is known, an analysis of the oxygen isotopic composition of xylem water provides information about the water sources being used by the plant at the time of study (Chimner & Cooper, 2004; Ehleringer & Dawson, 1992). We used stem water $\delta^{18}O$ values (rather than δD) as a measure of plant water sources, because xerophytic plants might be able to discriminate against deuterium during soil water uptake by roots (Ellsworth & Williams, 2007). Oxygen stable isotope ratio analyses were performed by headspace equilibration, on an Isoprime (Micromass, UK) SIRMS, coupled on continuous flow mode to a Multiflow (Micromass, UK) auto-sampler and sample equilibration system. Analytical uncertainty is <0.1‰.

Soil water at 10, 30 and 50 cm depth are from now on named SW_{10}, SW_{30} and SW_{50}, respectively.

Quantification of water sources used by plants

The relative contribution of different water sources to the composition of the xylem water was estimated by Bayesian stable isotope mixing models using the graphical user interface and model framework MixSIAR for R (Parnell et al., 2013; Stock & Semmens, 2013). In our study, the model used as input the individual isotope values of xylem water $\delta^{18}O$ of each plant (‘mixture’ raw data), all sources described in *water sources*, and a matrix for potential fractionation (set to 0). We calculated the water-use proportion in the two seasons and in each site and plot separately, using an individual-basis model to obtain separate estimates for each of the plants. We ran models using the individual plant (‘ID’ within the ‘plot’) values of xylem water $\delta^{18}O$ (raw data), the sampling plot water sources’ mean (plus standard deviation), and the ‘process only (n=1)’ error structure option. We set the Markov Chain Monte Carlo to ‘long’ or ‘very long’ model run, until the diagnostics were satisfying. Spring precipitation was aggregated with SW_{10} in both sites (because their isotopic composition was similar). Thus, the spring mixing model ran with four sources (Precipitation+SW_{10}, SW_{30}, SW_{50} and Groundwater), except in cases of groundwater depth ≤50 cm (which only happened in spring, in the semi-arid site, at two plots). In these cases, the model ran with appropriate water sources (Precipitation+SW10, SW30 and SW50 or Precipitation+SW10 and SW30). The summer mixing model ran with the 4 sources sampled (SW_{10}, SW_{30}, SW_{50} and Groundwater), since it didn’t rain in the two weeks prior to sampling.

Statistical analysis

We analysed differences in xylem water $\delta^{18}O$ between species, using mixed linear, random intercept models in which season (spring and summer), functional type (xerophytic shrub, hygrophytic shrub and conifer tree) and site (humidMed and semi-aridMed) were used as fixed effects, plus their interaction term (season*functional type*site), and individual plants as random effects, using the R package ‘nlme’ (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2013). Differences in seasonal variation (summer–spring, in % or ‰) of physiological variables (WI, PRI, CHL and $\delta^{13}C$) between species were analysed using analysis of variance (ANOVA). All-pair comparisons post-hoc analyses were performed with Tukey’s HSD.

Since data did not fulfill linear regression assumptions even after *log* transformed, Spearman correlations were performed by species to investigate possible correlations between: (i) groundwater depth and water used by plants (water source proportions %: SW_{10}, SW_{30}, SW_{50}, GW); (ii) water used by plants and physiological variables (WI, PRI, CHL and $\delta^{13}C$); and (iii) groundwater depth and physiological parameters. To compute the proportion of water use from deep soil layers we made the distinction between shallow and deep soils considering deep soil as the region in the soil profile below 50 cm, and shallow soils to be the region in the soil profile above 50 cm (Dawson, Mambelli, Plamboeck, Templer, & Tu, 2002; Rossatto et al., 2012). Accordingly, we used the percentage of groundwater use as the proportion of water use from deep soil layers (> 50 cm). In cases of a significant Spearman correlation ($p<0.05$), locally weighted smoothing (lowess) lines were used for visualization purposes only (depicting relationships between variables).

All statistic analyses were performed in R 3.2.3 (R Core Team, 2015).

3.1.4 Results

Plant water sources usage along the gradient of groundwater depth under different water availability conditions

To test our first hypothesis, we first explored the influence of site and season on the water-use patterns of the different functional types. We found a significant interaction (season*functional type*site) effect on xylem water δ^{18}O (F-value = 24.18, p-value = <.0001, Table S3). Accordingly, plant functional types presented different water sources depending on the site and season (Fig. 2, Fig. S3). In spring, in both sites, the composition of xylem water was better explained by precipitation mostly present at SW_{10}, except in semi-arid conifer trees that used mainly SW_{50}+SW_{30} (Fig. 2a,c). During summer, the estimated SW_{10} use by hygrophytic shrubs decreased in both sites, and the use of deeper soil layers and groundwater increased (Fig. 2b,d). Conifer trees in humidMed (*P. pinaster*) presented a higher contribution of SW_{10} during summer, contrasting with semi-aridMed conifer trees (*P. pinea*) that showed a considerable contribution of groundwater (Fig. 2b,d). In the dry season, xylem water of the xerophytic shrub *C. album* was mainly composed by SW_{10} and extremely enriched water, not changing to deeper soil layers (Fig. 2b,d; Fig. S3). Plants from the humidMed site presented a higher seasonal similarity on water use when compared to the semi-aridMed site (Fig. 2, Fig. S3).

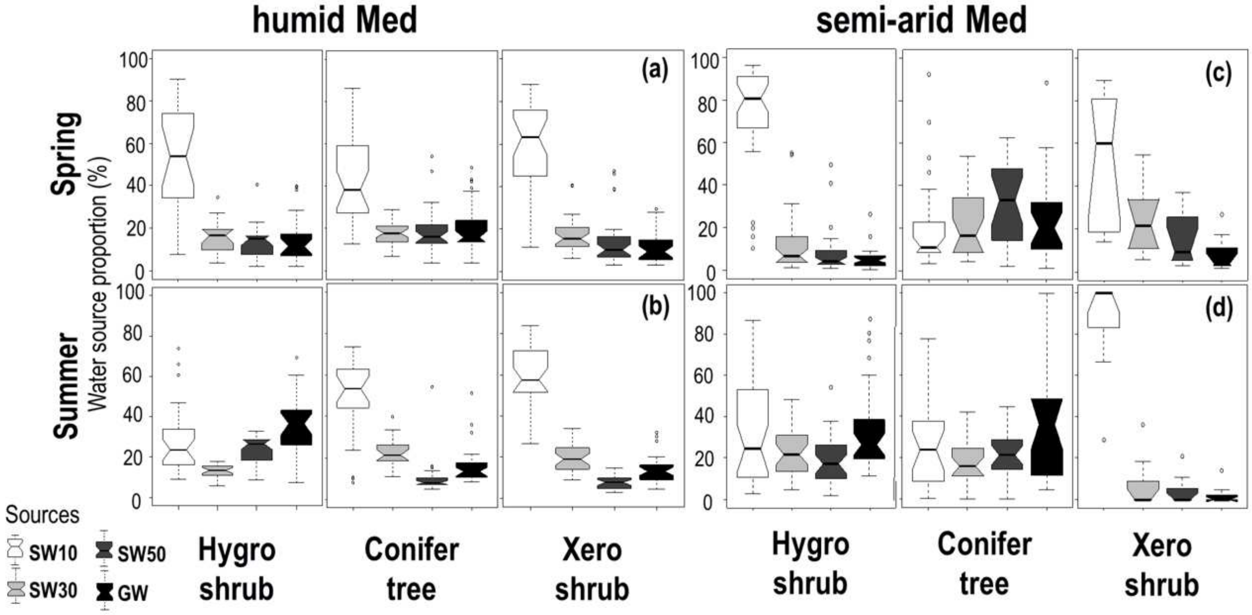

Figure 2. Boxplots of water source use proportion (estimated by Bayesian stable isotope mixing models) considering the two climatic sites: (a), (b) humid and (c), (d) semi-arid Mediterranean; three functional types: Hygrophytic shrub, Conifer tree and Xerophytic shrub; two seasons: (a), (c) spring and (b), (d) summer; and four sources (soil water at 10 cm – SW10, 30 cm – SW30, 50 cm – SW50, groundwater – GW). Sources have different colors as shown in inner legend. Precipitation in spring (for both sites) was aggregated with soil water at 10 cm depth since it was highly similar to it. Species considered in each site are: humidMed - *S. repens*, *P. pinaster* and *C. album*; semi-aridMed - *E. scoparia*, *P. pinea* and *C. album* (Hygrophytic shrub, Conifer tree and Xerophytic shrub, respectively).

Despite the complexity, we found clear soil water profiles, particularly in summer, with ^{18}O enrichment associated with shallower layers of the soil (Fig. S3, Fig. S4). The seasonal variation in soil water $\delta^{18}O$ was stronger in the semi-aridMed site comparing to the humidMed site, and semi-aridMed site showed higher soil water $\delta^{18}O$ values in summer (Fig. S3). Additionally, in semi-arid summer conditions, groundwater depth was significantly positively correlated with isotopic composition of SW_{30} and SW_{50} (i.e. greater groundwater depth is associated with less negative SW_{30} and SW_{50} $\delta^{18}O$) (rho=0.80, rho=0.78, respectively), and negatively correlated with soil moisture measured at 10 cm depth (rho=-0.54) (Table S4, Fig. S4). Higher similarity between SW_{50} $\delta^{18}O$ and groundwater $\delta^{18}O$ was found where water table was closer to soil surface (Fig. S4).

Since plant functional types presented different water sources depending on the site and season, we explored the correlation between water-use proportions and groundwater depth by site and season separately. These correlations showed different water-use responses of the functional types to groundwater depth (Table 2). Xerophytic shrubs didn't change their water use in both climatic sites, maintaining the use of superficial water throughout the groundwater depth gradient (Table 2, Fig. S5). Contrastingly, in summer, the conifer trees of the semi-aridMed site responded to groundwater depth: greater groundwater depth led to lower SW_{10} use (rho= -0.59, $p<0.001$) and higher groundwater use (rho= 0.57, p=0.004) (Table 2, Fig. S5). In both sites, hygrophytic shrubs showed no significant correlation with groundwater depth in spring, while in summer they responded significantly. As depth to groundwater increased (falling groundwater table), this functional type decreased SW_{10} use and increased the contribution of either SW_{50} (in the humidMed site; rho = 0.45, p=0.02) or groundwater (in the semi-aridMed site; rho= 0.47, p=0.005) (Table 2, Fig. S5). Accordingly, in the semi-aridMed site, we observed a high groundwater-use proportion (>35%) in sampling points where the water table was deeper (Fig. S5). The percentage of use of deeper soil layers of the hygrophytic shrub *E. scoparia* and the conifer tree *P. pinea* increased with increasing groundwater depth (Table 2). Comparatively, plants of the humiMed site tended to maintain a shallower water use along the groundwater depth gradient (Table 2).

Table 2. Spearman correlations (rho) between water used by plants from each soil layer (in % contribution) and groundwater depths in the humidMed and semi-aridMed sites and in two seasons (spring and summer). Three functional types considered: Hygrophytic shrub (Hygro shrub), Conifer tree and Xerophytic shrub (Xero shrub). Bold represents significant correlations; *p<0.05, **p<0.01, ***p<0.001.

	Spring				**Summer**				
	SW_{10}	**SW_{30}**	**SW_{50}**	**GW**	**SW_{10}**	**SW_{30}**	**SW_{50}**	**GW**	***n***
Humid site									
Hygro shrub (*S. repens*)	-0.30	0.40	0.25	0.19	**-0.57****	0.24	**0.45***	0.27	*27*
Xero shrub (*C. album*)	-0.30	0.32	0.20	0.25	0.19	-0.21	-0.11	0.06	*33*
Conifer tree (*P. pinaster*)	-0.06	0.04	-0.05	**0.40***	-0.05	-0.02	0.07	0.27	*23*
Semi-arid site									
Hygro shrub (*E.scoparia*)	-0.19	0.16	0.18	0.24	**-0.55*****	**-0.37***	0.19	**0.47****	*33*
Xero shrub (*C. album*)	0.09	-0.19	-0.21	-0.08	0.14	-0.14	-0.14	-0.10	*12*
Conifer tree (*P. pinea*)	-0.07	0.37	-0.08	-0.28	**-0.59*****	**-0.44***	0.20	**0.57****	*23*

Relationships between use of water sources, depth to groundwater and plant physiological performance

First, we identified changes in the plants' physiology relative to the type of water they used, by correlating spectral and isotope data with groundwater use, considering the seasonal data that revealed previous water-use changes along the groundwater depth gradient (dry season). We found that increasing proportion of groundwater use was significantly correlated with decreasing WI for the semi-arid site hygrophytic shrub (*E. scoparia*) and conifer tree (*P. pinea*), but no significant correlations were observed for PRI, CHL and $\delta^{13}C$ (Table 3, Fig. S6).

Table 3. Spearman correlations (rho) between groundwater use and physiological variables in the humidMed and semi-aridMed sites, in dry season (summer). Three functional types considered: Hygrophytic shrub (Hygro shrub), Conifer tree and Xerophytic shrub (Xero shrub). Plant reflectance indices: WI - Water index, PRI - Photochemical index, CHL - Chlorophyll content index. Bold represents significant correlations; *p<0.05, **p<0.01, ***p<0.001.

summer	**$\delta^{13}C$**	**WI**	**PRI**	**CHL**	***n***
Humid site					
Hygro shrub (*S. repens*)	-0.04	-0.13	0.06	0.17	*27*
Xero shrub (*C. album*)	-0.29	0.33	0.32	0.23	*33*
Conifer tree (*P. pinaster*)	-0.35	-0.17	0.03	0.07	*23*
Semi-arid site					
Hygro shrub (*E.scoparia*)	-0.18	**-0.37***	0.03	0.16	*33*
Xero shrub (*C. album*)	-0.05	0.13	0.17	0.07	*12*
Conifer tree (*P. pinea*)	-0.02	**-0.43***	-0.10	0.03	*23*

Secondly, we tested how the physiological parameters varied with increasing groundwater depth. Increasing depth to groundwater led to a significant decline in WI of all functional types in the semi-arid site (Fig. 3b). Greater groundwater depth did not influence any photosynthetic parameter (PRI, CHL and δ^{13}C) of hygrophytic shrubs and conifer trees in the semi-aridMed site (Fig. 3c). PRI and CHL decreased significantly with increasing groundwater depth in the semi-arid xerophytic shrub *C. album* (Fig. 3c).

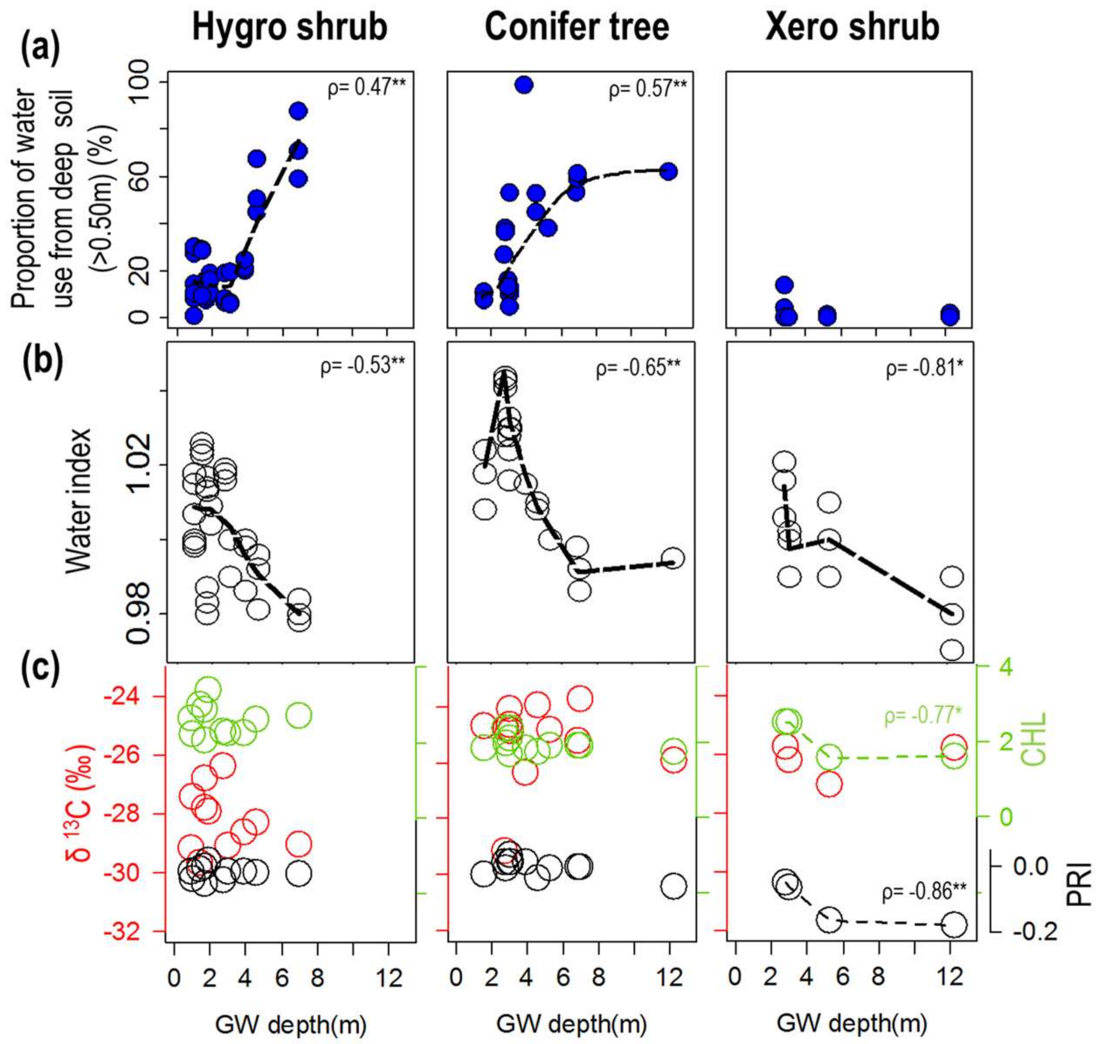

Figure 3. Summer in semi-aridMed site: (a) proportion of water uptake from deep soil layers (%), (b) water index and (c) physiological parameters, along the groundwater table depth gradient (GW depth), considering the three functional types: Hygrophytic shrub E. scoparia (left), Conifer tree P. pinea (middle) and Xerophytic shrub C. album (right). (a) Proportion of water uptake from deep soil layers (below 0.50 m depth) of each individual (blue dots); (b) Water index of each individual plant, as a proxy of plant water content, is represented by open black dots; (c) Physiological parameters considered are: δ13C (red open dots and left y-axis), photochemical reflectance index (PRI - black open dots and right y-axis) and chlorophyll index (CHL - green open dots and right y-axis); dots indicate the species' average value in each sampling plot. (a,b,c) Spearman rho (ρ) and respective p-value (*p<0.05, **p<0.01, ***p<0.001) are presented in the figure if the correlation between variables was significant (p<0.05); for visualization purposes, lowess lines were used to fit the data that showed significant spearman correlations (dashed lines).

At the humidMed site, with increasing groundwater depth, we observed a significant increase of: WI in the hygrophytic shrub *S. repens*, CHL in both the hygrophytic shrub and the conifer tree *P. pinaster*, and PRI in the three functional types (Fig.4bc). At this site, $\delta^{13}C$ decreased significantly with increasing groundwater depth in both conifer trees and xerophytic shrubs (Fig. 4bc). Additionally, plants from the semi-aridMed site suffered a higher summer ^{13}C enrichment and a greater summer lowering of PRI and CHL, compared to humidMed plants (Fig. 5, Fig. S7).

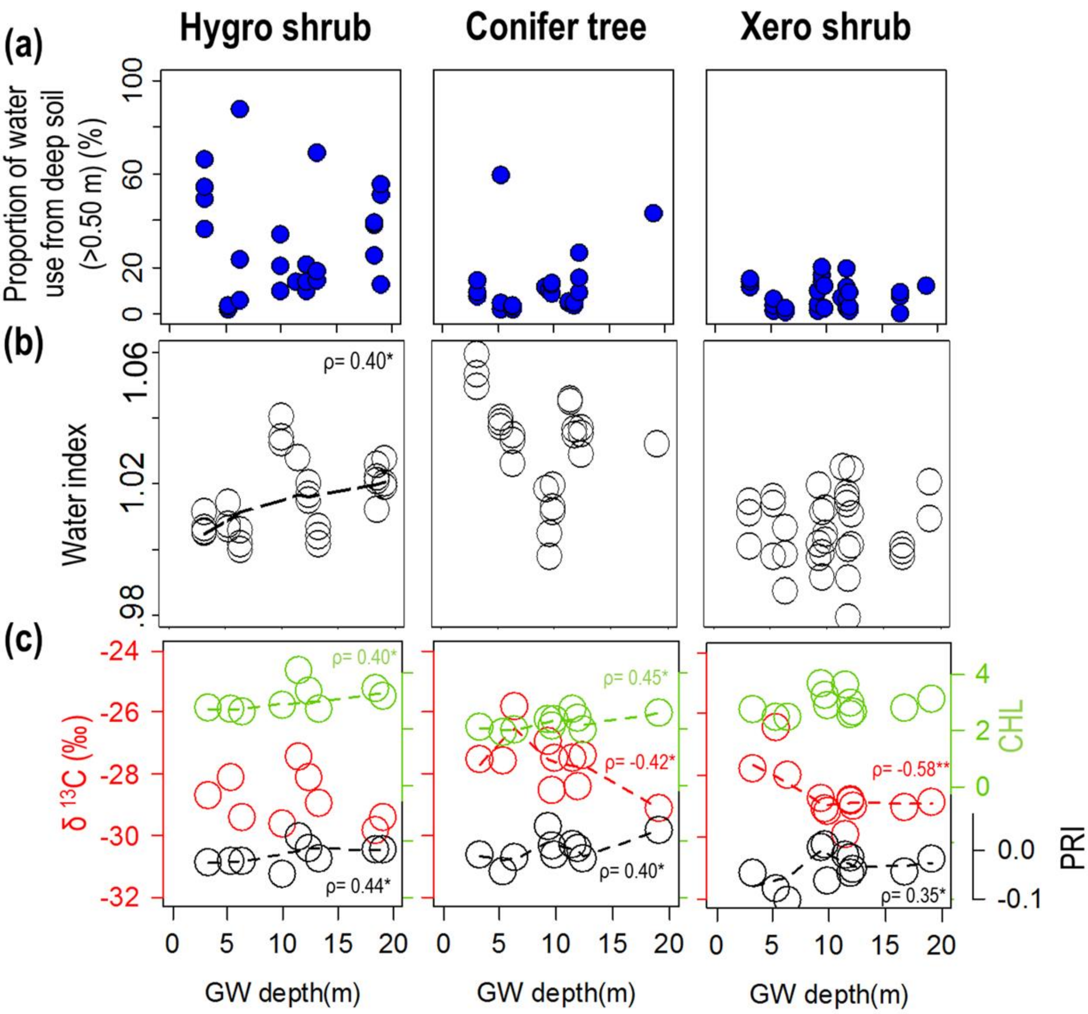

Figure 4. Summer in humidMed site: (a) proportion of water uptake from deep soil layers (> 0.5 m deep) (%), (b) water index and (c) physiological parameters, along the groundwater table depth (GW depth) gradient, considering the three functional types: Hygrophytic shrub *S. repens* (left), Conifer tree *P. pinaster* (middle) and Xerophytic shrub *C. album* (right). Please check Fig. 3 legend for further details.

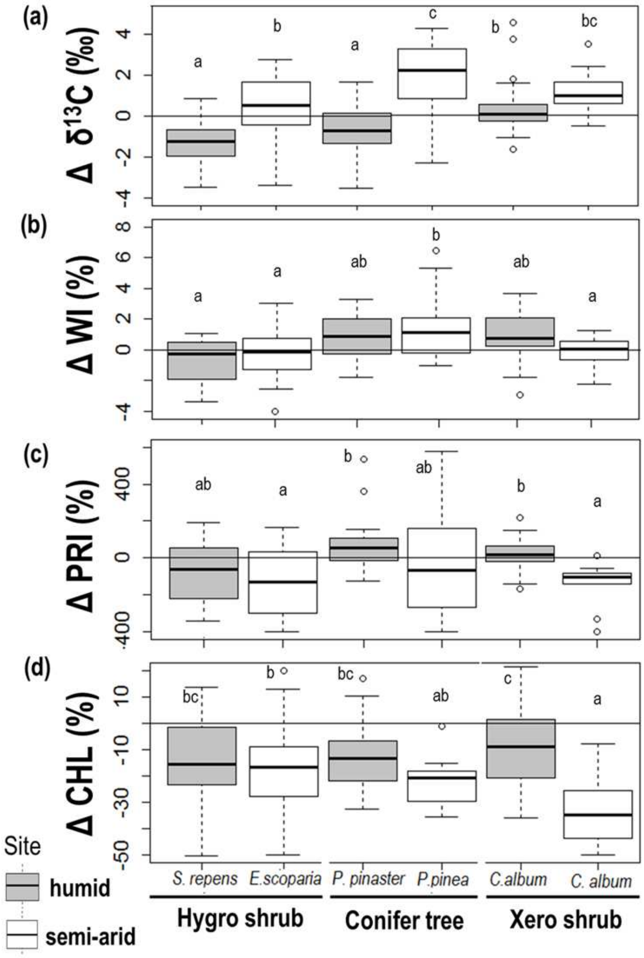

Fig. 5 Seasonal variation (Δ = Summer – Spring) of physiological parameters in the three plant functional types (hygrophytic shrub, conifer tree and xerophytic shrub) in both climatic sites: (a) $\delta^{13}C$ (‰); (b) water index (WI, %); (c) photochemical index (PRI, %); (d) chlorophyll index (CHL, %). Humid site represented by grey and semi-arid site by white. Species considered in each functional type are represented in the figure. Different letters stand for significant differences between species.

3.1.5 Discussion

As hypothesized, only under drier conditions (summer in semi-arid site) did increasing groundwater depth trigger water uptake adjustments toward deeper soil layers of conifer trees, but not of xerophytic shrubs. Contrastingly to our hypothesis, under dry conditions, hygrophytic shrubs increased groundwater use with increasing groundwater depth. Similarly to the conifer tree *P. pinea*, this functional type showed water uptake adjustments towards deeper soil layers with greater depths to groundwater. The water-sources-use changes of both hygrophytic shrubs and conifer trees in summer were associated to a decrease in water content only under semi-arid conditions. However, increasing groundwater depth did not negatively impact the photosynthetic activity of these functional types. In contrast, a greater groundwater depth implied a decrease in chlorophyll content and photosynthetic activity of xerophytic shrubs *C. album* under summer in semi-arid, while maintaining a high reliance on top-soil water.

Plant water-use under increasing depth to groundwater

The increase in groundwater depth modified the water sources used by plants, but the responses to groundwater limitation were conditioned by precipitation availability and species characteristics (Table 2, Fig. 2). As expected, groundwater use and responses to groundwater lowering were more evident when water availability was scarce (summer and semi-arid conditions) (Barbeta et al., 2015; Grossiord et al., 2017; Saha, Sternberg, & Miralles-Wilhelm, 2009; Voltas, Lucabaugh, Chambel, & Ferrio, 2015; West et al., 2012; West, Hultine, Burtch, & Ehleringer, 2007; Zencich et al., 2002). As the surface soils became recharged with moisture from spring rainfall, all functional types used more water from the upper zone of the soil profile, irrespective of proximity and presence of groundwater (except *P. pinaster* in the humidMed site, probably due to a competition effect). Under low water availability (summer conditions), the water-use of the xerophytic shrub *C. album* was completely uncoupled from access to groundwater in both climatic sites, and this species used a minor proportion of water from deeper soil layers. The highly enriched δ^{18}O values presented by this species in the semi-arid site during summer may be related to: (i) the use of highly enriched water sources not characterized in this study (such as dew, fog, or water available in <10cm soil layers (Alessio, de Lillis, Brugnoli, & Lauteri, 2004; Álvarez-Cansino, Zunzunegui, Díaz Barradas, & Esquivias, 2010; Dawson, 1998; Ingraham & Matthews, 1995); or (ii) the occurrence of xylem water isotopic enrichment through the occurrence of water-depleted leakage by bark evaporation or leaf shedding and/or exchange of xylem water with phloem, enriched bulk leaf water or xylem air bubbles under restricted water flow (Alessio et al., 2004; Cernusak, Farquhar, & Pate, 2005; Dawson & Ehleringer, 1993; Ellsworth & Williams, 2007; Gan, Wong, Yong, & Farquhar, 2003; Martín-Gómez, Serrano, & Ferrio, 2017). In contrast, the semi-arid hygrophytic shrub *E. scoparia* and the conifer tree *P. pinea* did rely on groundwater. During the drought periods, these species changed water uptake accordingly towards deeper soil layers with declining groundwater levels. Surprisingly, these species increased groundwater-use proportion with increasing groundwater depth. Plants occurring in places with a relatively deep water table extracted significantly more water from deeper soil layers than plants growing at places with a shallow water table, where water uptake tended to be restricted to superficial soil layers (as in Rossatto et al., 2012). This water-use pattern enhances the importance of shallow soil layers' wetness driven by the close presence of groundwater. In spring, rain events provide water at top-soil layers, but at summer dry conditions, when precipitation is no longer available, the proximity of groundwater will play an important role in increasing soil moisture at shallow soil layers (Chen & Hu, 2004). This may be closely related with important processes such as hydraulic lift and/or capillary rise, common in sandy soils in dry environments, making deeper water available in upper soil layers (Burges, Pate, Adams, & Dawson, 2000; Caldwell, Dawson, & Richards, 1998; Horton & Hart, 2017; Jackson, Sperry, & Dawson, 2000; Naumburg et al., 2005). Thus, shallower groundwater will lead to higher soil water availability at 30 and 50 cm depth, where isotopic fractionation is expected to occur. Our observations of higher similarity between GW and SW_{50} isotopic water composition, more depleted soil $\delta^{18}O$ values and higher soil water content at 10 cm with shallower groundwater support this hypothesis. Hence, species may establish a shallow root system in response to a high frequency of shallow soil's water supply (water-table capillary rise), and thus rely on superficial unsaturated zone of the soil profile, even in the dry season (Busch, Ingraham, & Smith, 1992; Gaines et al., 2016; Gries et al., 2003; Rossatto et al., 2012; Zencich et al., 2002).

Our results of seasonal water sources usage are consistent with the presence of a dimorphic root system in *E. scoparia* and *P. pinea*. While in spring they tap rain water at superficial soil layers, in summer deep sinker roots, with larger hydraulic conductivity to tap groundwater (Dawson & Pate, 1996), may follow the decline of the groundwater table (and of the capillary fringe) towards deeper soil layers (Fan, Miguez-Macho, Jobbágy, Jackson, & Otero-Casal, 2017). Accordingly, both species' water-use is strongly dependent on groundwater, and an investment in root development to compensate the lowering of the groundwater table was probably made by these species.

Effects of groundwater depth on plant physiological performance

Unexpectedly, all studied species from the humid site revealed an increase of physiological performance (i.e. increasing WI, PRI and CHL, and declining $\delta^{13}C$) with increasing depth to groundwater (Fig. 4). Thus, rather than groundwater depth, other environmental or biotic factors can be contributing to reduce plant physiological status at the humid site. These factors can be (i) salinity and wind stress (greater in plots closer to the sea) (Ciccarelli, Picciarelli, Bedini, & Sorce, 2016; Naumburg et al., 2005), and (ii) differential abundance of tree species and canopy cover (lower organic matter accumulation, greater evaporative losses at soil level in the dry season, and lesser protection from wind and salt deposition of understory shrubs, with decreasing canopy cover) (Potts, Scott, Bayram, & Carbonara, 2010). Contrastingly, under more xeric conditions, all the studied species declined their WI with increasing groundwater depth. Even with the capacity to accompany the groundwater table lowering with increasing use of deeper soil layers, the hygrophytic shrub *E. scoparia* and conifer tree *P. pinea* declined their WI (Table 3, Fig. 3a,b). Thus, these functional types were not fully buffered from groundwater limitation. However, no significant decline of photosynthetic parameters (such as PRI and CHL), with increasing depth to groundwater (and accompanying reliance on water from deeper soil layers), was observed for these plant types (Fig. 3c). Thus, the amount of water was still enough to sustain dry season photosynthetic activity of these plants throughout the groundwater depth gradient.

Plants from the semi-arid site showed a considerable seasonal variation in photosynthetic parameters (Fig. 5), indicating a summer drought effect. Consistent with a drought avoidance strategy and isohydric behavior of deep-rooted conifer trees (Calama, Puértolas, Madrigal, & Pardos, 2013; Garcia-Forner et al., 2016; Martínez-Vilalta & Garcia-Forner, 2017), *P. pinea* showed ^{13}C enrichment and lowering of PRI and CHL under seasonal drought, while maintaining leaf water content at spring levels. In contrast, the hygrophytic shrub *E. scoparia* showed a seasonal maintenance of both δ^{13}C and leaf water content. Despite their different leaf-level drought strategies, both species adjust water uptake (towards deeper soil layers) and experience a decline in water content where groundwater is deeper and where water was generally scarce, by possibly taking up a lower amount of water. Within the studied range of depth to groundwater, *E. scoparia* and *P. pinea* acclimation to greater groundwater depth through modifications on the utilization of water resources appears to be a key response. Through these adjustments, they partly compensate, for water stress caused by decreasing soil water availability. However, such changes in belowground traits and the decline of WI may lead to physiological implications in the long-term capacity of responding to limiting water availability (Gries et al., 2003).

Under semi-arid conditions, the xerophytic species *C. album* was shown to be vulnerable to a lack of upper soil moisture when groundwater is deep. Unexpectedly, this xerophyte (with the absence of water-use changes) was not completely uncoupled from groundwater depth, showing a decline in physiological parameters (WI, PRI and CHL) with groundwater table lowering (Fig. 3). This underpins the impact that a greater depth to groundwater can have on vegetation in this semi-arid ecosystem.

3.1.6 Conclusion

In conclusion, the present findings contributed to gain an insight into how different species are acclimating to groundwater lowering in a *region* experiencing climatic drought and a scarcity in soil water availability due to anthropogenic groundwater extraction. In coastal dune systems, greater depth to groundwater combined with low soil water availability will cause important shifts in plant water-use, including greater proportion of water uptake from deeper soil layers and/or physiological adjustments. These responses will depend on the plant functional type and drought intensity. Under humid Mediterranean climate, the lowering of groundwater table did not negatively influence the studied species. Thus, the effects of greater depths to groundwater should be less severe in more mesic conditions. Under high drought intensity (as in semi-arid summer), dimorphic-rooted species and shallow-rooted species showed different responses to increasing groundwater depth. The hygrophytic shrub *E. scoparia* and conifer tree *P. pinea* (dimorphic root system) made belowground adjustments (such as water uptake adjustments towards deeper soil layers and higher groundwater use probably coupled with enhanced root development), at the cost of declining water content, but maintaining their photosynthetic activity. The xerophytic shrub *C. album* (shallow root system), with an absence of water-use adjustments towards deeper soil layers, showed leaf-level physiological adjustments (showing a decline of WI, CHL and PRI). Accordingly, under strong drought conditions, groundwater drawdown is, direct or indirectly, driving water-use changes and physiological adjustments in species belonging to different functional types in the studied semi-arid coastal dune system.

3.1.7 Authors' Contributions

C Antunes and C Máguas conceived the ideas and designed methodology; C Antunes, A Anjos and A Pereira collected the data; A Pereira and MJ Pereira conducted the groundwater modelling; C Antunes analysed data; C Antunes, MC Díaz-Barradas, C Máguas, S Vieira, M Zunzunegui and O Correia led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data Accessibility

Isotopic and reflectance data available at the DRYAD Digital Repository: http://doi.org/10.5061/dryad.6vr0666.

3.1.9 References

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3.1.10 Supporting Information

Table S1. Detailed description of the study areas: humid and semi-arid Mediterranean sites.

Table S2. Details of groundwater absolute level modeling and DTM calibration.

Table S3. Summary of linear mixed model of xylem water $\delta^{18}O$.

Table S4. Spearman correlations between groundwater depth and soil water $\delta^{18}O$ (at three depths) and top-soil water content in the humid and semi-arid Mediterranean sites and in both seasons.

Figure S1 Climatic and groundwater patterns of each study site along the year.

Figure S2 Maps of groundwater depth seasonal variation in humid and semi-arid Mediterranean sites.

Figure S3 Boxplots of water sources and xylem water $\delta^{18}O$ (‰) of the three plant functional types studied in both climatic sites.

Figure S4: Relationship between groundwater depth at summer semi-arid site and (a) Mean soil $\delta^{18}O$ and (b) Volumetric soil moisture content.

Figure S5: Relationship between groundwater depth and water use proportion for the three plant functional types in spring and summer and in the two climatic sites.

Figure S6: Relationship between water use proportions and photosynthetic parameters for the semi-arid plant functional types in the dry season.

Figure S7: Boxplots of spring and summer leaf-level physiological factors measured in the three plant functional types at both climatic sites.

Supporting Information

Table S1. Detailed description of the study areas: humid and semi-arid Mediterranean sites.

Site	Location	Climatic region	Description
Humid Med	Osso da Baleia, centre of Portugal (40.006146 N, -8.904036 W)	meso-Mediterranean, humid, AI =0.91 (Atlas 1992, Quezel & Médail, 2003)	Facing the Atlantic Ocean, the area consists of West-East dune belts and of a series of trending North-South dunes separated by lows. The secondary dune is covered by a well conserved coastal pine forest (with dominating *Pinus pinaster* originally planted in the XIII century). The land elevation increases from west to east, to a maximum topographic height of 77 m, with many dune-slacks appearing throughout the area and considered as a Natura 2000 habitat: 2170 – Dunes with *Salix repens* ssp. *argentea* (ICNB, 2006). The dune-slacks present a particular flora dominated by hygrophytes and dune ridges and slopes comprise drought adapted woody species (such as *Pinus pinaster, Myrica faya*, *Corema album, Helichrysum picardii* and *Halimium halimifolium*). In this area a national electric company (REN S.A.) is exploiting the ground caverns for gas storage. To access these caverns the water table is being explored since 2001. For that, 20 pumping wells are distributed in a 4 km north-south pumping line, at approximately 600 m from the coast, to induce the extraction of groundwater. Maximum water extraction of all wells is limited to 600 m³/h and maximum lowering of the groundwater table is restricted to 5 m in each well (Abrunhosa 2002; Máguas *et al.* 2011).
Semi-arid Med	Biological Reserve of Doñana, southwest of Spain (36.983903 N, -6.489620 W)	thermo-Mediterranean, , semi-arid, AI=0.47 (Atlas 1992, Quezel & Médail, 2003; Metzger *et al.* 2013). semi-arid (0.20 < AI < 0.50)	The stabilized sands correspond to a coastal plain fronting the Mediterranean Sea originated in the Pliocene which was covered by aeolian mantles in the Holocene. The present-day topography shows a series of arch-formed ridges (old dunes) separated by flat depressions. In this landscape, a fairly shallow water table results in the frequent flooding of depressions in rainy periods, which means that temporary ponds arise, and the presence of permanentt ponds (Zunzunegui *et al.* 1998; Manzano & Custodio, 2006). Within the Doñana Biological Reserve the sand mantle is mostly covered by Mediterranean scrub with a species composition closely following water availability which depends on groundwater flow systems of different spatial scales (Muñoz-Reinoso *et al.* 2005). The vegetation is dominated by Mediterranean shrubland with local patches of the original *Juniperus* and *Quercus* woodlands. The area is influenced by an intense groundwater pumping for urban water supply to Matalascañas tourist centre, in the northwest part of the study zone, and for irrigation of the south areas of La Rocina and northeast of the Park (Manzano & Custodio, 2006; Serrano & Zuzunegui, 2008). The intense groundwater withdrawal (for over twenty five years) has caused accumulated local drawdown of the deep piezometric levels and as a result, a reduction of the natural groundwater seepage at the sandy-clay interface, the drying of temporary ponds and the decrease in hygrophyte vegetation (Serrano & Serrano, 1996; Serrano & Zunzunegui, 2008).

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Table S2. Details of monthly groundwater absolute level's kriging and DTMs calibration.

Kriging with external drift is appropriate to estimate in space, a variable, Z(u) that despite not having spatial stationarity, they can be decomposed into a trend (mean), m(u) and a residue, r(u) with spatial stationarity.

$Z(u)^*=m(u)+r(u)$

The trend can be calculated by a function of auxiliary variables and the residue is the difference between the mean and the "real" value. Thus, residues are not measured but they can be derived from observed values and must have spatial continuity. In this particular case, a trend related to the distance to the coastline was detected on the monthly groundwater absolute level, i.e., in average the groundwater absolute level increased with the distance to the coastline. For sake of simplicity, a trend was fitted using the geographic coordinates of the piezometers, for each monthly groundwater absolute level. The application of the method to estimate a monthly groundwater absolute level surface, can be summarized in the following steps:

i) Fit a trend to the monthly groundwater absolute value based on the respective observed values at the piezometers and its location coordinates, using a least squares regression method.
ii) Define a grid of MxN nodes, were we want to calculate the monthly groundwater absolute level, to build a map (surface);
iii) Use the previous fitted function to calculate the trend value at each piezometer locations and for each grid node location.
iv) Calculate the residues for each piezometer location, denoting them as "observed" residues, as well as the respective experimental variograms. To proceed the residues must have a spatial structure and spatial stationarity, allowing for the fitting of a variogram model to the experimental variogram.
v) Interpolate the residues in each grid node applying ordinary kriging taking into account the previous fitted variogram model and the "observed" residues.
vi) In each grid node, add the previous calculated trend value to the kriged residue value, to obtain the estimated value of the monthly groundwater absolute value.

In this case study all experimental variograms were fitted by a spherical isotropic variogram function.

To calibrate the DTMs, a correction was made using terrain measures with high precision GPS with corrections in some of our sampling points. For this correction, the differences between the altitudes measured at the sample points and the altitude of the DTM were calculated. These differences were then krigged (ordinary kriging) and summed to the original DTM to generate a new DTM corrected with the field information

Table S3. Summary of linear mixed model of xylem water $\delta^{18}O$ [full model = lme(d18O ~ site*season*fg, random = ~1|ID,data = mixm1)]. Bold represents significant effects (p<0.05). fg = plant functional group.

	numDF	denDF	F-value	p-value
(Intercept)	1	183	628.00	**<.0001**
site	1	183	0.08	0.7734
season	1	131	23.37	**<.0001**
fg	2	183	29.53	**<.0001**
site:season	1	131	25.79	**<.0001**
site:fg	2	183	6.01	**0.003**
season:fg	2	131	41.41	**<.0001**
site:season:fg	2	131	24.18	**<.0001**

Table S4. Spearman correlations (rho) between groundwater depth and soil water $\delta^{18}O$ (at three depths) and top-soil water content in the humid and semi-arid Mediterranean sites and in both seasons. Bold represents significant correlations; * p<0.05, **p<0.01, ***p<0.001.

		humidMed		semi-aridMed	
variable	depth (cm)	spring	summer	spring	summer
Soil water $\delta^{18}O$	10	**-0.53***	-0.32	0.07	0.41
	30	0.02	-0.42	0.09	**0.8*****
	50	-0.19	-0.46	0.26	**0.78****
Soil moisture	10	-	-	-0.09	**-0.54*****

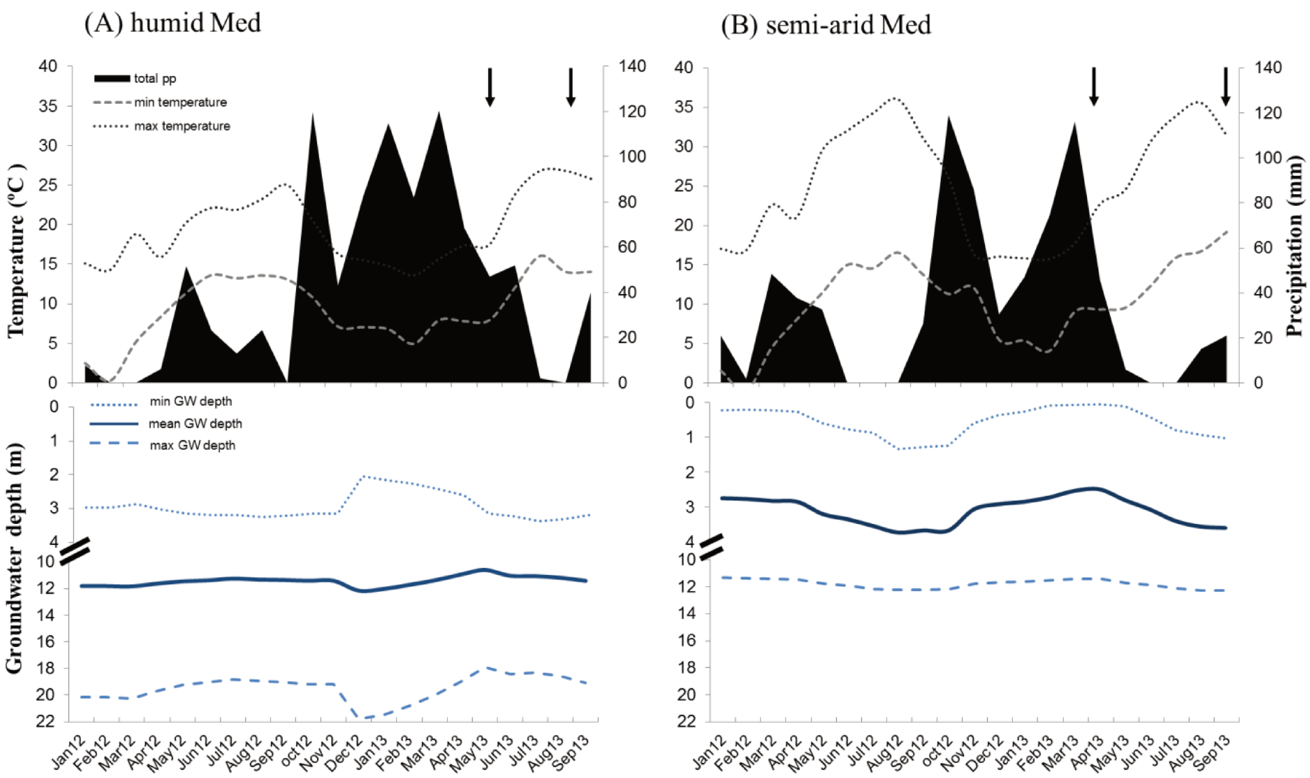

Figure S1. Climatic and groundwater depth of each study site (from January 2012 to September 2013): (A) humid Mediterranean and (B) semi-arid Mediterranean. Monthly total precipitation (secondary y-axis), maximum and minimum temperature (primary y-axis) are shown in upper panels. Ecophysiological sampling campaigns marked with down arrows. Minimum, maximum and mean groundwater depth are shown in bottom panels, considering only the sampling points (n=18) (note that y-axis was cut to better fit the data, and reverse order was applied for an easier interpretation of GW depth, i.e. 0 m indicates surface soil).

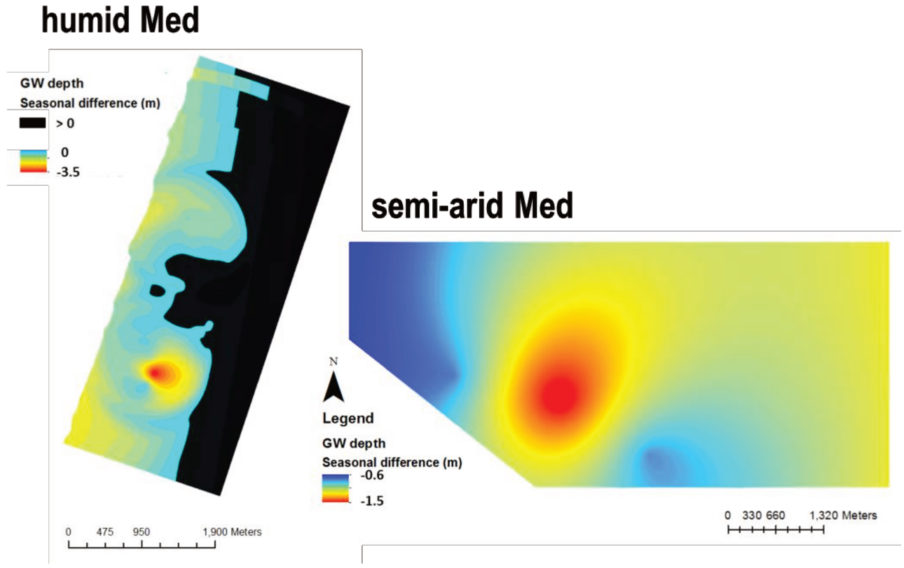

Figure S2. Groundwater depth seasonal difference (GW depth April – GW depth August) in humid and semi-arid Mediterranean sites. Specific legends in figure. Minus signal represents summer groundwater lowering, seasonal differences higher than zero (no GW lowering, i.e either stable or summer rising water table) are masked with black color.

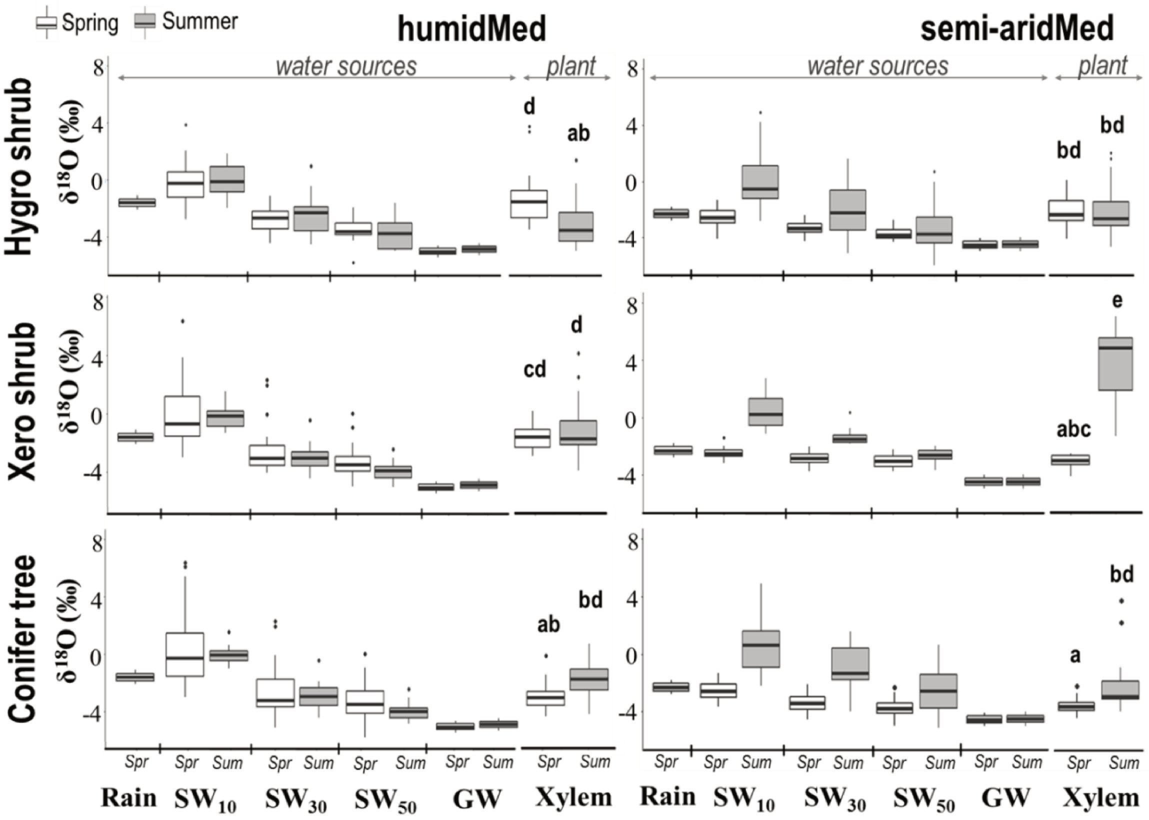

Figure S3. Boxplots of water sources $\delta^{18}O$ (‰) (soil water at three different depths, rain and groundwater) and xylem water of the three plant functional types (xerophytic shrub, hygrophytic shrub and conifer tree). White boxes represent spring and grey represents summer data (*Spr* and *Sum*). Significant differences in xylem water $\delta^{18}O$ across seasons and plant types are represented by different letters.

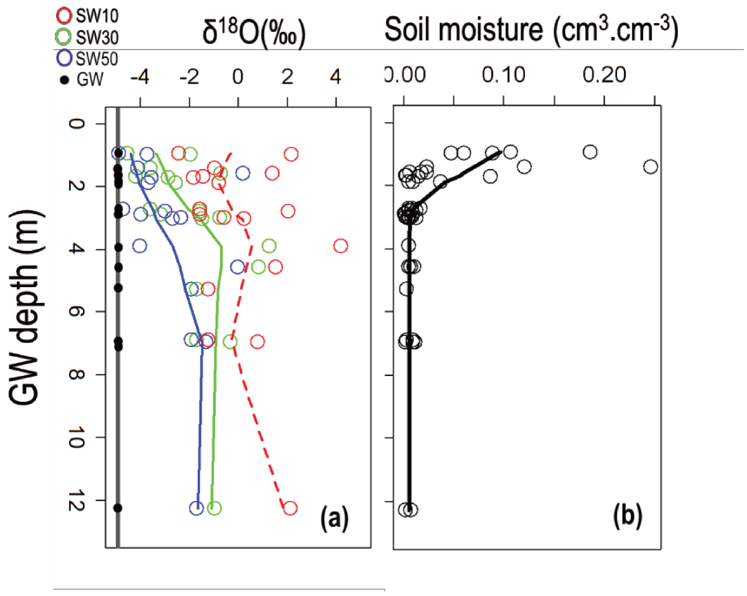

Figure S4. Summer in semi-arid site: relationship between groundwater (GW) depth and (a) Mean soil $\delta^{18}O$ at three depths (different water sources are represented by different colors as showed in the inner legend), and (b) Volumetric soil moisture content ($cm^3.cm^{-3}$). (a) Fitted lowess lines: dashed for non-significant and continuous line for significant spearman correlations between variables ($p<0.05$). Check Table S4 for detailed spearman correlations between variables (rho and p-value).

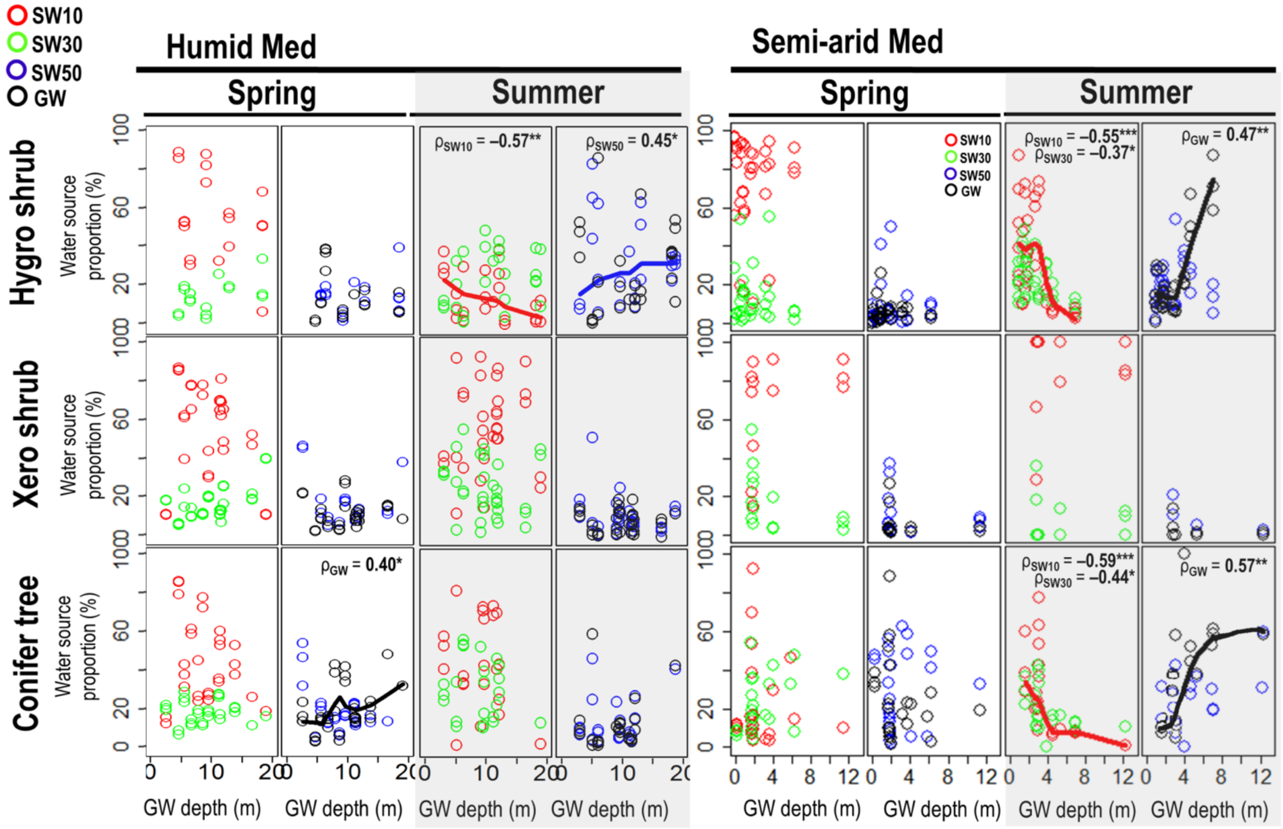

Figure S5. Water use proportions of the three plant functional types (hygrophytic shrub, xerophytic shrub and conifer tree) along the groundwater depth gradient in spring and summer, at two climatic sites (humid and semi-arid Mediterranean). Different water sources are represented by different colors as showed in the inner legend. Spearman rho (ρ) and respective p-value (*p<0.05, **p<0.01, ***p<0.001) is presented in the figure if the correlation between variables was significant (p<0.05). For visualization purposes, lowess lines were used to fit the data that showed significant spearman correlations. Check Table 2 (main manuscript) for more details on (spearman) correlations between variables.

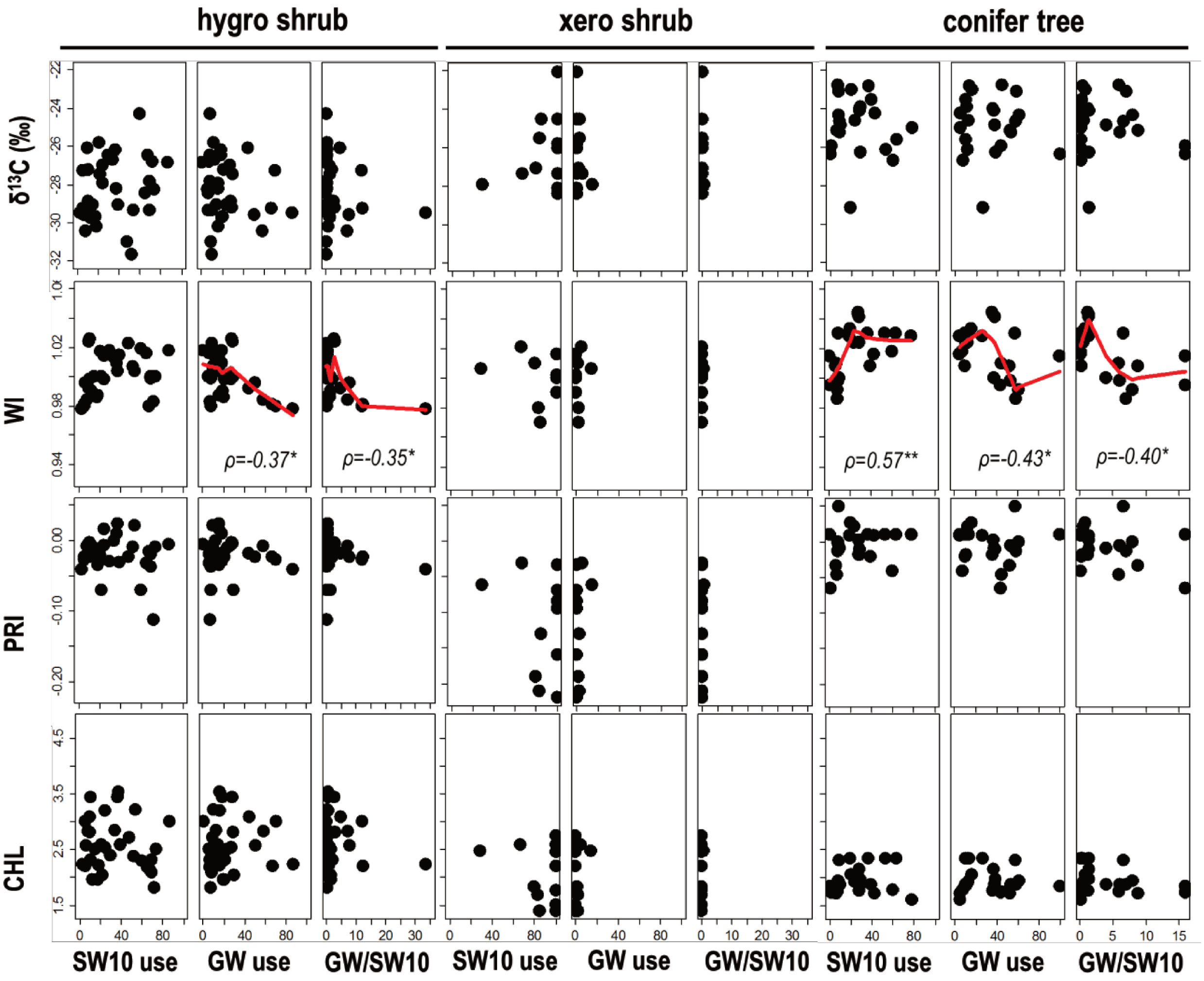

Figure S6. Relationship between water use proportions and physiological parameters for the three plant functional types (hygrophytic shrub, xerophytic shrub and conifer tree) considering data of summer semi-arid site. Spearman rho (ρ), respective p-value (*$p<0.05$, **$p<0.01$, ***$p<0.001$) and lowess line (for visualization purposes) are represented in the figure only if the correlation between variables was significant ($p<0.05$). Check Table 3 (main manuscript) for more details on (spearman) correlations between groundwater use and physiological variables.

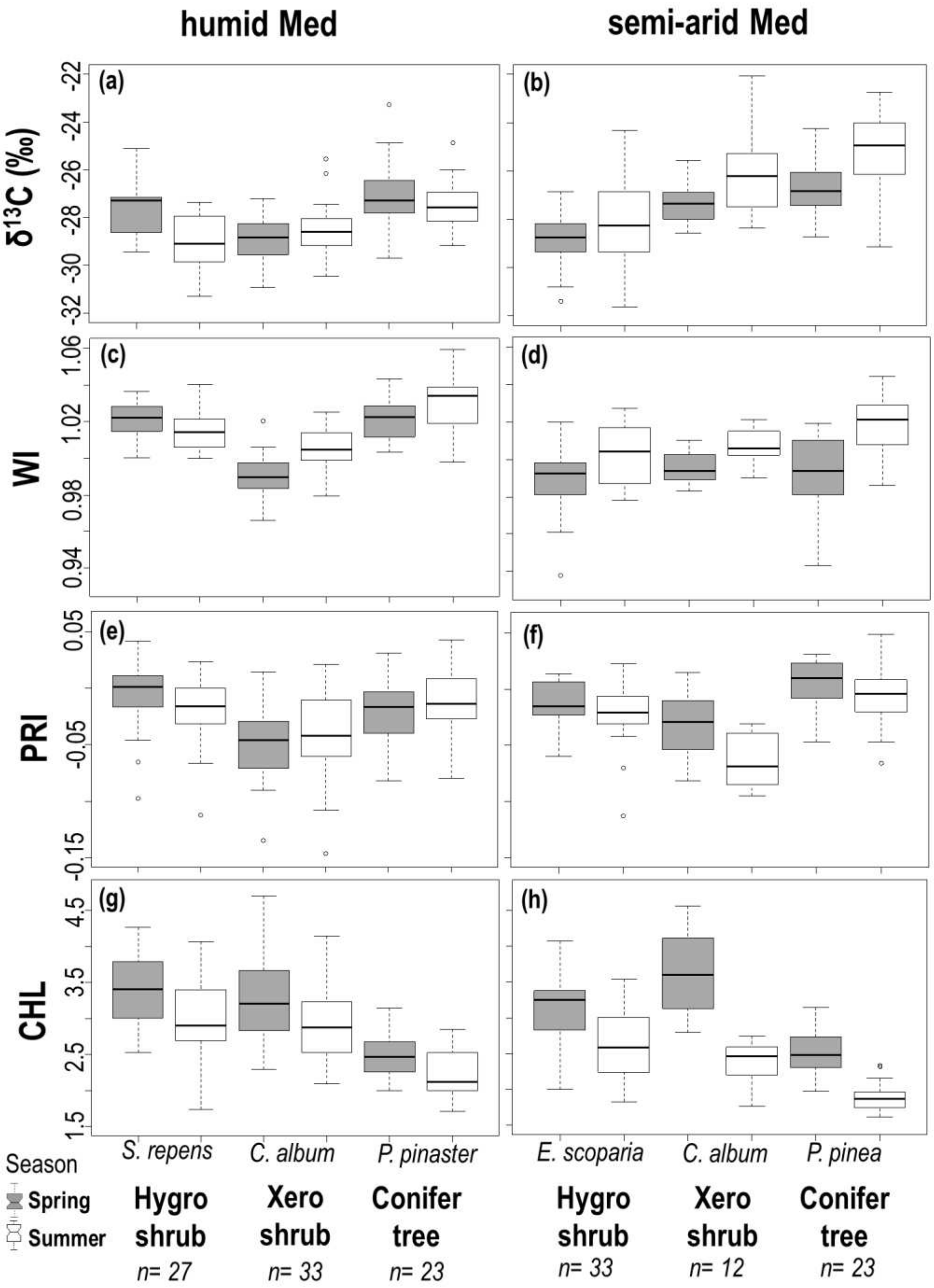

Figure S7. Spring and summer physiological parameters of the three plant functional types (hygrophytic shrub, xerophytic shrub and conifer tree): (a), (b) $\delta^{13}C$; (c), (d) water index (WI); (e), (f) photochemical reflectance index (PRI); (g), (h) chlorophyll index (CHL); in (a), (c), (e), (g) humid and (b), (d), (f), (h) semi-arid site. Grey boxplots represent spring and white boxplots summer. Number of plants considered (N) of each functional type within a site and season is represent in the figure.

3.2 Groundwater drawdown drives ecophysiological adjustments of woody vegetation in a semi-arid coastal ecosystem

Antunes C., Chozas S., West J., Zunzunegui M., Diaz Barradas M.C., Vieira S., Máguas C. (2018) Groundwater drawdown drives ecophysiological adjustments of woody vegetation in a semi-arid coastal ecosystem. *Global Change Biology*. https://doi.org/10.1111/gcb.14403

3.2.1 Abstract

Predicted droughts and anthropogenic water use will increase groundwater lowering rates and intensify groundwater limitation, particularly for Mediterranean semi-arid ecosystems. These hydrological changes may be expected to elicit differential functional responses of vegetation either belowground or aboveground. Yet, our ability to predict the impacts of groundwater changes on these ecosystems is still poor. Thus, we sought to better understand the impact of falling water table on the physiology of woody vegetation. We specifically ask (i) how is woody vegetation ecophysiological performance affected by water-table depth during the dry season?, and (ii) does the vegetation response to increasing depth to groundwater differ among water-use functional types?

We examined a suite of physiological parameters and water-uptake depths of the dominant, functionally distinct woody vegetation along a water-table depth gradient in a Mediterranean semi-arid coastal ecosystem that is currently experiencing anthropogenic water extraction pressure.

We found that groundwater drawdown did negatively affect the ecophysiological performance of the woody vegetation. Across all studied environmental factors, depth to groundwater was the most important driver of ecophysiological adjustments. Plant functional types, independent of groundwater dependence, showed consistent declines in water content and generally reduced C and N acquisition with increasing depths to groundwater. Functional types showed distinct operating physiological ranges, but common physiological sensitivity to greater water table depth. Thus, although differences in water-source use exists, a physiological convergence appeared to happen among different functional types. These results strongly suggest that hydrological drought has an important impact on fundamental physiological processes, constraining the performance of woody vegetation under semi-arid conditions.

By disentangling the functional responses and vulnerability of woody vegetation to groundwater limitation, our study establishes the basis for predicting the physiological responses of woody vegetation in semi-arid coastal ecosystems to groundwater drawdown.

Key-words: groundwater drawdown; plant functional types; coastal dune ecosystems; photosynthetic activity; plant water status; water-sources-use; water uptake depth; hydrological changes.

3.2.2 Introduction

The seasonal cycle of water availability drives variation in Mediterranean ecosystem function. Water availability is considered to be the main factor limiting vegetation growth in this region, and changes in precipitation are already inducing changes in these ecosystems (e.g. Jump et al., 2006; Peñuelas and Boada 2003; Allen et al., 2010; Barbeta et al., 2013). Climate models broadly predict a decrease in water resources and negative impacts on ecosystem integrity, particularly on semi-arid ecosystems (Kirtman et al., 2013). These predicted meteorological droughts will diminish recharge and increase groundwater lowering rates, intensifying groundwater limitation of plant performance (Taylor et al., 2012). Additionally, groundwater will be affected by the changing patterns of anthropogenic use, as increasing withdrawal combined with reduced recharge can decrease groundwater levels significantly (Kopp et al., 2013; Kløve et al., 2014). These trajectories raise concerns, since they can lead to insufficient supplies for both human society and ecosystems (Taylor et al., 2012).

Climate and human-induced changes in groundwater will directly affect groundwater-dependent ecosystems (Naumburg et al., 2005; Cooper et al., 2006; Colón-Rivera et al., 2014). Groundwater can serve as an important water resource for woody vegetation in semiarid landscapes (Dawson & Pate, 1996; Miller et al., 2010; Barbeta et al., 2015; Barbeta & Peñuelas, 2017; Garcia-Forner et al., 2016; Evaristo & McDonnell, 2017; Palacio et al., 2017). Particularly in sandy soils, where water retention is low, there is strong soil moisture limitation in the top soil during dry seasons. Thus, limited plant access to groundwater caused by groundwater depth changes is expected to have major impacts on plant physiological performance (Zencich et al., 2002; Rossatto et al., 2012). However, due to niche differentiation among species in response to ecohydrological factors, not all species are expected to respond in the same way (Silvertown et al., 1999, 2015; Araya et al., 2011).

Plant responses to changes in water availability are generally linked to varying capacities for water uptake and tolerance of water stress (Chaves et al., 2002; West et al., 2012; Pivovaroff et al., 2016; Grossiord et al., 2017). Both meteorological drought and hydrological drought might initiate differential species responses either belowground or aboveground (Zunzunegui et al., 1998, 2000; Imada et al., 2008; Máguas et al., 2011; Antunes et al., 2018a). After drawdown events, regional water tables commonly continue to decline, with the vegetation usually responding through progressive and unidirectional changes in abundance and composition (Froend & Sommer, 2010; Sommer & Froend, 2011). Variations in composition can be manifested as a shift towards species not dependent on specific hydrological conditions (e.g., relatively shallow groundwater), with the progressive change in hydrology continuing to force a transition in the flora towards an alternative ecohydrological state (Sommer & Froend, 2011, 2014). These variations are expected to be primarily based on ecophysiological processes of acclimation and stress tolerance. Phenotypic plasticity and physiological adjustments in plants allow some species to sustain (or increase) resource-use efficiency rates, subsequently maintaining their fitness under varying environmental conditions (Ernande & Dieckmann, 2004; Valladares et al., 2014). Still, there are some species that, by lower tolerance of water stress, decrease their physiological performance and ultimately face die-back and a decline in the community (Horton et al., 2001; Zunzunegui et al., 2005; Padilla & Pugmaire, 2007; Lloret & Granzow-de la Cerda, 2013). Interestingly, ecophysiological processes of acclimation and structural changes forced by (long-term) cyclic droughts may further mitigate the negative effects of extreme droughts and of human-induced hydrological changes (Barbeta et al., 2013, 2015; Matesanz & Valladares, 2013; Helman et al., 2017). However, there are costs and limits to the functional benefits that acclimation processes can deliver in a given environment (Valladares et al., 2007; McDowell et al., 2008).

Our ability to predict with confidence the impacts of hydrological droughts, on ecosystems with high human and climate pressure such as coastal semi-arid woodlands is still poor. Understanding current water, N and C use responses to declining water table and soil moisture in the semi-arid Mediterranean region is essential for predicting, and thus anticipating or perhaps mediating, the altered functioning of these key ecosystems under scenarios of future groundwater limitation (Schröter et al., 2005). The evaluation of physiological adjustments can improve the categorization of plant functional responses to hydrological drought, ultimately helping to better inform and make more accurate predictions regarding plant responses to future changes in groundwater availability. Through an ecophysiological perspective, we can potentially identify mechanisms underlying the hydrological impacts that threaten the persistence of ecosystems in their current structures and compositions.

Thus, in this study, we sought to better understand the impact of groundwater drawdown (i.e falling water table) on the physiology of overlying woody vegetation. Accordingly, we aimed to assess functional responses of woody species to increasing water table depth in a semi-arid Mediterranean coastal dune ecosystem under anthropogenic groundwater abstraction. We specifically ask: how is woody vegetation ecophysiological performance affected by water table depth during the dry season? Are there other environmental factors that influence variation in ecophysiological responses in the dry season? Does the vegetation response to increasing depths to groundwater differ among water-use functional types?

3.2.3 Methods

Study site

The study was conducted at a semi-arid Mediterranean coastal dune ecosystem at Biological Reserve of Doñana, southwest of Spain (Fig. S1a). The site has a typical thermo-Mediterranean climate, with a hot-dry summer. During the hydrological year of 2012-2013 total annual rainfall was 566 mm (weather station at BRD: 36°59'19"N; 6°26'35"W), close to the long-term average of annual rainfall, and the evapotranspiration was higher than precipitation (with a negative water balance of -197 mm). Top-soil moisture was reduced by 85% from spring to summer, reaching a mean value of 0.017 cm3.cm-3 in the dry season.

Topography defines a spatial gradient of depth to groundwater which has been exacerbated due to human water extraction and rainfall reduction. The excessive pumping for human use led to groundwater table lowering in the site, which is threatening Doñana habitats (De Castro Ochoa & Muñoz-Reinoso, 1997; Muñoz-Reinoso, 2001; Muñoz-Reinoso & de Castro, 2005; Muñoz-Reinoso & García Novo, 2005; Serrano & Zunzunegui, 2008; Díaz-Paniagua & Aragonés, 2015).

The water table level (height relative to sea level) was measured every month with a portable probe in PVC piezometers (diameter 6 cm) installed at 7 sampling points (Serrano & Zunzunegui 2008). The belowground vertical distance from the soil surface to the water table (i.e. depth to groundwater), across the study area, was estimated using a digital terrain model (DTM) and through a geo-statistical approach as in Antunes et al. (2018a). We observed a shallower groundwater table depth in winter months and a deeper summer water table.

Nineteen sampling plots (20 m x 20 m), separated by at least 200 m, were distributed throughout the study area, which presented a spatial gradient of depths to groundwater (Fig. S1b). The spatial gradient of depth to groundwater across the plots ranged from 0.94 to 12.28 m during the summer (August).

As all sampling plots were in similar sandy soils and under the same meteorological conditions during the dry season, we can focus on specific responses of vegetation to water table depth and top-soil moisture variations.

Plant species

The vegetation is primarily Mediterranean scrub with local patches of Juniperus phoenicea and Quercus suber woodlands, and the presence of Pinus pinea (Muñoz-Reinoso & García Novo, 2005). We focused our study on woody plant species. In each plot, we sampled two dominant scrub species and the dominant tree species (either one or two species). When possible, we sampled three individuals per species per plot. Fifteen woody plant species, with distinct functional characteristics (Díaz Barradas et al., 1999), and a total of 191 plants were sampled (Table 1). In a previous study (Antunes et al., 2018b) these species clustered in five water-use functional groups, with distinct summer water-use patterns, exploring soil water from shallower to deeper soil layers. The species in this study and their respective functional type classification are described in Table 1.

Table 1. Species names, number of individual plants sampled (n), functional type classification (based on water used by plants in the dry season), and water-uptake depth for each functional type (mean ± standard deviation and maximum estimated depth). Different letters stands for significant differences between functional types.

Species	***n***	**Summer water-use functional type***	**Description**	**Water sources used (dry season)***	**Potential water-uptake depth (m) [mean ± SD (max)]**
Cistus libanotis	6	NXs	Narrow-leaved xerophytic shrubs	Shallow	0.17 ± 0.15^{a} (0.7)
Halimium calycinum	11				
Rosmarinus officinalis	9				
Corema album	9				
Cistus salviifolius	6	Os	Aphyllous/spike or semi-deciduous broad-leaf shrubs		0.6 ± 0.38^{ab} (1.4)
Lavandula stoechas	6				
Stauracanthus genistoides	3				
Ulex australis	3				
Halimium halimifolium	42	sMs	Semi-deciduous mesophytic shrub		0.86 ± 1.2^{b} (5.5)
Erica scoparia	33	eH	Evergreen hygrophytic shrubs and trees		0.96 ± 1.25^{b} (6.12)
Phillyrea angustifolia	7				
Salix atrocinerea	3				
Quercus suber	5				
Pinus pinea	24	eXt	Evergreen xerophytic trees		1.9 ± 1.61^{c} (5.79)
Juniperus phoenicea	24			Deep	

*based on Antunes et al., 2018b.

Physiological parameters

Ecophysiological traits measured included leaf C and N concentrations and isotope ratios and spectral reflectance indices from plants sampled at the 19 sampling plots (Fig. S1). In each sampling plot, all physiological measurements were carried out during the dry season, in late summer, September 2013.

Leaf carbon and nitrogen isotope composition

Mature leaves (2-10, depending on leaf size) were collected from each plant. The bulk leaf samples were dried at 60 ºC for at least 48 h, and milled to fine powder in a ball mill (Retsch MM 2000, Germany) for isotopic analysis. Leaf $\delta^{13}C$ and $\delta^{15}N$ and C and N concentrations were determined by continuous flow isotope ratio mass spectrometry (CF-IRMS) on a Sercon Hydra 20-22 (Sercon, UK) stable isotope ratio mass spectrometer, coupled to a EuroEA (EuroVector, Italy) elemental analyser. Uncertainty of the isotope ratio analysis, calculated using values from 6 to 9 replicates of secondary isotopic reference material interspersed among samples in every batch analysis, was ≤ 0.1‰.

Reflectance indices

We used a nondestructive optical method, based on the reflectance of light by an intact leaf. Spectral reflectance was measured using a UniSpec Spectral Analysis System (PP Systems, Haverhill, Massachusetts, USA) and carried out in 6 different leaves per plant. The mean value of the 6 measurements per plant was considered. The following reflectance indexes were calculated:

Chlorophyll index (CHL) = $R750/R705$, where $R750$ and $R705$ are the reflectance at wavelengths of 750 nm and 705 nm, respectively (Peñuelas *et al.*, 1995). This index has been found to be well correlated with leaf chlorophyll content on a number of plant species, and can provide information about photosynthetic potential, primary production and nutrient status (Richardson *et al.*, 2002).

Photochemical Index (PRI) = $(R531-R570) / (R531+R570)$, where $R531$ and $R570$ are the reflectance at wavelengths of 531 nm and 570 nm, respectively (Peñuelas, Llusia, Pinol, & Filella, 1997). There is an emerging consistency in the relationship between PRI and light use efficiency, which can be used as an index of photosynthetic activities (Wong & Gamon, 2015).

Water Index (WI) = $R900/R970$, where $R900$ and $R970$ are the reflectance at wavelengths of 900 nm and 970 nm, respectively (Peñuelas *et al.*, 1997). This index is highly related to plant water content, so it can be used as a proxy for water status in the plant (Claudio *et al.*, 2006).

Normalized difference vegetation index (NDVI) = $(R900-R680) / (R900+R680)$, where $R900$ and $R680$ are the reflectance at wavelengths of 900 nm and 680 nm, respectively (Gamon *et al.*, 1995). NDVI allows the assessment of biomass "greenness," and therefore of plant photosynthetic capacity (Mänd *et al.*, 2010).

Estimation of potential water uptake depth

To determine the isotopic composition of available water sources, samples were collected from two distinct water pools: shallow soils (region in the soil profile above 60 cm) and groundwater (deep water-source) (Dawson *et al.*, 2002; Berry *et al.*, 2017), on the same days of physiological sampling. In each sampling plot, soil samples were collected at 3 depths: 10 cm, 30 cm, and 50 cm, with 3 replicates per depth. Groundwater samples were collected in piezometers (sampled after bailing to remove stored casing water) or wells reaching the water table (n=3) (Newman *et al.*, 2010). Since it didn’t rain in the two weeks prior to sampling, we didn’t consider rainwater as an available water source for plants during the period of sampling. Lignified woody stems of each individual selected for physiological measurements were also collected at the same dates of soil sampling. Soil and twig sampling, storage and water extraction by vacuum distillation followed the methodology detailed in Antunes et al. (2018) (Ehleringer & Dawson, 1992; West *et al.*, 2008a). Oxygen stable isotope ratio analysis of all water samples was performed by headspace equilibration, on an Isoprime (Micromass, UK) IRMS, coupled in continuous flow mode to a Multiflow (Micromass, UK) auto-sampler and sample equilibration system. Analytical uncertainty was <0.1‰.

The relative contribution of different water sources to the composition of the xylem water was estimated by individual-based Bayesian stable isotope mixing models, using the graphical user interface and model framework MixSIAR for R (Parnell *et al.*, 2013; Stock & Semmens, 2013), as described in Supporting information - Appendix 1 (Antunes *et al.,* 2018).

After the quantification of water sources used by plants, we estimated the potential maximum water uptake depth of each individual plant. For that we used a weighted average of the contribution of the different soil layers to the xylem water (derived from MixSIAR), as follows:

$$Potential\ WUD\ (m) = \frac{(\mathrm{SW10} * 0.10) + (\mathrm{SW30} * 0.30) + (\mathrm{SW50} * 0.50) + (\mathrm{GW} * \mathrm{GWdepth})}{100}$$

being SW_{10}, SW_{30}, SW_{50} and GW the % of contribution of the soil layers; 0.10, 0.30 and 0.50 the soil layer depths in m and GW depth the summer water table depth in meters (minus the hypothetical capillary fringe of 0.5 m) (see Appendix 2 for further details). For water table depth, information based on depth to groundwater maps (obtained as in Antunes et al., 2018) was used.

Environmental variables

(i, ii) Spring and summer groundwater depths were calculated per sampling plot using the groundwater depth maps of April and August, respectively.

(iii) Annual mean groundwater depth was obtained per sampling plot by calculating the mean of monthly groundwater depths from October 2012 to September 2013 (hydrological year).

(iv) Seasonal variation of groundwater (GW) depth was calculated per sampling plot, as GW diff (m) = summer GW depth – spring GW depth, yielding an estimation of groundwater lowering from April to September.

(v, vi) Spring and summer top-soil water contents were obtained by measuring the soil water moisture ($cm^3.cm^{-3}$) in April and September using a ML3 ThetaProbe Soil Moisture Sensor (Delta-T Devices). Soil moisture (~10 cm deep) was measured at three points per sampling plot and a mean value considered.

(vii) Seasonal variation in surface soil water content (SWC_{10}) was calculated at each sampling point, as SWC_{10} diff (%) = summer SWC_{10} – spring SWC_{10}.

(viii) Distance to the sea was calculated as the linear distance (m) from the sampling point to the nearest shore line (in ArcGIS 10.4.1).

Statistical analysis

Multicollinearity among potential environmental explanatory variables was handled by dropping collinear covariates when correlated at Pearson $|r| > 0.7$ (Dormann *et al.*, 2013) (Table S1). This selection resulted in a reduced set of five potential explanatory variables: Summer groundwater depth (GW depth), Seasonal variation of GW depth (GW diff), Spring top-soil water content ($SWCsp_{10}$), Summer top-soil water content ($SWCs_{10}$) and Distance to the sea (Sea Distance).

A multivariate principal component analyses (PCA) was performed with the individual physiological traits measurements aiming to integrate the complete set of physiological traits measured in all woody species, and defined the patterns of physiological performance of the dominant vegetation (accounting with specific relative position within the community physiological axis). Then, Spearman correlations between the environmental variables and PCA (meaningful) axes were performed to examine the relationships between those variables and the vegetation physiological traits (Serrano *et al.*, 2015). All environmental variables that showed a significant correlation ($p<0.05$) were considered for further analysis.

We tested if the measured ecophysiological traits were different among water-use functional types (considering each physiological trait separately and the PCA axis factor scores), using an analysis of variance (ANOVA). Post-hoc pairwise comparisons were performed with Tukey's HSD.

In order to select the most meaningful environmental variables, and describe their influence on the functional responses of the vegetation, we performed a stepwise multiple regression model selection by Akaike Information Criterion (AIC) (backward elimination of variables), using (i) PC1 factor scores, (ii) WI (reflectance-based water index), and (iii) Potential water-uptake depth as response variables, separately. To identify the most important independent variable(s) in the regression models, we inferred the relative importance of regressors using the 'relaimpo' package of R (Grömping, 2006). The two most important environmental predictors were selected for further analysis.

To further characterize the relationship between the most important selected environmental predictors and the functional responses of (i) vegetation, and (ii) of each plant functional type, we performed Generalized Additive Models (GAMs), using the function "gam" of the ''mgcv'' R package (Wood 2017). For both vegetation and functional groups, the responses considered were (i) individual PC1 factor scores (extracted from the PCA performed with the physiological traits), (ii) WI (reflectance-based water index), and (iii) Potential water-uptake depth. For regression models and GAMs, $SWCs_{10}$ was log-transformed.

All statistical analyses were performed in R version 3.4.3 (R Core Team, 2017).

3.2.4 Results

Influence of environmental factors on ecophysiological patterns of woody vegetation

Through a multivariate approach, we integrated the complete set of physiological traits measured in all woody species and defined the patterns of physiological performance of the dominant vegetation (accounting with their specific relative position within the overall vegetation's physiological axis, Fig. 1 and Fig. S2). The first two axes of the principal component analysis (PCA), PC1 and PC2, accounted for 40.6% and 18.6% of the variance, respectively. PC1 reflected a gradient of physiological performance, from low to high values of chlorophyll content index (CHL), normalized difference vegetation index (NDVI) and leaf δ^{15}N; and high to low values of δ^{13}C and leaf C/N (Fig. 1; Table S2), while PC2 reproduced a plant water status gradient, from low to high values of plant water index (WI) and Photochemical Index (PRI) (Fig. 1; Table S2). Since PC2 was mainly reflecting WI (Table S2), plus the WI was the most significant physiological trait associated with groundwater depth (Fig. S5), and a previous study showed that WI could be an important response factor to water table depth (Antunes *et al.*, 2018), we used WI as a response variable in the subsequent analysis.

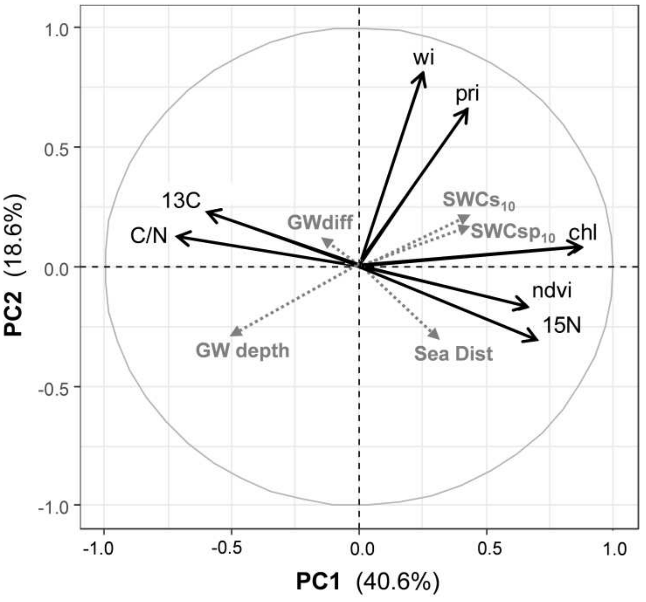

Figure 1. First and second axis of a principal component analysis (PCA) based on individual physiological measurments (n=191). For physiological parameters considered see Methods section. The first axis (PC1) explains 40.6% of the variance and reflects a gradient of photosynthetic activity and nitrogen acquisition, while the second one (PC2) explains 18.6% and reproduces a water status gradient. Abiotic (non autocorrelated) variables were included as supplementary variables (grey dashed arrows). Abiotic supplementary variables considered: depth to groundwater (GW depth), Distance to the sea (Sea Dist), Spring soil water content at 10 cm depth (SWCsp$_{10}$), Summer soil water content at 10 cm depth (SWCs$_{10}$), and Seasonal difference of GW depth (i.e. seasonal GW lowering) (GWdiff).

Thus, using PC1 as a proxy of *physiological performance* related with photosynthetic capacity and both carbon and nitrogen acquisition, and WI as a proxy of plant *water status*, we explored the influence of the environmental variables on plants' physiology. Additionally, as described in the methods, we also considered the potential water uptake depth as a response variable. There was a significant correlation between the five candidate environmental predictors and the physiological performance axis (PC1) (Table 2).

Table 2. Spearman correlations (rho coefficient) between the axes of the principal component analysis performed with the physiological variables (PC1, 40.6% and PC2, 18.6%) and the (selected) environmental variables: Summer groundwater table depth (GW depth), Seasonal variation of GW depth (GW diff), Spring top-soil water content (SWCsp10), Summer top-soil water content (SWCs10) and Distance to the sea (Sea Distance). *** p<0.001; ** p<0.01; *p<0.05.

	PC1		**PC2**	
GW depth	-0.51	***	-0.29	***
GW diff	-0.14	*	0.13	
SWCsp10	0.45	***	0.17	*
SWCs10	0.46	***	0.23	**
Sea Distance	0.34	***	-0.32	***

The multiple regression analysis showed that only spring SWC_{10} did not significantly influence PC1, and 47.4% of PC1 deviance was explained by GW depth, log($SWCs_{10}$), seasonal difference of GW depth (GW diff) and distance to sea (Sea Dist) (Table 3). The variables GW depth, log($SWCs_{10}$) and Sea Dist explained 39.3% of WI's variance, while GW diff and spring SWC_{10} were non-significant variables (and thereby excluded from the WI model). Potential water-uptake depth was mostly explained by groundwater related variables (Table 3).

Table 3. Results of multiple regression between ecophysiological response variables and the predictors obtained by stepwise selection. Deviance explained (%) by each abiotic predictor, deviance explained by the model, adjusted R^2, F value and the model are represented in the table. The ecophysiological response variables considered were the first axis of the PCA (PC1, as a proxy of physiological condition related with photosynthetic activity and nitrogen acquisition), Water Index (WI, a proxy of plant water status) and Potential water uptake depth (W-uptake depth). Predictor variables not selected ($p>0.05$), and not included in the model, are denoted as '*ns*'. Predictors that explain more than 10% of the deviance are in bold, and considered the most important variables in the regression model. *** $p<0.001$; ** $p<0.01$; *$p<0.05$.

Predictors	**PC1**	**WI**	**Water-uptake depth**
GW depth	**16.1%**	**27.8%**	**13.40%**
log(SWCs10)	**18.9%**	7.75%	*ns*
Sea Distance	9.11%	3.74%	5.58%
GW diff	3.32%	*ns*	9.01%
SWCsp10	*ns*	*ns*	3.70%
Deviance explained	47.4% [a]	39.3% [b]	31.7% [c]
adjusted R^2	0.463***	0.384***	0.301***
F	41.96	40.43	18.72

[a] $PC1=3.74-0.21\mathit{GW}+1.30\mathit{SWCs}-1.74\mathit{GWdiff}+0.4e^{-4}\mathit{SeaDist}$

[b] $WI=1.05-3.79e^{-3}\mathit{GW}+7.8e^{-3}\mathit{SWCs}-6.38e^{-6}\mathit{SeaDist}$

[c] $WUD=2.46+0.16\mathit{GW}+4.28\mathit{SWCsp}-1.56\mathit{GWdiff}-4e^{-4}\mathit{SeaDist}$

Overall, the most important explanatory variables of ecophysiological variation were GW depth and summer SWC$_{10}$ (Table 3). Therefore, these were the predictors considered for further detailed relationships analysis. Both PC1 and WI were significantly, and non-linearly, affected by the selected predictors: negatively by GW depth and positively by summer SWC$_{10}$ (Fig. 2). Potential water-uptake depth was weakly correlated with GW depth (adjusted $R^2=0.23$, $p<0.001$), and not significantly influenced by summer SWC$_{10}$ ($p=0.07$) (Fig. 2). The significant non-linear relationship between both PC1 and WI and GW depth presented two inflection points: at ~3 m and ~7.5 m groundwater depth (Fig. 2).

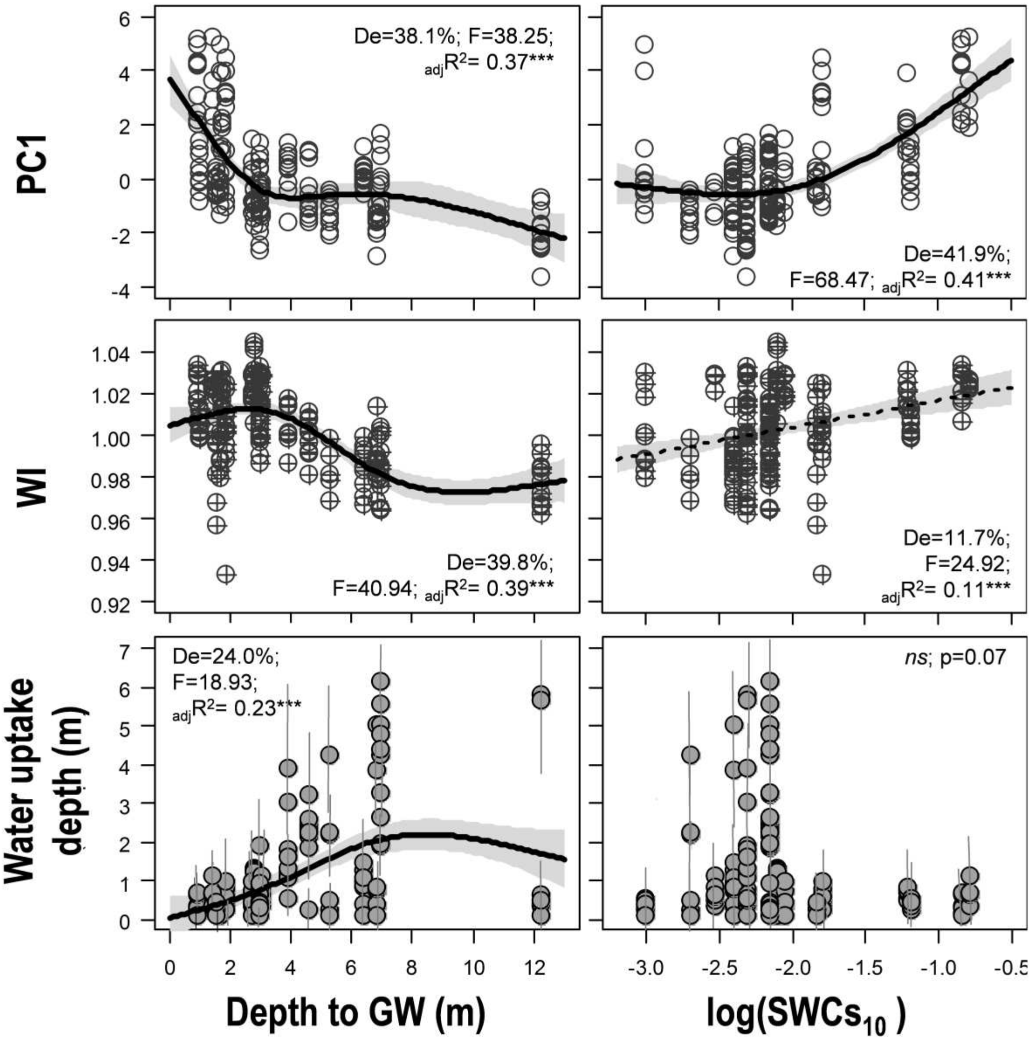

Figure 2. Relationships between the selected (most important) environmental variables and plant physiological variables. Environmental variables considered: depth to groundwater (Depth to GW) and soil water content in summer at 10 cm [log(SWCs10)]. Ecophysiological variables considered: scores of the first axis of PCA (PC1), reflectance water index (WI) and potential water-uptake depth (Water-uptake depth). PC1 represents a proxy of C and N acquisition and WI the plants' water status. Solid and dashed lines represent the main trend of a Generalized Additive Model (GAM) explaining more than 20 % of the variance and less than 20 %, respectively. Grey bands represent 95 % confidence intervals of a GAM. Statistical information shown in the figure: deviance explained (De), F- value (F), adjusted R2 (adjR2) and p-value (*** p<0.001; ** p<0.01; *p<0.05'). Grey vertical bars in water-uptake depth panels represent the standard deviation. n=191.

Adjustments of plant functional types along a groundwater depth gradient and to top-soil water content

We first tested if the vegetation physiological performance were different among water-use functional types (see Table 1). We found significant differences in physiology (PC1 and PC2) between the functional types (F=69.41, p<0.001; and F=22.10, p<0.001, respectively) (Fig. S3, S4ac). We further realized that *J. phoenicea* and *P. pinea*, although from the same functional type, showed a different physiological pattern, especially on water status (Fig. S2, S3, S4bd). Thus, we subdivided this functional type, considering these species separately in the following analysis. The considered six plant types clustered in the PCA, showing different summer physiological patterns (Fig. 3).

The evergreen hygrophytic species (that explore deeper soil layers and rely on groundwater) showing the higher PC1 scores (i.e. higher photosynthetic capacity and both carbon and nitrogen acquisition), while narrow-leaved xerophytic shrubs (that rely on water from shallower soil layers) showing the lower ones (Fig. 3). The evergreen conifer tree *P. pinea* (dimorphic rooted species that are able to explore deeper soil layers) showed the highest values of water status (PC2, WI), while xerophytic shrubs the lowest ones (Fig. 3, Fig. S3, Fig. S4d).

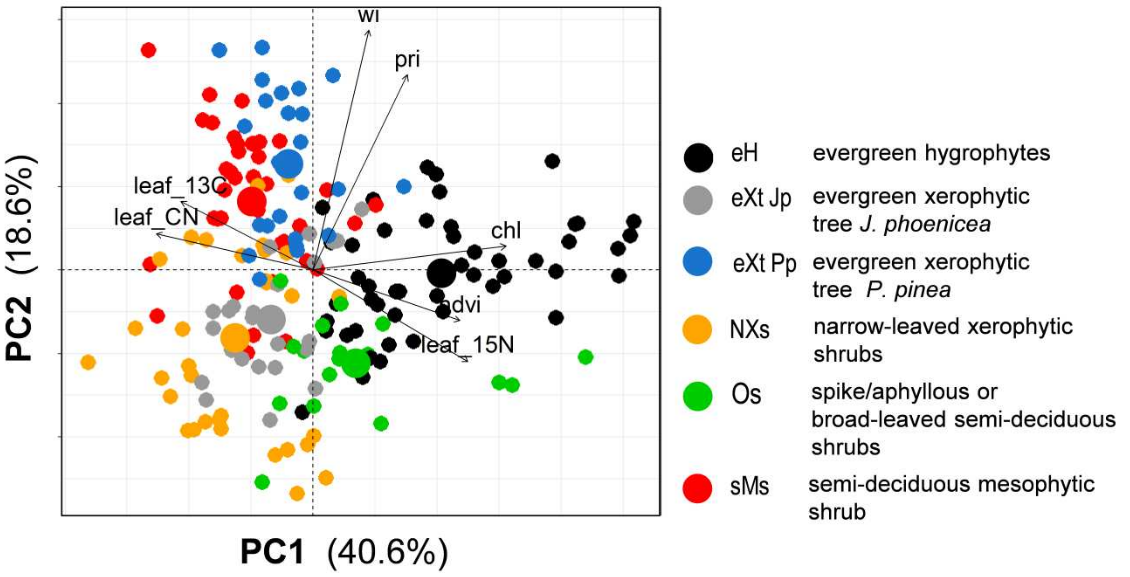

Figure 3. Principal component analysis (PCA) based on physiological variables showing the individual factor scores and plant functional types (principal component axes 1 and 2). For physiological parameters considered see methods section. Plant functional types are listed in the inner legend. The mean value of each functional type is represented by a bigger symbol. For functional classification based on plant water-use and its effects on PC1, see Table 1 and Table S2. n=191

Secondly, we explored the responses to the identified most important environmental drivers, i.e. summer groundwater depth (GW depth) and top-soil water content (SWC_{10}), among water-use functional types. For that, we used the PC1 factor scores of each functional type as a proxy of the variation of physiological performance within functional type (considering their relative physiological position within the community). The significant positive correlation observed between physiological performance and SWC_{10} considering the overall woody vegetation (Fig. 2, Fig. S5) did not hold up when considering different functional types (Fig. 4). Evergreen hygrophytic species was the only group that showed a significant positive trend (Fig. 4). Conversely, we found a significant decrease of physiological performance (PC1) with increasing depths to groundwater among several functional types (Fig. 4, Fig. S6). Nevertheless, the photosynthetic conditions of the xerophytic tree *P. pinea* were not affected by groundwater lowering (Fig. 4d, Table S4). Although hygrophytic species also made water uptake adjustments towards deeper soil layers when groundwater was deeper, unlike *P. pinea*, their physiological performance (PC1) values declined (Fig. 4a, Table S4). This decline was not linear, showing an inflection at ~ 3m of depth to groundwater, after which the PC1 values don't change.

Shrubby species that did not adjust their water uptake depth towards deeper soil layers with increasing depths to groundwater, linearly declined their PC1 (Fig. 4b,f).

We further confirmed the high variation in water uptake depth responses and water-sources-use strategies among the different functional types (Fig. 4, Fig. S3). The relevant water-source-use adjustments observed in both evergreen xerophytic tress and hygrophytic species were driven by groundwater depth, but not SWC_{10} (Fig. 4a,d,e).

We found a significant decrease of WI with increasing depths to groundwater among all functional types (except for aphyllous species) (Fig. 4). Because this common trend was shared by functional types differing in water-sources-use, and no significant correlations were found between WI and water-uptake depth in five functional types (Table S3), WI changes seem to be independent of water sources used by plants. The only plant types that additionally changed their WI in function of SWC_{10}, were narrow-leafed xerophytic shrubs and hygrophytic species (Fig. 4a,b).

A summary of how hydrological changes affected the woody vegetation and the different functional types (i.e. their ecophysiological responses to groundwater depth) is shown in Table 4.

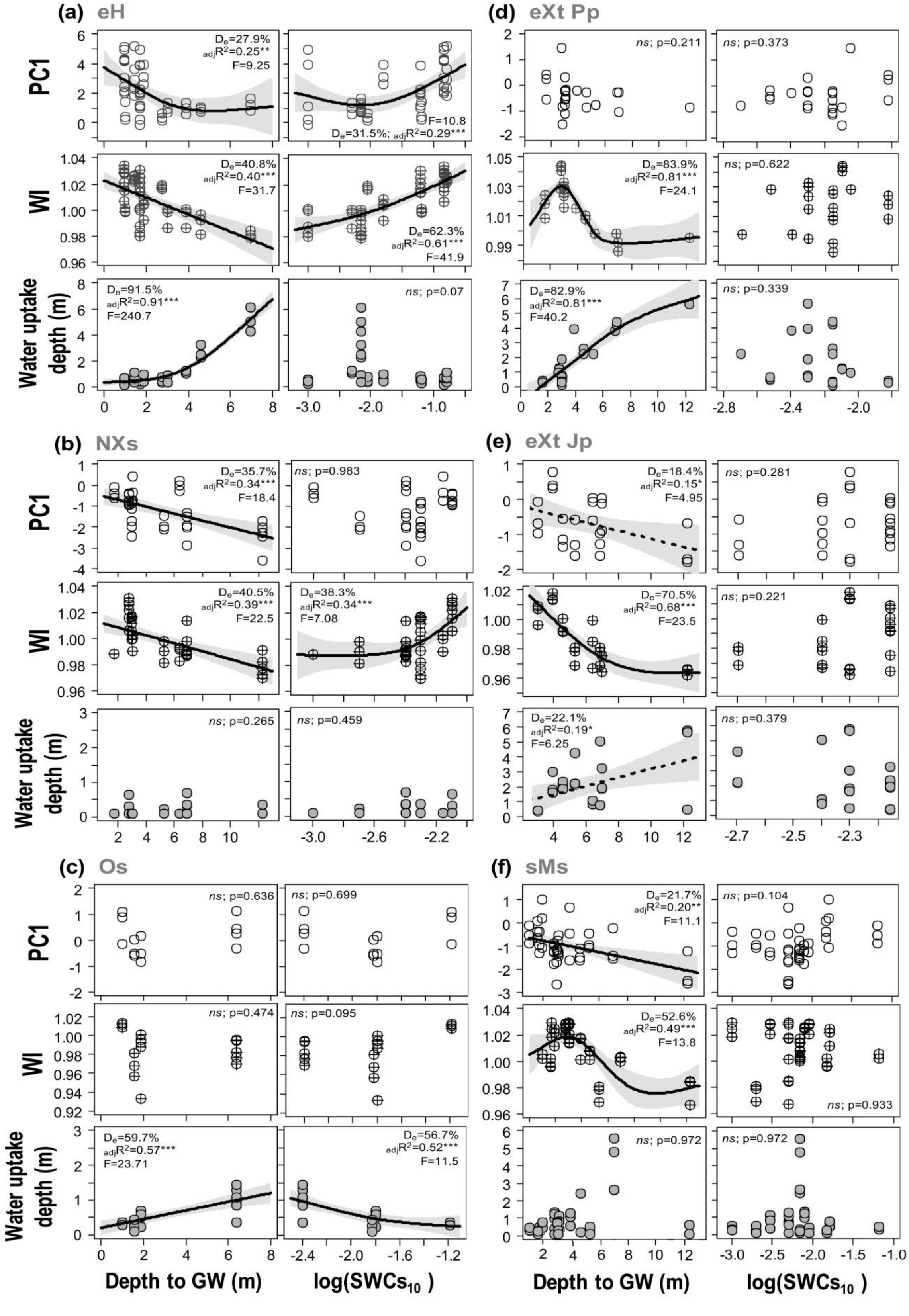

Figure 4. Relationships between the selected environmental variables and plant physiological variables per functional type. Environmental variables considered: depth to groundwater (Depth to GW) and top-soil water content in summer [log(SWCs10)]. Ecophysiological variables considered: scores of the first axis of PCA (PC1), water index (WI) and potential water-uptake depth (Water uptake depth). PC1 represents a proxy of C and N acquisition and WI the plants' water status. Plant functional types considered as in Table 1: (a) eH

(n=48), (b) NXs (n=35), (c) Os (n=18), (d) eXt Pp (n=24), (e) eXt Jp (n=24), (f) sMs (n=42). Y-axis of the different functional types can present different scales. Solid and dashed lines represent the main trend of a Generalized Additive Model (GAM) explaining more than 20 % of the variance and less than 20 %, respectively. Grey bands represent 95 % confidence intervals of a GAM. Statistical information shown in the figure: deviance explained (De), F-value (F), adjusted R^2 ($adjR^2$) and p-value (*** $p<0.001$; ** $p<0.01$; *$p<0.05$).

Table 4. Functional responses to increasing water table depth of woody community (woody species) and of the different functional types in the dry season. Direction of the relationships between functional traits and depth to groundwater are denoted as arrows: down if negative (↓), up if positive (↑); null symbol if no significant correlation occurs (Ø).The functional types considered were: narrow-leaved xerophytic shrubs (NXs), semi-deciduous mesophytic shrub (sMs), evergreen hygrophytic shrubs and trees (eH), evergreen xerophytic tree *P. pinea* (eXt Pp) and evergreen xerophytic tree *J. phoenicea* (eXt Jp). The functional type that included aphyllous/spike species ('Os', see Table 1) was excluded from the table, since there was no significant correlations in either PC1 or WI.

	C and N acquisition	Water status	Water-uptake depth
Woody species	↓[a]	↓	↑
NXs	↓	↓[a]	Ø
sMs	↓	↓	Ø
eH	↓[a]	↓[a]	↑
eXt Jp	↓	↓	↑
eXt Pp	Ø	↓	↑

[a] Additional significant effect of top-soil moisture

3.2.5 Discussion

Our results showed that increasing depth to groundwater does negatively affect the ecophysiological performance of woody vegetation in the dry season. We found that there was a convergence of physiological responses among various plant functional types, independent of water-uptake depth responses. Accordingly, this hydrological factor significantly, either direct or indirectly, influenced functional traits related to both water status and photosynthetic capacity of woody plants, irrespective of their water-sources-use strategy. We observed non-linear relationships which imply the existence of a groundwater depth threshold after which there is a steep physiological response in the vegetation. We also revealed that pre-determined functional groups did sort along a physiological trait axis. Accordingly, they showed distinct operating physiological ranges, but common physiological responses to increasing depth to groundwater. They consistently declined their water content and generally reduced carbon and nitrogen acquisition with falling water table (Table 4). These results strongly suggest that groundwater drawdown has an impact on physiological fundamental processes, constraining the physiology of woody vegetation in this semi-arid ecosystem.

Woody vegetation physiological responses to increasing depth to groundwater

Through a multivariate approach we integrated into a single dimension traits related to photosynthetic activity and N assimilation and consequently obtained a proxy of the vegetation's C and N acquisition (Fig. 1). Considering this proxy and the reflectance water index (related to plant water status), we revealed that the woody vegetation's physiology was strongly affected by groundwater table depth during the dry season (Table 3). Under greater depth to groundwater and lower top-soil moisture, plants decreased their C and N acquisition, regulating water loss through stomatal control (indicated by increasing $\delta^{13}C$), while reducing chlorophyll content and NDVI (Fig. 2). Also linked to increasing depths to groundwater, we found both an increase in leaf C/N and a decrease in $\delta^{15}N$, reflecting varying N acquisition along the gradient of depth to groundwater. The impossibility of taking up nutrients can be caused by the low soil moisture, as soil water content below wilting point significantly constrains plant capacity to take up nutrients. This is strongly experienced by plants that depend on shallow water layers and where water table is deeper. Moreover, nutrient availability is known to change along soil moisture gradients, with a maximum in mesic soils and minimum in very dry conditions (Saha et al., 2009; Araya et al., 2013). Thus, the fine-scale hydrological gradient present in our study area can be also linked to N conditions (Gallardo & Merino, 1993; Martínez et al., 1998; Serrano et al., 2003), which plant $\delta^{15}N$ could reflect (Handley et al., 1999; Bedard-Haughn et al., 2003). As we observed intraspecific variation in leaf $\delta^{15}N$ linked to groundwater table depth (i.e. within species with the same nitrogen strategy) we suspect that soil water and N availability are the main causes of the $\delta^{15}N$ changes (and not mycorrhizal associations (Hobbie & Högberg, 2012)). Thus, in addition to the intrinsic limitation of nutrient acquisition under low water availability, by low water and N uptake, the decline in soil nutrient availability can be additionally promoting the decline in nitrogen content and $\delta^{15}N$ along the groundwater table depth gradient (Kulmatiski et al., 2017). Furthermore, we observed a strong link between the water status of the woody community and variations in depths to groundwater. This extends the patterns previously observed for some woody species (Antunes et al., 2018a). Interestingly, we observed a non-linear physiological pattern along the groundwater table depth gradient. At the community level, from 0 to 3 m water table depths there is a steep decline in C and N acquisition, and at water table depth of 3 m we observed a sharp decrease in plant water content. In these threshold responses, trade-offs seem to occur: although C and N acquisition decreases, water status can be maintained; while when photosynthetic activity is maintained (probably through the maintenance of stomatal conductance) water status declines (through possibly both water loss and low water uptake, at places where groundwater is deeper than 3 m). .

Ecophysiological adjustments to falling water table among plant functional types

Exploring the ecophysiological differences among water-use functional types we have found that they separated according to different physiological status (Fig. 3). Their relative position along the C and N acquisition and water status axes reflected the inter-specific variability of the woody community. These results are in accordance with the differential water-use strategies and distinct responses and adaptations to summer drought among coexisting semi-arid Mediterranean species (Filella & Peñuelas, 2003; Veneklaas & Poot, 2003; Peñuelas et al., 2011; Zunzunegui et al., 2011; West et al., 2012). Nevertheless, the individuals within each functional type varied in their ecophysiological performance, resulting in a considerable intra-specific variation. This variation within functional type was strongly associated with the depth of the water table (Fig. 4). Unlike top-soil water content, increasing depth to groundwater negatively affected all studied plant functional types. Particularly, the impact of falling water-table on water status was consistent throughout the different functional types studied. Thus, regardless of their water-sources-use strategy and different leaf-level drought strategies, plants experienced a decline in water content where groundwater is deeper and therefore less available to plants. This convergent result strongly suggests that there are a limited number of physiological responses to extremely low availability of groundwater and soil water in this woody community (Bucci et al., 2004).

There was also a generality of declines of the C and N acquisition, however this was particularly steep for those functional types that don’t root more deeply. The groundwater depth gradient produced clear root adjustments only in evergreen hygrophytic species and the coniferous tree P. pinea, an acclimation process especially relevant for species dependent in groundwater as a water source (Dawson & Pate, 1996; Zencich et al., 2002; Naumburg et al., 2005; Barbeta & Peñuelas 2017; Fan et al., 2017). In comparison to shallowly-rooted species, it seems that this strong water-source-use adjustment towards deeper soil layers compensates, although only partly, for water stress caused by decreasing groundwater availability. In fact, these belowground changes are expected to buffer plants from drought stress (Nardini et al., 2016; Johnson et al., 2018). Accordingly, *P. pinea* showed no impact on photosynthetic activity along the groundwater gradient (in accordance with Antunes et al., 2018a); and the hygrophytic species stopped exhibiting drops in C and N acquisition once an exponential rise in water-uptake depth begins (when groundwater was over 3 m deep). However, despite evidence of the use of deeper soil water across the range of water table depths, increasing depth to groundwater induced a significant decline in plant water content in these functional types. Thus, and similarly to other studies in dry areas (Horton et al., 2001; Zencich et al., 2002; Gries et al., 2003; Miller et al., 2010), water-uptake depth adjustments towards deeper soil layers allowed the woody vegetation to subsist during the dry summer but not to flourish. Comparatively, the similarly deep-rooted species *J. phoenicea*, showed a weaker response in water-uptake depth through the groundwater depth gradient. Apart from the possibility that groundwater is not the main driver of adjustments in this species, this might be caused by physiological trade-offs resulting in more variable responses along the groundwater gradient. In fact, juniper species can show a relatively high sensitivity to drought (West et al., 2007, 2008b; Grossiord et al., 2017; Johnson et al., 2018), but also great resistance (Zunzunegui et al., 2011; Dickman et al., 2015; Garcia-Forner et al., 2016).

Populations of the studied functional types might tolerate a wide range of depths to groundwater due to physiological and root trait plasticity. However, our results suggest that ecophysiological conditions can reach limiting values, with important implications for persistence of the existing community composition. In general, response to groundwater depth reflect the ecophysiological sensitivity of the vegetation. As such, higher correlation between the hydrological driver and the response implies stronger sensitivity and vulnerability, since small changes in the environment represent larger changes in the physiological condition (Esperón-Rodríguez & Barradas, 2015). From this perspective, woody vegetation in this semi-arid sandy ecosystem is expected to be more vulnerable to photosynthetic and N limitation until 3 m water table depth, and further vulnerable to water limitation at 3-8 m depth. Particularly, the narrow range of water table depths within which hygrophytes are in better physiological state suggests that future changes in the depth of water table could lead these species to high water stress levels and, ultimately, to transitions between vegetation states. Interestingly, the range reported here is in agreement with Sommer and Froend’s (2014) reported thresholds of transition of hydrotypes: at 3 m water table depth they observed a transition from hygrophytic dominant community to a more meso-xerophytic one.

3.2.6 Conclusion

We found that, during the dry summer, depth to groundwater is highly important in physiological structuring woody community in a semi-arid sandy ecosystem. We have highlighted the impact hydrological drought can have on plant water-related functional processes in this semi-arid ecosystem. Across all studied environmental factors, groundwater table depth was the most important driver of ecophysiological adjustments. Greater depths to groundwater affected all studied plant functional types, either on their photosynthetic related parameters, water status, or water-uptake depth (or a combination of all). Under these hydrological changes, a general increase in ecophysiological vulnerability of woody vegetation occurs, which strengthens the generality of hydrological drought impact on these ecosystems, where water stress is the rule. Although differing in their physiological range and limitations, and in their water-sources-use strategy, these acute physiological responses support the existence of systematic physiological constraints at the community level. Thus, the different plant functional types appeared to have distinct operating ranges along common physiological response curves dictated by groundwater table depth. Their physiological responses, caused by differing long-term groundwater limitation, reveal that water uptake depth adjustments are not enough to compensate for the experienced water deficits. From a longer-term perspective, it is expected that woody vegetation ecophysiology, and ultimately their survival, will be greatly impacted by groundwater drawdown as the water table continues to drop, depleted by prolonged drought and human consumption. We acknowledge that that this study does not establish the ecophysiological limits of the studied species, but observations of high mortality of plants in the last years in this ecosystem (Zunzunegui et al., 1998; Muñoz-Reinoso, 2001; Muñoz-Reinoso & de Castro, 2005; Lloret & Granzow-de la Cerda, 2013; Lloret et al., 2016) suggest vulnerability and fitness loss of woody vegetation under groundwater limitation, consistent with our observations. Our study contributes to efforts to predict physiological performance changes and state of woody vegetation that result from ongoing increased groundwater drawdown. These have clear implications for the conservation of plant communities that now face changing hydrological conditions caused by water extraction and climate change in this coastal ecosystem.

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The authors have no conflict of interest to declare.

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3.2.9 Supporting Information

Appendix 1. Bayesian stable isotope mixing models for quantification of water sources used by plants.

Appendix 2. Details on the estimation of potential water-uptake depth.

Figure S1. Study site and sampling points over summer groundwater table depth map.

Figure S2. PCA based on physiological measurements (n=191), showing species' individual scores.

Figure S3. Boxplots of physiological traits and water-uptake depth by functional types.

Figure S4. Boxplots of PCA factor scores of first and second dimension by functional type.

Figure S5. Relationships between physiological traits and both groundwater depth and top-soil water content considering the overall woody vegetation.

Figure S6. PCAs of physiological traits of each functional type and relationships between PCA axis and both groundwater depth and top-soil water content.

Table S1. Pearson correlations between potential predictor variables.

Table S2. Spearman correlations between the PCA axes (PC1 and PC2) and the leaf-level physiological variables.

Table S3. Spearman correlations between plant water-uptake depth, water index and PC1.

Table S4. Spearman correlations between physiological traits measured and both groundwater depth and top-soil water content, for the functional types: eH and eXt_Pp.

Supporting Information

Appendix 1 – Bayesian stable isotope mixing models for quantification of water sources used by plants

In our study, the model used as input the individual isotope values of xylem water $\delta^{18}O$ of each plant (‘mixture’ raw data), all sources described in *water sources*, and a matrix for potential fractionation (set to 0). We calculated the water-use proportion in each plot separately, using an individual-based model to obtain separate estimates for each of the plants. We ran models using the individual plant (‘ID’ within the ‘plot’) values of xylem water $\delta^{18}O$ (raw data), the sampling plot water sources’ mean (plus standard deviation), and the ‘process only (n=1)’ error structure option. We set the Markov Chain Monte Carlo to ‘long’ or ‘very long’ model run, until the diagnostics were satisfying. The summer mixing model ran with the 4 sources sampled (SW_{10}, SW_{30}, SW_{50} and Groundwater), since it didn’t rain in the two weeks prior to sampling.

Appendix 2 – Details on the estimation of 'potential water uptake depth'

We acknowledge that the isotopic composition of soil water below 50 cm depth was not measured in this study and is therefore unknown. However, it seems reasonable to suspect that the isotopic composition of soil water between 51 cm and the capillary fringe zone might very well be different to that of the groundwater (GW). This assumption is supported by the observed increasing isotopic depletion of soil water at 50 cm deep (SW_{50}) with increasing influence of groundwater table (i.e. shallower water table depth implies a higher depletion of SW_{50} ^{18}O) (Fig. SS1A). SW_{50} reaches similar isotopic values when groundwater is at ~ 1m depth (Fig. SS1A). The influence of groundwater table level on water content, through capillary rise, is also observed: till GW is at 3 m deep it influences the soil moisture of soil surface (Fig. SS1B). We considered the capillary fringe as the saturated soil zone above water-table, with $\delta^{18}O$ similar to GW, and the capillary rise region the (unsaturated) soil zone influenced by (bottom-up) soil moisture input by GW (Fig. SS1C). We assume that the isotopic and soil moisture observations along the GW depth gradient would reflect soil patterns in depth and hypothesized the capillary fringe and capillary rise region (Fig.SS1D).

We then calculated the potential water uptake depth considering groundwater isotopic signal to be present at various depths (i.e. five different scenarios of capillary fringe depth) (Fig. SS2). Notice that, considering an equal evaporation demand at soil surface, 'scenario' B, D and E of Fig. SS2 is inconsistent with the isotopic pattern observed at SW_{50}. Having into account the hydrologic framework of Fan et al. (2017) for semi-arid coarse-textured sandy soils, we chose to present in this study the hypothetical maximum value of water uptake depth calculated with scenario 'A' (Fig. SS1D, SS2A). Accordingly, we used the summer GW depth minus the hypothetical capillary fringe of 0.5 m (Fig. SSA) to calculate the 'potential water uptake depth' described in methods section.

Fan Y, Miguez-Macho G, Jobbágy EG, Jackson RB, Otero-Casal C (2017) Hydrologic regulation of plant rooting depth. Proceedings of the National Academy of Sciences, 114, 10572 LP-10577.

Liu Q, Yasufuku N, Miao J, Ren J (2014) An approach for quick estimation of maximum height of capillary rise. Soils and Foundations, 54, 1241–1245.

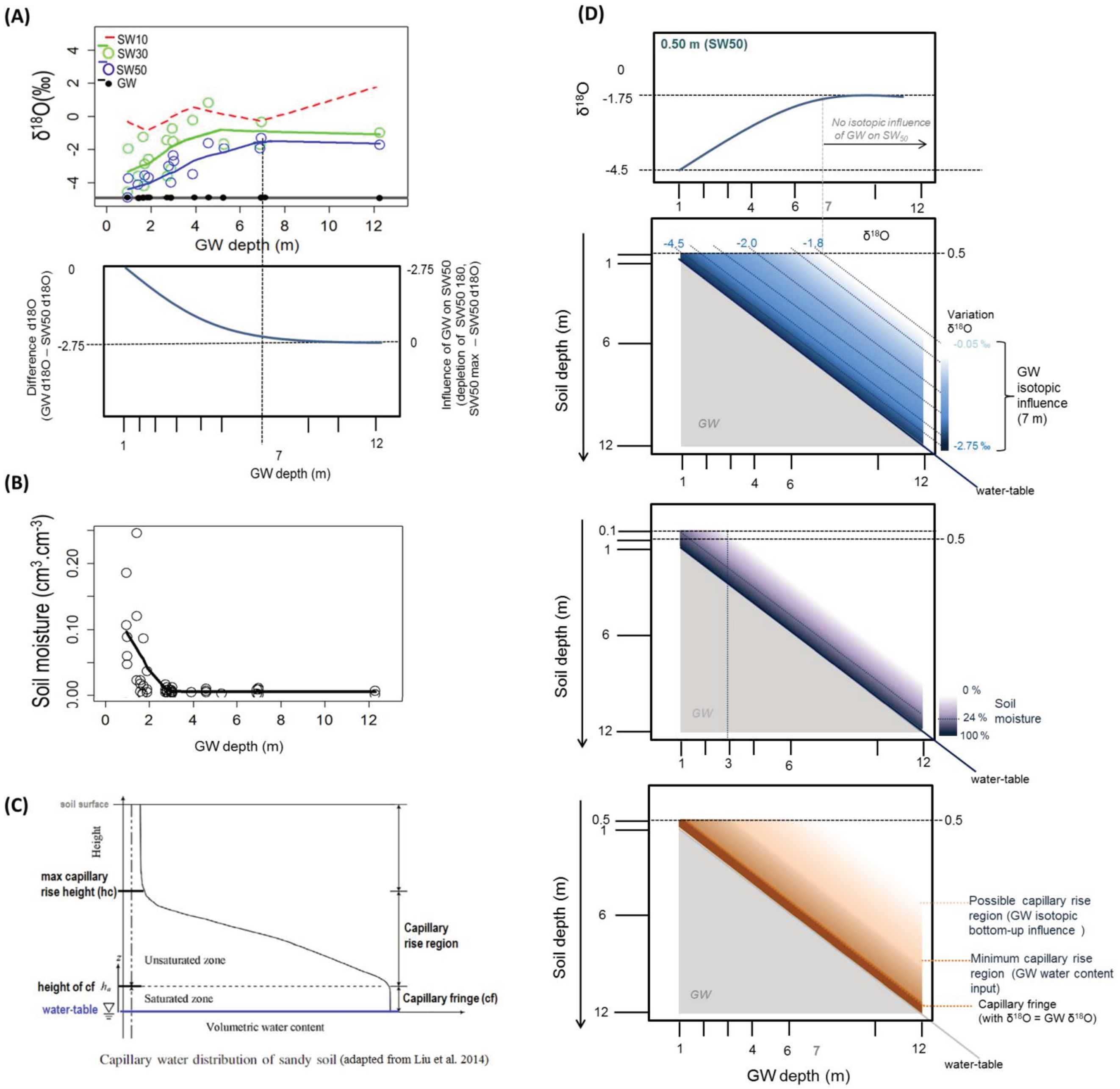

Figure SS1. (A) Relationship between groundwater depth and mean soil water δ^{18}O at 0.10, 030 and 0.50 m deep (different water sources are represented by different colors as showed in the inner legend). (B) Relationship between groundwater depth and volumetric soil water content at 10 cm depth (cm3.cm-3). Fitted lowess lines of (A) and (B): dashed for non-significant and filled line for significant spearman correlation ($p<0.05$). (C) Water content variation due to capillary rise, and capillary fringe of typical sandy soils (Liu et al. 2014). (D) From top to bottom - isotopic variation with depth along the groundwater depth gradient [using information of (A)]; soil moisture variation with depth [using information (B)]; hypothetical capillary fringe and capillary rise region [based on (A) and (B)].

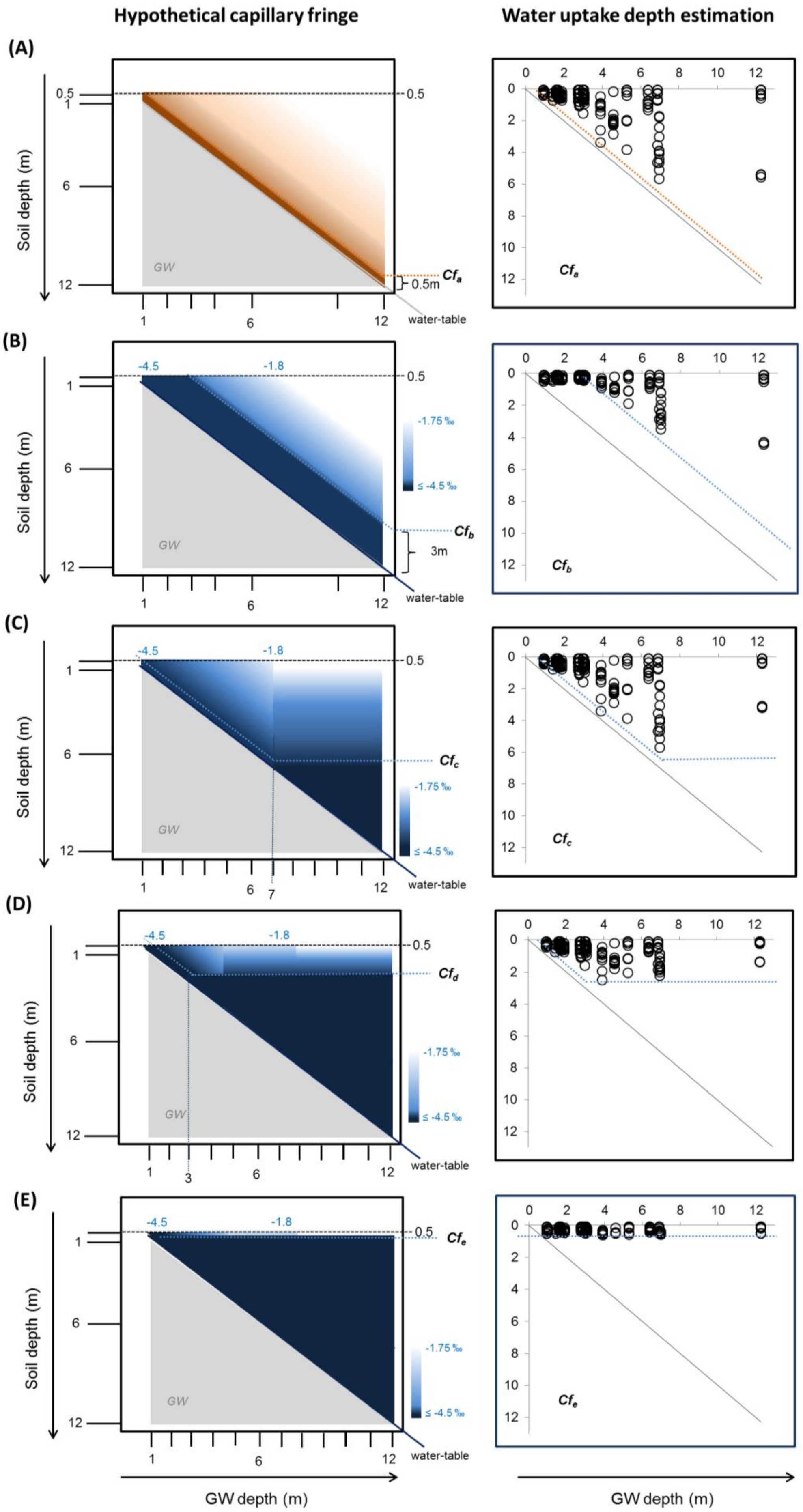

Figure SS2. Hypothetical capillary fringe (soil zone above water table, saturated with water due to capillary rise and with δ^{18}O similar to GW, *Cf*), and plants' water uptake depth (WUD) calculated considering various scenarios. (A) *Cf* based on isotopic patterns observed at SW$_{50}$. WUD of deep of dimorphic-rooted plants follows groundwater level (GW) decline. (B) *Cf* based on minimum capillary rise region (3m). (C) *Cf* based on *Cf*a till 7 m and *Cf* depth = 6.5 m when GW is deeper than 7 m. (D) *Cf* based on *Cf*a till 3 m and Cf depth = 2.5 m when GW is deeper than 3 m. (D) *Cf* based on the unknown values of deeper than 0.5 soil layers (*Cf* = 0.60 m throughout the GW gradient).

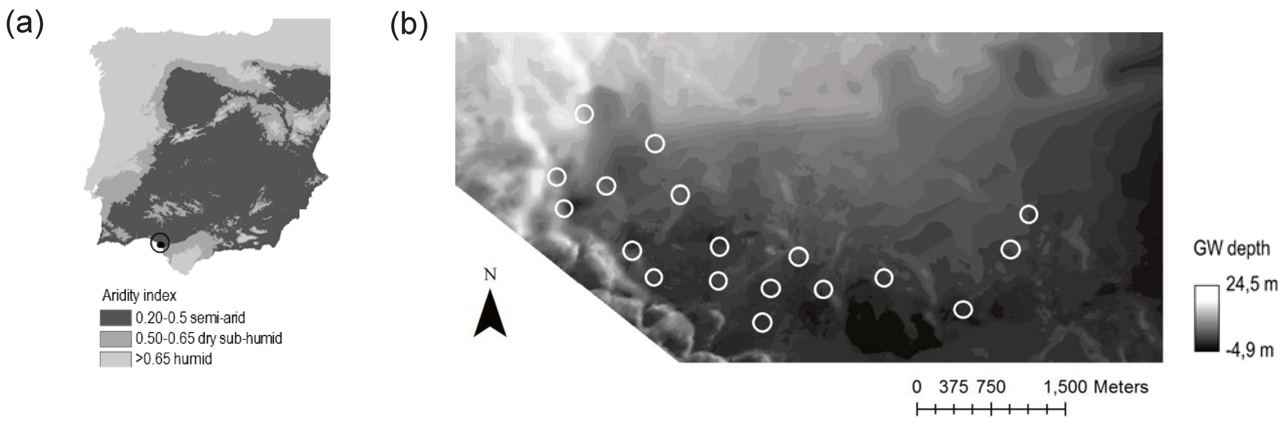

Figure S1. Study (a) site and (b) sampling points (white circles) over summer groundwater table depth (GW depth) map.

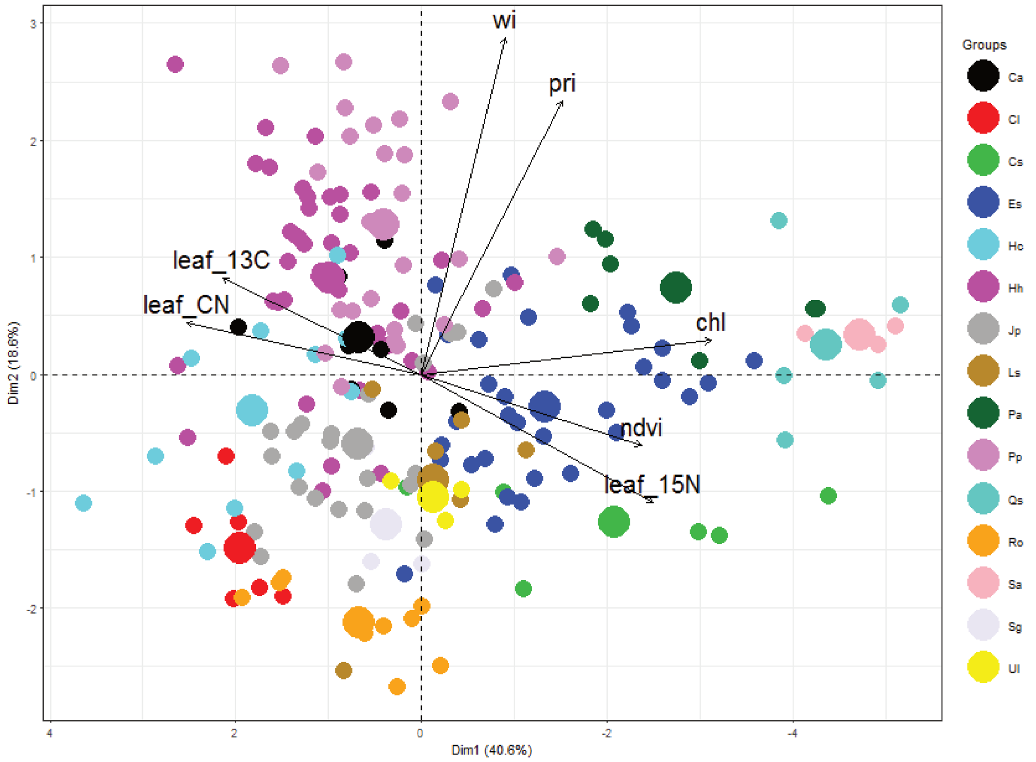

Figure S2. Principal component analysis (PCA) based on physiological measurements (n=191), showing the individual scores by species. Species are represented by different colors as shown in the inner legend: see Table 1 for Species names. The mean value of each species is represented by a bigger symbol. For physiological parameters considered see Methods section.

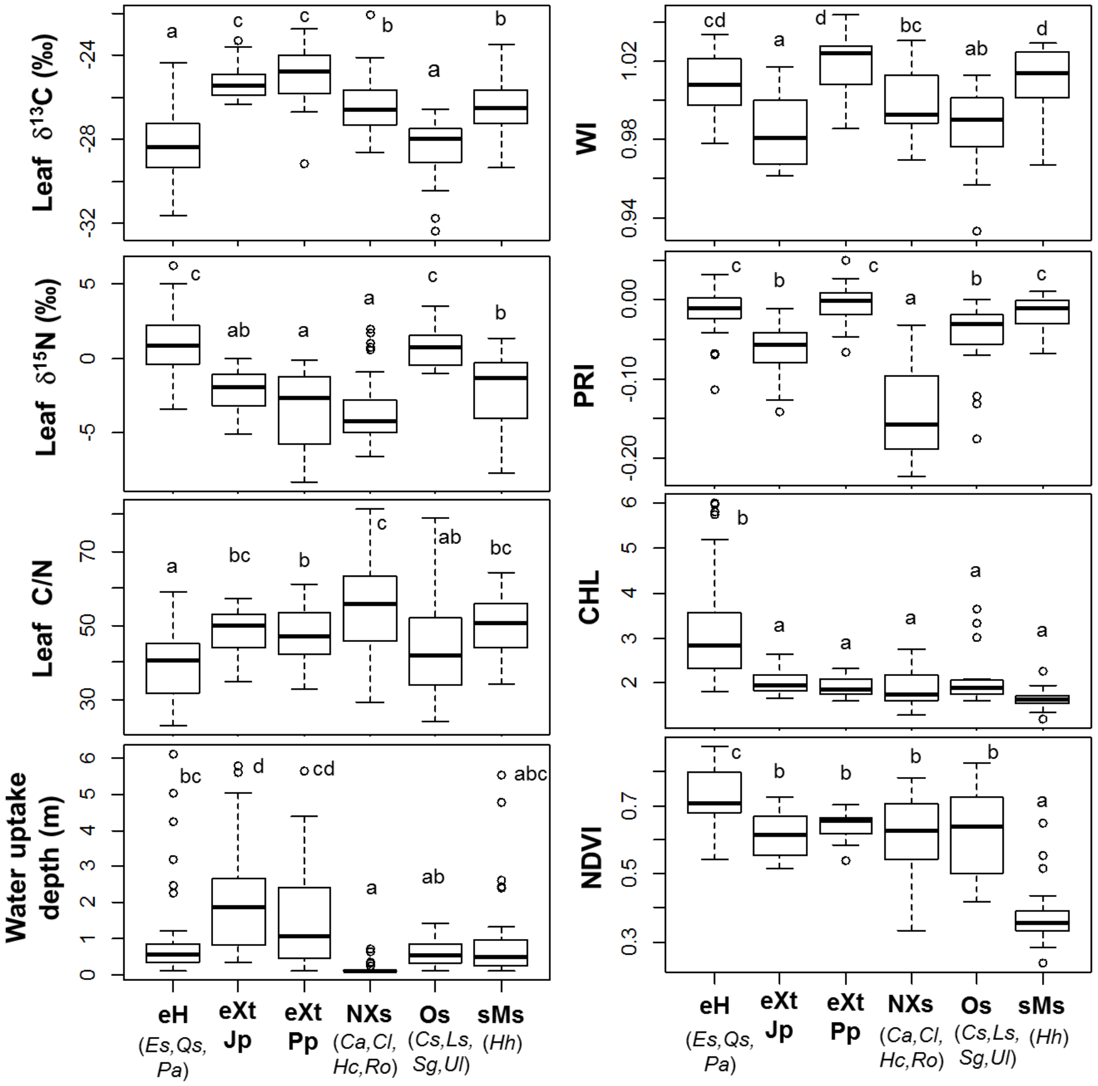

Figure S3. Boxplots of physiological traits and water uptake depth by functional types. NXs: narrow-leaved xerophytic shrubs; Os: aphyllous/spike or semi-deciduous broad-leaf shrubs; sMs: semi-deciduous mesophytic shrub; eH: evergreen hygrophytic shrubs and trees; eXt: evergreen xerophytic trees. eH (n=48), eXt Jp (n=24), eXt Pp (n=24), NXs (n=35), Os (n=18), sMs (n=42). Species included in the functional types are presented between parentheses below each functional group. See Table 1 for species and functional type's description. Different letters denote significant differences between functional types.

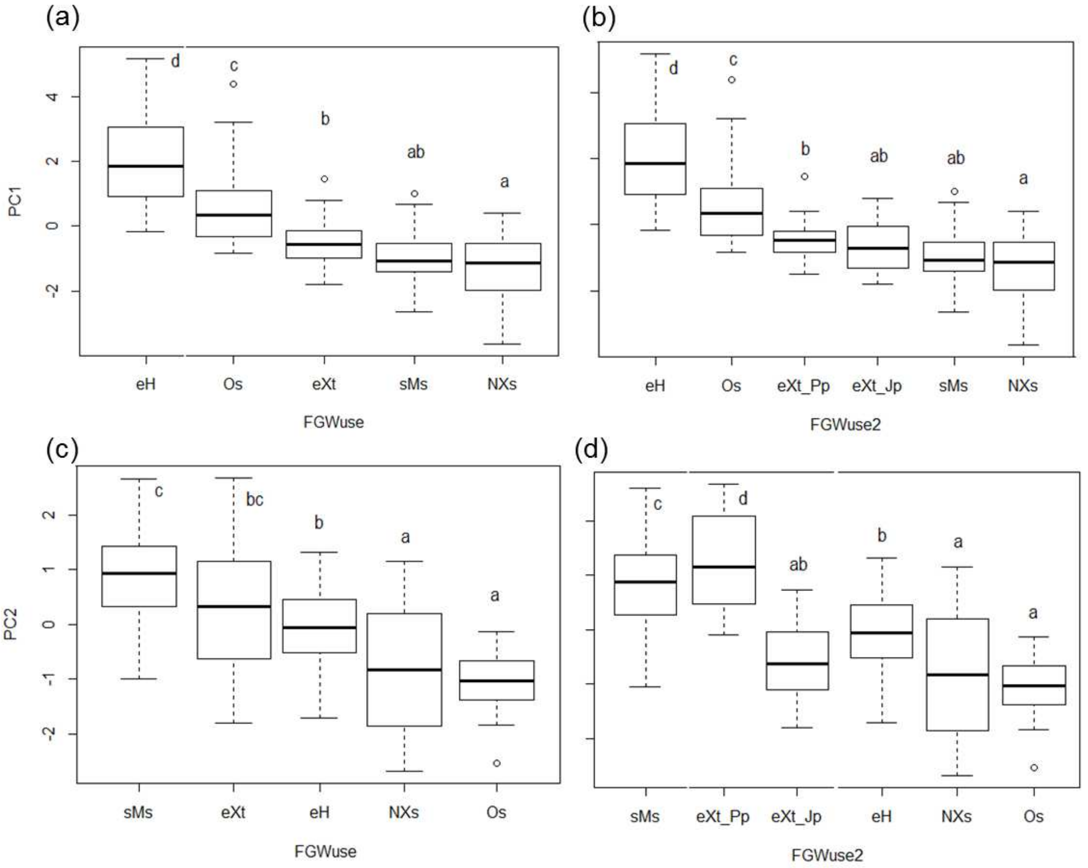

Figure S4. Boxplots of Principal component analysis factor scores of (a,b) first dimension (PC1), and (c,d) second dimension (PC2), by (a,c) functional type (FGWuse) and (b,d) sub-divided functional types (FGWuse2). NXs: narrow-leaved xerophytic shrubs; Os: aphyllous/spike or semi-deciduous broad-leaf shrubs; sMs: semi-deciduous mesophytic shrub; eH: evergreen hygrophytic shrubs and trees; eXt: evergreen xerophytic trees. Different letters denote significant differences between functional types.

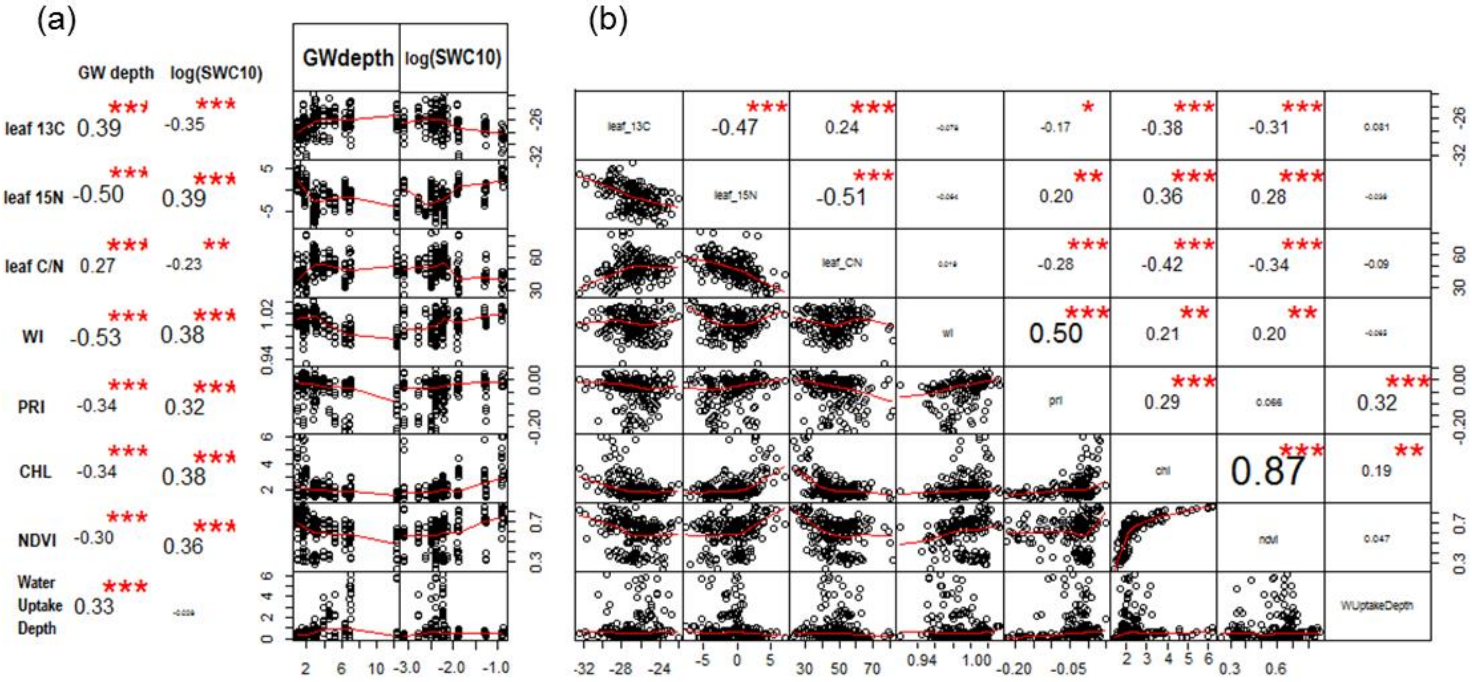

Figure S5. Relationships between (a) physiological traits and both groundwater depth (GW depth) and top-soil water content (log(SWC10)); (b) between all physiological traits measured, considering the overall woody vegetation, N=191. Spearman ρ and its significance (***p< 0.001, **p< 0.01,*p<0.05); and lowess lines, representing the trend of the relationship, are presented in the figure.

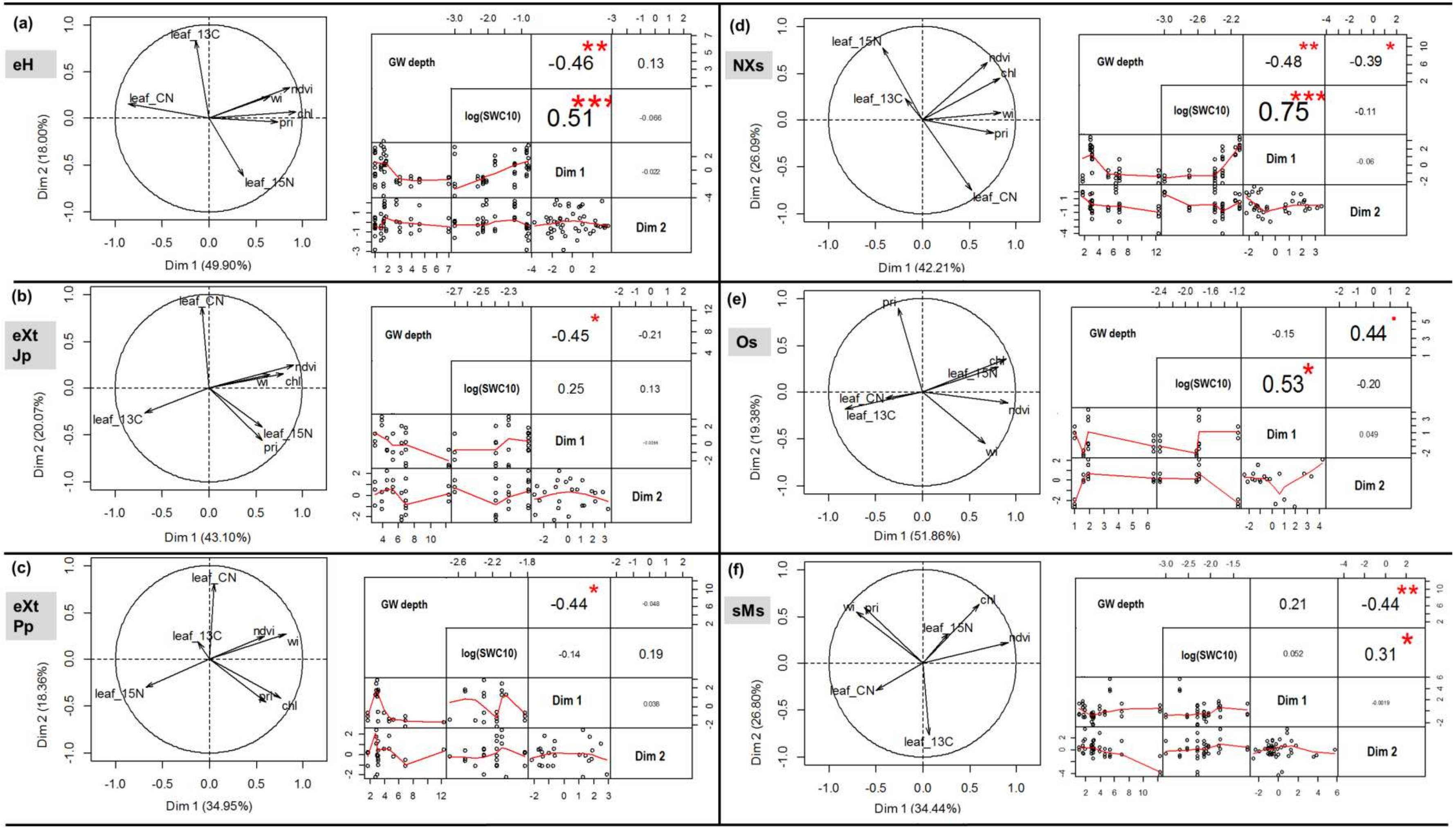

Figure S6. Principal component analysis of physiological traits of each functional type and relationships between 1st and 2nd PCA axis and both groundwater depth (GW depth) and top-soil water content (log(SWC10)). Functional types as described in Table 1: (a) eH (n=48), (b) eXt Jp (n=24), (c) eXt Pp (n=24), (d)NXs (n=35), (e) Os (n=18), (f) sMs (n=42). Spearman correlation rho (r), its significance (***p< 0.001, **p< 0.01,*p<0.05), and lowess lines (representing the trend of the relationship) are presented in the figure.

Table S1. Pearson correlations (r coefficients) between potential predictor variables. r > 0.70 denotes highly correlated variables and is highlighted in grey. The selected variables, i.e. the ones that are not highly auto-correlated (r <0.80) are represented in bold. *** p<0.001; ** p<0.01; *p<0.05.

	GWSp	GWSu	Gwam	Gwdiff	SWCSp	SWCSu	SWCdiff
GW depth Spring (GWSp)	1						
GW depth Summer (GWSu)	1.00***	1					
GW depth annual mean (Gwam)	1.00***	1.00***	1				
GW seasonal variation (Gwdiff)	-0.26***	-0.20**	-0.24***	1			
SWC$_{10}$ Spring (SWCSp)	-0.17*	-0.20**	-0.17*	-0.49***	1		
SWC$_{10}$ Summer(SWCSu)	-0.39***	-0.41***	-0.39***	-0.15*	0.49***	1	
SWC$_{10}$ seasonal variation (SWCdiff)	-0.37***	-0.37***	-0.37***	0.03	0.06	0.84***	1
Sea Distance(SeaDist)	-0.22**	-0.23**	-0.21**	-0.10	0.36***	0.29***	0.14

Table S2. Spearman correlations (ρ coefficients) between the PCA axes (PC1 and PC2) and the leaf-level physiological variables. *** p<0.001; ** p<0.01; *p<0.05.

variable	**PC1**		**PC2**	
$\delta^{13}C$	**-0.58**	***	0.19	**
$\delta^{15}N$	**0.70**	***	-0.26	***
C/N	**-0.70**	***	0.16	*
NDVI	**0.69**	***	-0.06	
PRI	0.42	***	**0.67**	***
CHL	**0.80**	***	0.02	
WI	0.19	**	**0.84**	***

Table S3. Spearman correlations (ρ coefficients) between the plant mean water-uptake depth, and water index (WI) and the factor scores of first principal component (PC1, a proxy of photosynthetic activity and nitrogen acquisition), considering the overall woody vegetation and each functional type. Functional types as described in Table 1: NXs: narrow-leaved xerophytic shrubs; Os: aphyllous/spike or semi-deciduous broad-leaf shrubs; sMs: semi-deciduous mesophytic shrub; eH: evergreen hygrophytic shrubs and trees; eXt: evergreen xerophytic trees. *** p<0.001; ** p<0.01; *p<0.05; *ns* if non-significant (p>0.05).

	WI		**PC1**	
Woody vegetation	-0.01	*ns*	0.13	*ns*
eH	-0.27	*ns*	-0.12	*ns*
NXs	-0.12	*ns*	0.08	*ns*
Os	0.02	*ns*	0.32	*ns*
eXt *P.pinea*	-0.42	*	-0.18	*ns*
eXt *J. phoenicea*	-0.28	*ns*	0.18	*ns*
sMs	0.18	*ns*	-0.25	*ns*

Table S4. Spearman correlations (ρ coefficients) between physiological traits measured and both groundwater depth (GW depth) and top-soil water content (log(SWC10)), in eH (n=48) and eXt Pp (n=24). *** p<0.001, ** p<0.01, *p<0.05; bold denotes ρ> 0.40.

	eH		**eXt_Pp**	
	GW depth	log(SWC10)	GW depth	log(SWC10)
leaf δ^{13}C	0.02	-0.08	0.02	0.04
leaf δ^{15}N	**-0.69***	**0.46**	0.07	0.28
leaf C/N	**0.39**	**-0.41**	0.00	-0.07
WI	**-0.62***	**0.78***	**-0.64***	0.12
PRI	-0.14	0.16	-0.09	**-0.43***
CHL	**-0.37**	**0.43**	-0.17	-0.27
NDVI	**-0.31***	**0.40**	-0.40	0.24

Chapter 4

4 Ecophysiological performance of woody species in a tropical dune forest: the relative importance of water table depth

Antunes C., Máguas C., Joly, C.A., Vieira S.

4.1 Abstract

In tropical forests, both light and water are considered important drivers of variations in growth of tree species. In addition, biotic controls related to stand structure can also play a role in these highly dense and diverse ecosystems. However, in seasonally flooded tropical dune forests, as *restinga*, fine-scale groundwater availability can eventually overcome these factors. Thus, the aim of this study was to explore the relative importance of groundwater depth for the ecophysiological condition of *restinga* forest's woody vegetation under a less-wet period.

The study was conducted in a permanent plot of a *restinga* forest. We modelled water table depth, and sampled woody species along a spatial gradient of depth to groundwater in a rainless period. We measured plant traits related with photosynthetic activity, carbon and nitrogen acquisition and water use, and estimated the water-uptake depth through an isotopic approach. Considering 15 species, we calculated the relative importance of environmental factors (such as water table depth and light accessibility) and biotic factors (such as water-uptake depth, plant size, and stand structure) on shaping vegetation physiological condition.

We found that water table depth relative importance was negligible, explaining less than 7% of plants' physiological status. The physiological status of the overall woody community was mainly explained by light access (35%), and a great differentiation between tree and shrub species occurred. Contrastingly, even at fine-scale, greater depth to groundwater significantly influenced plants' water-uptake depth towards deeper soil layers. Trees showed higher photosynthetic capacity compared to understory shrubs, but did not show a more favourable water status when using deeper soil layers. Stand structure, density, biomass and viservity had minor influence on both below and above ground adjustments.

Our study highlights the role that groundwater availability has, under less-wet periods, on water-sources-use but not on carbon or nitrogen acquisition. We reinforce the ubiquity of groundwater depth as a driver of root adjustments. We further underpin the great importance that light has on shaping physiological performance in this ecosystem, particularly partitioning over and understory species.

Key-words: *restinga* forest; water-table depth; photosynthetic parameters; crown illumination index; biomass; over- and under-story plants

4.2 Introduction

In tropical ecosystems, both light and water are considered to be important drivers of variations in growth and survival of tree species (Poorter 2001; Wright 2002; Poorter 2002; Santiago et al. 2004; Vieira et al. 2004; Boisvenue & Running 2006; Markesteijn et al. 2007; Meinzer et al. 2013; Oliveira et al. 2014; Rosado et al. 2016; Fyllas et al. 2017). Species existence and physiological performance along gradients of water and light availability will, to a great extent, be determined by the ability of both water and light acquirement and tolerance to water shortage and/or shade. For example, if individual tropical plants are able to adjust their root systems to site-specific conditions such as variations in water table, their water stress can be alleviated (Zea-Cabrera et al. 2006; de Oliveira & Joly 2010; Fan et al. 2017). Indeed, functional traits such as root water-uptake depth systematically vary with water availability and soil fertility (Santiago et al. 2004; Fyllas et al. 2009). Deep rooting habit can be an important adaptation to seasonal drought, as access to more readily available water at greater depth can eventually allow the maintenance of a more favorable plant water status (Nepstad et al. 1994; Oliveira et al. 2005). However, exploring water in top-soil layers in lowland tropics, where flooding typically occurs, is crucial for plants' access to oxygenated soils (and avoidance of anoxia) (Stone & Kalisz 1991; Pavlis & Jeník 2000; Fan et al. 2017). Thus, plasticity in below-ground traits could be a major driver of water resource-use and, consequently, significantly influence plant physiological status (Jackson et al. 1990; Kulmatiski et al. 2017; Guderle et al. 2018). Additionally, individual structural features such as tree size can strongly influence plant water requirements, plant water-use patterns and, direct or indirectly, plant physiological status (Meinzer et al. 1999; Meinzer 2003; Rossatto et al. 2012; Brienen et al. 2017; Ledo et al. 2018).

Biotic controls related to stand structure and its functional composition can also have direct effects on plant physiological performance and primary productivity (Aerts 1999; Fyllas et al. 2017). As plants use the same basic resources (light, CO_2, water, nutrients and space for growth), co-occurring plants would likely compete (Silvertown 2004; Peñuelas et al. 2011). This competition would hence be greater with higher species overlapping, i.e. higher density, biomass and/or higher similarity of functional composition. Plants at low density are likely to survive and /or grow better than are plants at high density, due to reduced competition with conspecific neighbours (Weigelt et al. 2002; Skálová et al. 2013). However, the mechanisms of plant competition can be greatly controlled by which resource is limiting, unavoidable trade-offs in competition for above- and below-ground resources (encompassing differential allocation of biomass to structures involved in the acquisition of a resource) and the relative importance of traits related to resource acquisition and retention (Aerts 1999; Malhi et al. 2004). In turn, these biotic controls can be regulated by environmental factors (Fyllas et al. 2017), and thus work as 'bridges' between environmental controls and overall plant physiology (e.g. water, carbon and nitrogen status). This means that environmental conditions can also have an indirect effect on forest productivity and physiological performance by regulating the structure and/or the species/functional composition of the community.

To understand how forests ecophysiologically respond to changes in environmental conditions, and how functional traits mediate this response, is of great relevance. Environmental conditions change both in time and in space, and studies along environmental gradients can provide valuable insights into controls of ecosystem function. Specifically, studies along (spatial) gradients of groundwater depth could contribute to disentangle to what extent this water factor influences and constrains overall physiology of vegetation. This is particularly relevant in *restinga* forests, which are tropical coastal ecosystems that occur in poor sandy soils, where water availability changes can be very rapid, water retention is low, and little water is available within the top soil during dry periods while flooding occurs in wet periods (Assis et al., 2011; Magnago, Martins, Schaefer, & Neri, 2012; Joly et al. 2012). *Restinga* woody species have shown to re-adjust their water-sources use (as noted in the previous Chapter 2.2), belowground investments (Rosado et al. 2011; Silva 2015) and water-uptake (Rosado et al. 2016) to seasonal soil water availability. It comprises flood resistant species, but also flood sensitive species (Oliveria 2011), with some species showing adaptations to deal with low water and nutrient availability (Gessler et al. 2007; Rosado & De Mattos 2010; Rosado et al. 2013; Rosado et al. 2016). Even small changes in depth to groundwater within the same low precipitation availability (intra-season spatial variation due to micro-topographic features) can have implications on plants water-use and physiological status. Additionally, *restinga*'s woody species are also subjected to differential light accessibility and stand composition variations, which can show a combined effect on plant physiological status.

In this study, we explored whether and how *restinga*'s woody vegetation experience variations on physiological condition along a groundwater depth gradient. We examined functional responses to groundwater depth (i.e. variations in a suite of physiological parameters and water uptake depth) of the dominant woody vegetation during a less-wet period. We specifically ask: (i)

How are woody vegetation’s functional traits (such as water-uptake depth, photosynthetic activity, nitrogen acquisition and water status) affected by groundwater depth under a less-wet condition? (ii) What is the relative importance of environmental factors (such as groundwater and light accessibility) and biotic factors (such as water uptake depth, plant size, and stand structure) on molding vegetation physiological condition? (iii) Does the vegetation functional responses to increasing groundwater depth differ among over and understory woody species?

4.3 Methods

Study site

The study was conducted at Serra do Mar State Park, in the seasonally flooded coastal forest (known as *restinga* forest) that occurs at Praia da Fazenda, municipality of Ubatuba, São Paulo, Brazil, in a permanent plot previously established (1ha, 23° 21’ 22” S; 44° 51’ 03” O) (Joly et al., 2012). Its soil is sandy, acid and nutrient poor (Scarano 2002; Assis et al. 2011; Joly et al. 2012). A detailed description of soil, climatic data and stem density of the study site is given in (Assis et al., 2011; Joly et al., 2012; Scarano, 2002).

This sandy coastal ecosystem is subjected to seasonal or perennial waterlogging due to variations in precipitation along the year (Oliveira & Joly, 2010; Oliveira, 2011). Additionally, due to site micro-topography, the study area has a spatial gradient of groundwater depth. Following the methodology of Antunes et al. (2018) a map of depth to groundwater was created using piezometric data collected in a less-wet condition (November 2013) (Fig. 1). Within the study area (1 ha permanent plot), eighteen sampling (sub-)plots (10 m x 10 m) were randomly distributed (Fig. 1). The (sub-)plots end up distributed along a (spatial) gradient of depths to groundwater, which ranged from 0.87 to 1.41 m (i.e. distance from surface soil to water-table).

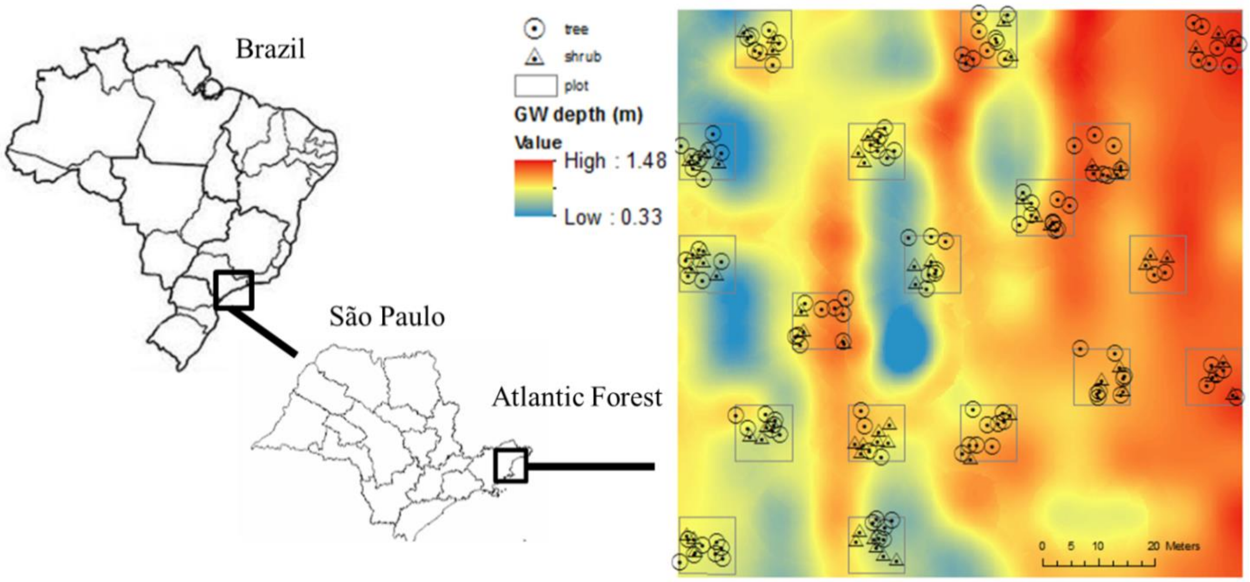

Figure 1. Study site, sampling plots within the study area, and individual plants sampled over less-wet condition groundwater table depth map (GW depth, i.e. distance from the surface soil to water table in m).

Study plant species

Thirty three woody species were considered, two understory shrubs and 31 trees (Table 1). In each plot, at least one shrub species and two tree species were sampled (Fig. 1, Table S1). A total of 171 individual plants were sampled for physiological assessment (Table 1).

Table 1 Woody species sampled and their code, growth form and number of individuals sampled (n). Bottom 15 species, separated by a dash line, are the sub-set of dominant species considered for abiotic and biotic variables effects' analysis.

Species	**Code**	**GF**	**n**
Andira fraxinifolia Benth.	Afra	tree	1
Byrsonima ligustrifolia Mart.	Blig	tree	1
Calyptranthes concinna DC.	Ccon	tree	1
Couepia venosa Prance	Cven	tree	1
Endlicheria paniculata (Spreng.) J.F. Macbr.	Epan	tree	1
Eugenia umbelliflora Berg.	Eumb	tree	2
Garcinia gardneriana (Planch. & Triana) Zappi	Ggar	tree	2
Inga sp	Isp	tree	1
Kielmeyera petiolaris Mart.	Kpet	tree	1
Lacistema pubescens Mart.	Lpub	tree	1
Margaritaria nobilis L.f.	Mnob	tree	1
Myrcia splendens (Sw.) DC.	Mspl	tree	1
Nectandra oppositifolia Nees	Nopp	tree	2
Ormosia arborea (Vell.) Harms	Oarb	tree	1
Rapanea ferruginea	Rfer	tree	1
Sloanea guianensis (Aubl.) Benth.	Sgui	tree	1
Tapirira guianensis Aubl.	Tgui	tree	1
Tibouchina sp1	Tsp1	tree	1
Euterpe edulis Mart.	Eedu	tree	8
Eugenia schuechiana Berg.	Esch	tree	4
Faramea pachyantha Müll.Arg.	Fpac	tree	4
*Guarea macrophylla (*Vell.) T.D.Penn.	Gmac	tree	7
Guapira opposita (Vell.) Reitz	Gopp	tree	4
Guatteria sp. 4	Gsp.	Tree	9
Jacaranda puberula Cham.	Jpub	tree	8
Myrcia brasiliensis Kiaersk.	Mbra	tree	4
Maytenus littoralis Carv.-Okano	Mlit	tree	9
Myrcia multiflora (Lam.) DC.	Mmul	tree	4
Myrcia racemosa (O.Berg) Kiaersk.	Mrac	tree	14
Marlierea tomentosa Cambess.	Mtom	tree	5
Pera glabrata (Schott) Poepp. ex Baill.	Pgla	tree	5
Psychotria sp1	Psp1	shrub	53
Psychotria sp2	Psp2	shrub	12
Total			171

A sub-set of 15 woody species was defined for further analysis (water-sources-use and regression analysis) (Table 1). We selected dominant woody species that occurred at least in three plots (Table 1, Table S1). The individuals growth form, diameter at breast height (DBH) and Crown illumination index (Keeling & Phillips, 2007; Joly et al., 2012; Vieira et al., 2008) were characterized. Understory shrubs with perimeters <15 cm were all attributed the 'DBH' value of 4.7 cm.

Physiological parameters

Ecophysiological traits measured included leaf C and N concentrations, leaf C and N isotope ratios and spectral reflectance indices from plants sampled at the 18 sampling plots (n=171) (Table 1). All the following physiological measurements were carried out in each plant individual during a less-wet period, November 2013.

(i) Leaf carbon and nitrogen isotope composition

Mature leaves were collected from each plant. The bulk leaf samples were dried at 60 ºC for at least 48 h, and milled to fine powder in a ball mill (Retsch MM 2000, Germany) for isotopic analysis. Leaf $\delta^{13}C$ and $\delta^{15}N$ (‰), and C and N (%) concentrations were determined by continuous flow isotope ratio mass spectrometry (CF-IRMS) on a Sercon Hydra 20-22 (Sercon, UK) stable isotope ratio mass spectrometer, coupled to a EuroEA (EuroVector, Italy) elemental analyser in SIIAF (FCUL). Uncertainty of the isotope ratio analysis, calculated using values from 6 to 9 replicates of secondary isotopic reference material interspersed among samples in every batch analysis, was ≤ 0.1‰.

(ii) Reflectance indices

We used a nondestructive optical method, based on the reflectance of light by an intact leaf. Spectral reflectance was measured using a UniSpec Spectral Analysis System (PP Systems, Haverhill, Massachusetts, USA) and carried out in different leaves per plant. The mean value of 6 measurements per plant was considered. The following reflectance indexes were calculated:

Chlorophyll index (CHL) = *R*750/*R*705, where *R750* and *R705* are the reflectance at wavelengths of 750 nm and 705 nm, respectively (Peñuelas et al. 1995). This index has been found to be well correlated with leaf chlorophyll content on a number of plant species, and can provide information about photosynthetic potential.

Photochemical Index (PRI) = (*R531-R570) / (R531+R570*), where *R531* and *R570* are the reflectance at wavelengths of 531 nm and 570 nm, respectively (Peñuelas, Llusia, Pinol, & Filella, 1997). There is an emerging consistency in the relationship between PRI and light use efficiency, which can be used as an index of photosynthetic activities (Wong & Gamon 2015).

Water Index (WI) = *R*900/*R*970, where *R900* and *R970* are the reflectance at wavelengths of 900 nm and 970 nm, respectively (Peñuelas et al. 1997). This index is highly related to plant water content or water status (Claudio et al. 2006).

Normalized difference vegetation index (NDVI) = (*R900-R680) / (R900+R680*), where *R900* and *R680* are the reflectance at wavelengths of 900 nm and 680 nm, respectively (Gamon et al. 1995). NDVI allows the assessment of biomass "greenness," and therefore of plant photosynthetic capacity.

Potential water uptake depth

Water-sources and stem sampling was carried out as Antunes et al. 2018. Oxygen isotopic composition ($\delta^{18}O$) of xylem water and water-sources were quantified for the (sub-set of) 15 species (Table 1) as in Antunes 2018 (Ehleringer & Dawson 1992; West et al. 2007). The relative contribution of different water sources to the composition of the xylem water was estimated by Bayesian stable isotope mixing models as in Antunes 2018.

After the quantification of water sources used by plants, we estimated their potential water uptake depth (WUD, in meters). For that we used a weighted average of the contribution of the different soil layers to the xylem water (derived from MixSIAR), as follows:

$$Potential\ WUD = \frac{(\mathrm{SW10} * 0.10) + (\mathrm{SW30} * 0.30) + (\mathrm{SW50} * 0.50) + (\mathrm{GW} * \mathrm{GWdepth})}{100}$$

being SW10, SW30, SW50 and GW the % of contribution of the soil layers; 0.10, 0.30 and 0.50 the soil layer depths in m and GW depth the summer groundwater depth in m. For groundwater depth, information extracted from depth to groundwater maps was used.

Abiotic and biotic variables

(i) Groundwater availability, as 'groundwater depth' calculated for the location of each sampled plant using the water table depth map developed for the less-wet condition (in ArcMap 10.4.1 ®), following Antunes et al. (2018) methodology.

(ii) Access to light, as the crown illumination index ('CII') for each plant.

(iii) Woody species density, as the number of individuals with DBH > 4.8 cm ('Density') present within the plot.

(iv) Woody species diversity, as the Shannon's equitability index ('Evenness') calculated per plot.

(v) Woody species total biomass, as the total biomass in kg ('Biomass'), calculated following Vieira et al. (2008).

Statistical analysis

Multicollinearity among potential environmental and biotic explanatory variables was handled by dropping collinear covariates when correlated at Pearson |r| > 0.7 (Dormann et al. 2013) (Fig. S1). This selection resulted in the dropping of 'Evenness' as a candidate predictor, excluding it from further analysis. Stepwise multiple regression models, selection by Akaike Information Criterion (AIC) were performed:

(A) To test the influence of both environmental controls and stand structure variables on potential water uptake depth (Fig. 2 – arrow A), using as response variable 'Water uptake depth', and as predictors: 'GW depth', 'CII', 'DBH', 'Density' and 'Biomass' (WUD~GWdepth+CII+DBH+Density+Biomass).

(B) To test the influence of both environmental and biotic controls on plant physiological status (Fig. 2 – arrow B), using a proxy of 'Physiological status' (see description below) as the response variable and the following predictors: 'GW depth', 'CII', 'Water uptake depth', 'DBH', 'Density' and 'Biomass' (Physio~GWdepth+CII+WUD+DBH+Density+Biomass).

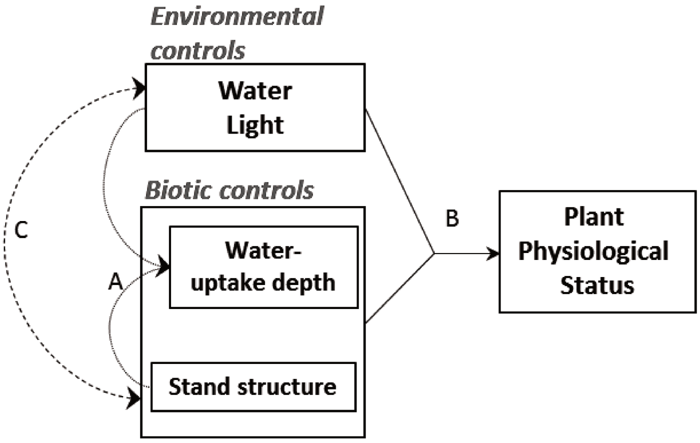

Figure 2. Environmental and biotic controls on plant physiological status. (A) Environmental factors, such as water and light, and aboveground biotic factors, such as plant size and stand biomass and density, can have direct effects (black arrows) on belowground characteristics, such as plant water-uptake depth. This can further reveal indirect effects on physiological status. (B) Plant overall physiology (photosynthetic activity and water status) can be directly influenced (black arrow) by both biotic factors, through processes of resources acquisition and competition, and environmental factors, such as access to light and groundwater availability. (C) Environmental conditions can additionally have an indirect effect on plant physiological status by regulating the structure and/or the species/functional composition of the community. Such distal effects tend to act on longer temporal scales.

For defining an integrated proxy of physiological performance, a multivariate principal component analyses (PCA) was performed with the individual physiological traits measurements (n=171). We aimed to integrate the complete set of physiological traits measured in all woody species and define the patterns of physiological performance of the dominant vegetation, accounting with specific relative position within the community physiological axis. We then extracted the individual factor scores of first principal component and used it as an integrated proxy of 'Physiological status'. Only the sub-set of 15 species was used for the multiple regression analysis previously described. The mean value of PC1 scores and water-uptake depth per species within plot was considered in cases of more than one individual of the same species sampled within a plot, resulting in n=87 for (A) and (B).

To identify the most important independent variable(s) in the regression models, we inferred the relative importance of regressors using the 'relaimpo' package of R (Grömping, 2006). We further tested if the physiological patterns were different between trees and understory shrubs (considering the PCA 1st axis scores), using an analysis of variance (ANOVA). As the differences between growth form were significant, we subsequently tested both (A) and (B) per growth form (tree and shrubs; n= 65 and n=22, respectively). A new physiological proxy was considered per functional type, by performing a PCA for each group (canopy trees and understory shrubs).

All statistical analyses were performed in R version 3.4.3 (R Core Team, 2017).

4.4 Results

Through a multivariate approach, we integrated the set of physiological traits measured in all woody species and defined the patterns of physiological performance of the dominant vegetation (accounting with their specific relative position within the overall vegetation's physiological axis, Fig. 3). The first axis (PC1), explaining 37.1% of the variance, reflected a gradient of physiological performance, from low to high values of chlorophyll content index (CHL), normalized difference vegetation index (NDVI), Photochemical Index (PRI), $\delta^{13}C$ and leaf carbon content (% C), and high to low values of leaf $\delta^{15}N$ (Fig. 3; Table S2). The second axis (PC2), explaining 18% of the variance, reflected plant water status and leaf $\delta^{15}N$, from low to high values of plant water index (WI) and leaf $\delta^{15}N$ (Fig. 3; Table S2).

The differences between trees and understory shrubs were significant (F=134.4, p <2.2e-16, n=171). Evaluating growth forms (i.e. trees and shrubs) separately, PC1 represented also a physiological axis related with photosynthetic activity (Fig. S2). Trees additionally incorporate in this axis their water status (WI).

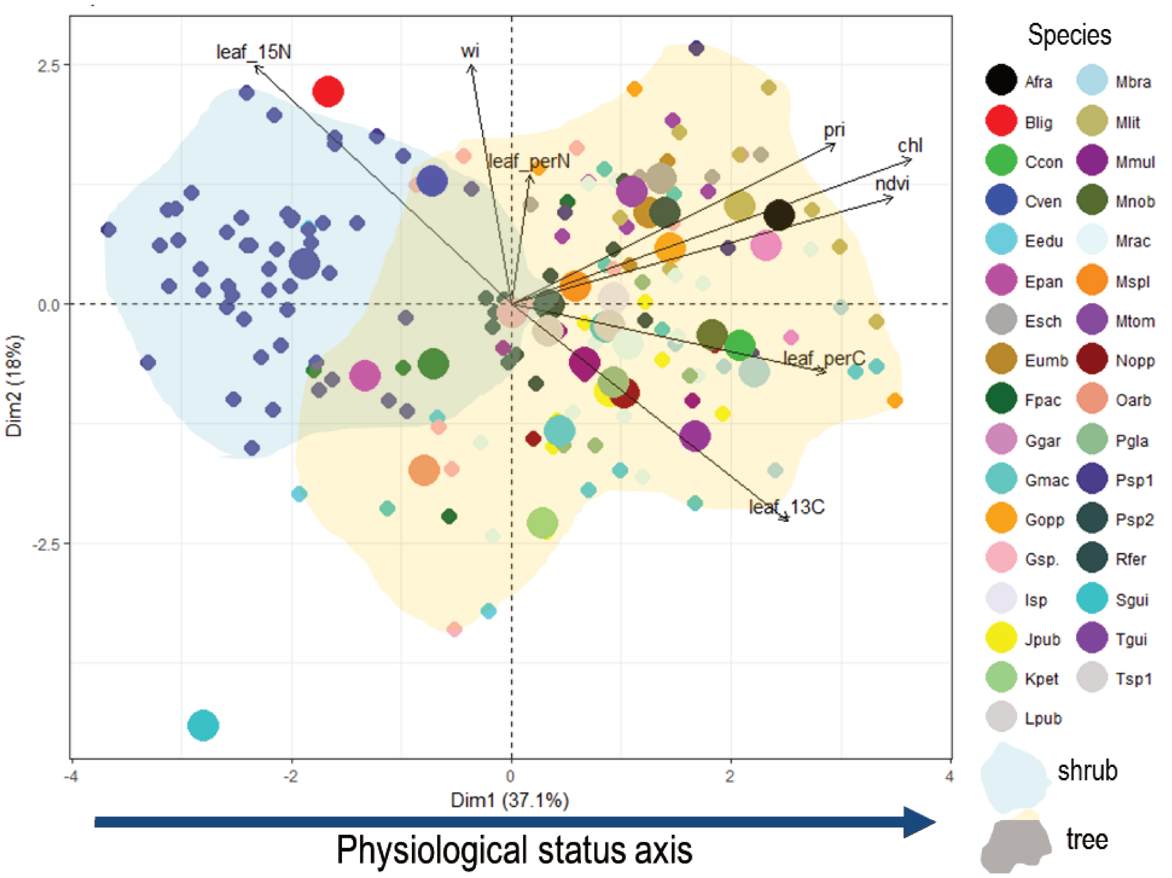

Figure 3. Principal component analysis (PCA) based on physiological measurements (n=190), showing the individual scores by species. Species are represented by different colors as shown in the inner legend: see Table 1 for Species names. The mean value of each species is represented by a bigger symbol. For physiological parameters considered see Methods section. The first axis (Dim 1) explains 37.1% of the variance and reflects a gradient of overall physiological status (low to high photosynthetic activity and carbon acquisition).

Thus, using PC1 (individual factor scores) as a proxy of *physiological status,* related with photosynthetic capacity and carbon acquisition, we explored how plants' physiology was influenced by both abiotic and biotic factors. The multiple regression analysis showed that the physiological status of the overall woody community, comprising tree and shrub species, was mainly explained by light access: 50.2% of its variance was explained mainly by crown illumination (35%) and DBH (12%) (Fig. 4a). 30.7% of trees' physiological status (photosynthetic activity, carbon acquisition and water status) was explained, and mostly by water-uptake depth (12%) and crown illumination (9%) (Fig. 4b). Water-uptake depth showed a negative effect on physiological status and crown illumination a positive effect (Fig. 4b). Thus, deeper water-uptake combined with low illumination implied a decrease in the physiological performance of trees in this ecosystem. The physiological status of shrubs was not explained by any of the studied variables (non-significant model) (Fig. 4c).

Biotic controls related with stand structure and potential resource competition were weakly positively affected by groundwater depth (Fig. 4), and had a negligible influence on plant physiological status (Fig. 4, Table S3).

Since water status, assessed through reflectance index WI, could be an important trait affected by both environmental and biotic factors (particularly groundwater depth), and because it was not represented in the first axis of the dominant woody vegetation's PCA, we further tested WI as a response factor. Less than 20% of WI was explained by the studied explanatory candidates and there was no significant effect of groundwater depth on plants' water status (Table S3). Among understory shrubs WI was not explained by any of the explanatory candidates (Table S3).

As described in the methods, we also considered the potential water uptake depth as a response variable, and thus the potential indirect effect that groundwater depth can have on plants physiological status through adjustments of water-sources-use. In all cases, i.e. considering the dominant woody community and life-forms separately, groundwater depth showed a significant positive effect on water-uptake depth, explaining more than 20 % of water-uptake depth variance (Fig. 4). Although the range of depths (soil layers) was minor (0.35 to 0.65 m), there was the common tendency for exploring deeper soil layers with increasing depths to water-table (Fig. 4, Table S3). Light accessibility, size or stand structure variables had minor or non-significant influence on water-uptake depth (Fig. 4, Table S3).

4.5 Discussion

Woody vegetation water-uptake depth is affected by groundwater depth

Our results showed that water-uptake depth of the woody community was affect by groundwater depth under less-wet conditions. From all the possible controls evaluated in this study, groundwater depth was the most relevant one for belowground adjustments. This is in agreement with the global trends of deeper root depths with increasing groundwater depths (Rossatto et al. 2012; Kulmatiski et al. 2017; Evaristo & McDonnell 2017; Fan et al. 2017). However, we did not observe a very strong association, and groundwater depth explained less than 30% of the water-uptake depth variance. Because neither stand or individual structural parameters nor light accessibility had a significant influence on the patterns of water-uptake depth, other water related factors, such as soil water content at shallow soil layers, may also play an important role. Although in a small range, the changes in groundwater depth drove, changes on the main water-source use and on the depth at which woody plants are extracting water. Thus, the changes in the reliance on deeper soil layers observed seasonally (previous Chapter 2.2) are further confirmed along the groundwater depth spatial gradient.

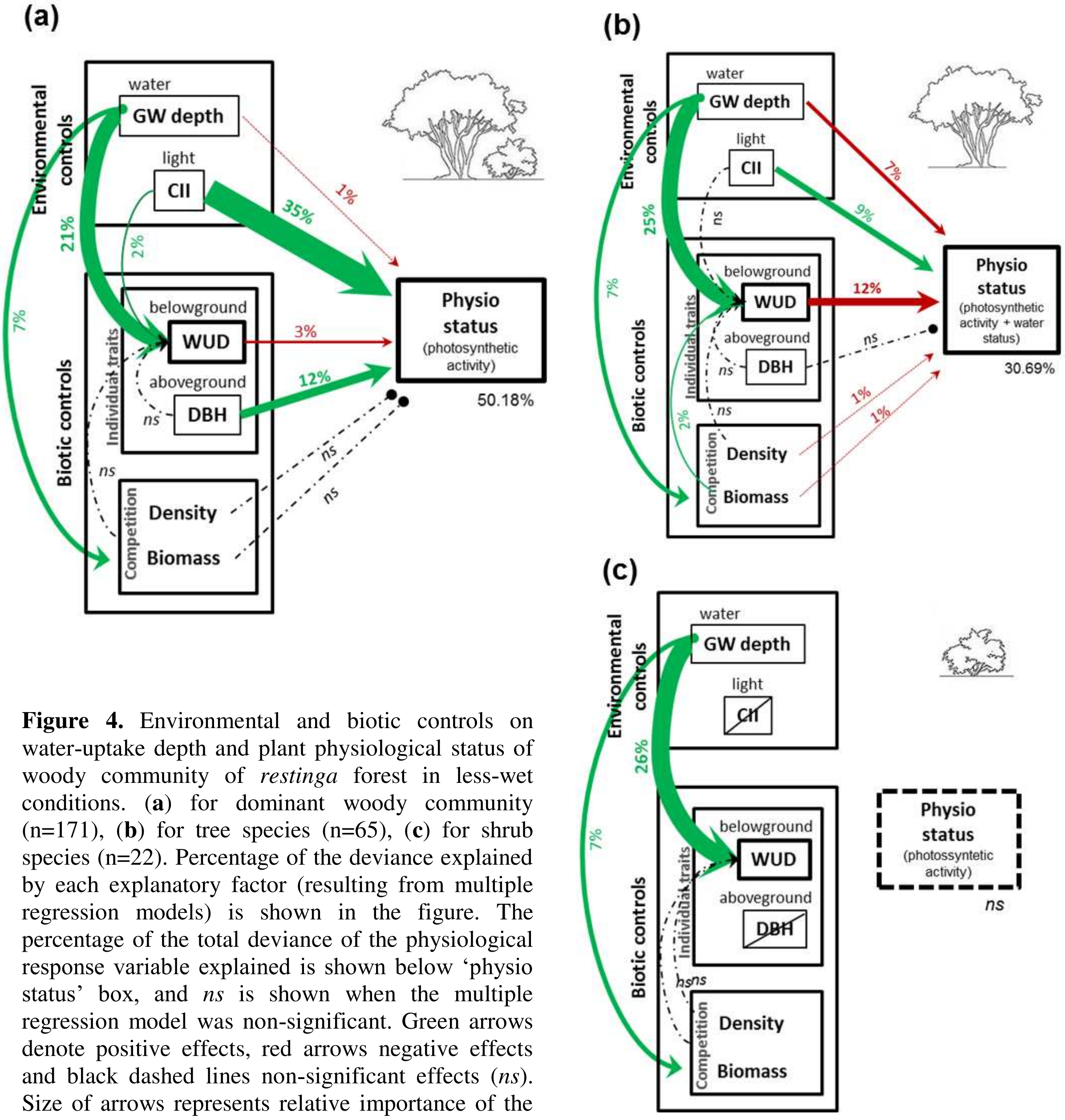

Figure 4. Environmental and biotic controls on water-uptake depth and plant physiological status of woody community of *restinga* forest in less-wet conditions. (**a**) for dominant woody community (n=171), (**b**) for tree species (n=65), (**c**) for shrub species (n=22). Percentage of the deviance explained by each explanatory factor (resulting from multiple regression models) is shown in the figure. The percentage of the total deviance of the physiological response variable explained is shown below 'physio status' box, and *ns* is shown when the multiple regression model was non-significant. Green arrows denote positive effects, red arrows negative effects and black dashed lines non-significant effects (*ns*). Size of arrows represents relative importance of the variables' in explaining the independent variable. See further statistical details in Table S3.

Both over and understory species increased their depth of water-uptake with increasing depth to water-table. Although as been reported that tree size affect water uptake in tropical forest tree species (Meinzer et al. 1999; Jackson et al. 1999; Meinzer et al. 2013; Bennett et al. 2015), we did not found any influence of structural factors on plant water-uptake depth. The results of this community level study confirm the general patterns of water extraction depth among life-forms, which are dependent on the depth of the water table. At *restinga* forests, a more superficial groundwater associated with shallower soil profiles seems to strongly restrict root growth, affecting the potential range of water uptake strategies. These restrictive conditions would also tend to limit biomass accumulation, reflected on the lower values of stand tree biomass at shallower groundwater depth. The increase in soil volume to be explored by the roots, that results from deeper water tables, could also affect a multitude of factors, e.g. nutrient uptake or the fixation of plants in the soil (Rossatto et al. 2012). The common adjustment in water uptake depth implies that woody community has a homogeneous response to water availability and similar strategies of water-resources root acquisition, possibly resulting in woody community root water-uptake overlap. This functional convergence of the woody community would thus be sustained by enough water supply, other physiological/water-use strategies or further water-uptake plasticity (Bucci et al. 2004; Zea-Cabrera et al. 2006; Zeppel 2013; Schwendenmann et al. 2015; Guderle et al. 2018).

Our results enlighten the importance of groundwater availability on plant water-sources use and possibly on belowground differential investments. As the relative importance of above and belowground investments may change with differential resource availability, and trade-offs related to the allocation of biomass are likely to occur, our results point to the possibility of further impacts of changing groundwater-resources at the community level (Shipley et al. 2016).

Light accessibility, not groundwater availability, drives differentiation in woody vegetation physiological condition

The photosynthetic activity of the overall woody community, comprising tree and shrub species, was mainly explained by light access under less-wet conditions. Groundwater depth showed no direct effects on the physiological status of woody plants. Contrastingly to water-uptake depth, physiological conditions were significantly different between trees and understory shrubs.

Shrubs showed lower values of photosynthetic (greenness) related reflectance indices (PRI, CHL, NDVI) and lower values of leaf $\delta^{13}C$ and carbon content. Substantial physiological variation, particularly foliar $\delta^{13}C$, among local species may exist due to canopy heterogeneity and differences among species in carbon assimilation (e.g. Martinelli et al. 1998). Higher values of $\delta^{13}C$ in trees are probably linked to higher assimilation (A), reflected in the observed higher carbon content. Thus, intrinsic water use efficiency is expected to be higher in trees (high $\delta^{13}C$, given the high carbon assimilation with similar stomatal regulation). Tropical tree species can show higher transpiration rates and greater leaf-level water demand, therefore necessitating an increased water supply to the leaf and increased stem- and leaf-specific hydraulic conductivity (Tyree et al. 1998; Sack 2004; Chave et al. 2009; Markesteijn et al. 2011). Leaf $\delta^{13}C$ patterns of shrubs are possibly also associated with vertical gradients of CO_2, as more depleted C inputs in understory environment due to soil respiration results in a more deleted signature of the carbon fixed in lower leaf layers (Medina et al. 1991; Sternberg et al. 1997).

Moreover, the physiological responses were more evident among trees than in shrubs. Trees' physiological status (photosynthetic activity, carbon acquisition and water status) was mostly influenced by water-uptake depth and crown illumination, while the physiological status of shrubs was not explained by any of the studied variables. In trees, deeper water-uptake combined with lower canopy illumination implied a decrease in the physiological performance of trees in this ecosystem. Thus, although indirectly, groundwater depth can influence in some degree the physiological status of trees through water-uptake depth regulation. In tropical ecosystems, light-demanding species tend to have different growth rates and root biomass allocation compared with shade-tolerant species (Markesteijn & Poorter, 2009; Poorter, 2001; Poorter & Bongers, 2006). Light conditions can shape community behavior and two main environments were identified: understory and overstory. In fact, a high inter-specific variation was observed in the physiological patterns associated with contrasting light environments of over and understory, and within the lower light conditions of understory lower light variability occurred. The canopy can intercept or reflect a very high percentage of photosynthetically active radiation, and low light levels limit all understory plants (Wright 2002; Poorter 2002; Poorter et al. 2005; Markesteijn et al. 2007; Li & Shipley 2018). Shade competition from canopy plants seems to have suppressed photosynthetic activity of understory plants. Shrubby species able to tolerate the understory environment (as *Psychotria* sp.) can better explore a specific light niche and thrive in shade environments. Under a more homogenous low light environment (i.e. understory), *Psychotria* plants can show similar shoot relative growth rates and lower phenotypic plasticity (Valladares et al. 2000). Contrastingly to other studies (Wright et al. 1992; Jackson et al. 1995), in evergreen shade-tolerant *Psychotria* shrubs, water-uptake depth was not correlated with photosynthetic activity nor with water status, indicating that plants using deeper, possibly more abundant water resources, did not show a more favorable leaf water status.

4.6 Conclusion

In conclusion, the comparison of functional trait values along a groundwater availability gradient, and including biotic components of these, allowed us to understand how different environments affected below and aboveground adjustments of *restinga* woody species in a less-wet period. As expected, groundwater depth variations mostly influenced plants water-uptake depth (belowground adjustment), and variation in light availability was the main driver of photosynthetic traits. Both over and understory species increased their depth of water-uptake with increasing depth to water-table, but the physiological responses were more evident among trees. Our study highlights the role that groundwater availability has, under less-wet periods, on water-sources-use but not on carbon or nitrogen acquisition. We reinforce the ubiquity of groundwater as a driver of root adjustments. We further underpin the great importance that light has on shaping physiological performance in this ecosystem, particularly partitioning over and understory species. Trees showed higher photosynthetic status compared with understory shrubs, but did not show a more favorable water status when using deeper soil layers. Differences in physiology, carbon allocation and water resources use, may affect the ability of individuals to grow, survive, reproduce, and disperse in a given environment. Thus, affect the relative abundance of specific functional types. Further works would help to disentangle the capture, loss, and cycling of energy and water resources regulated by woody functional types in this *restinga* ecosystem.

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4.9 Supporting Information

Table S1. Individuals of the 33 woody species sampled per sampling plot.

Table S2. Spearman correlations (ρ coefficients) between the PCA axes (PC1 and PC2) and the leaf-level physiological variables.

Table S3 Results of the multiple regression between ecophysiological response variables and the six predictors considered in this study, considering the dominant woody community, the shrubs and the trees.

Figure S1. Correlations between the explanatory variables

Figure S2. Principal component analysis of physiological traits of each functional type (tree, shrub).

Supporting Information

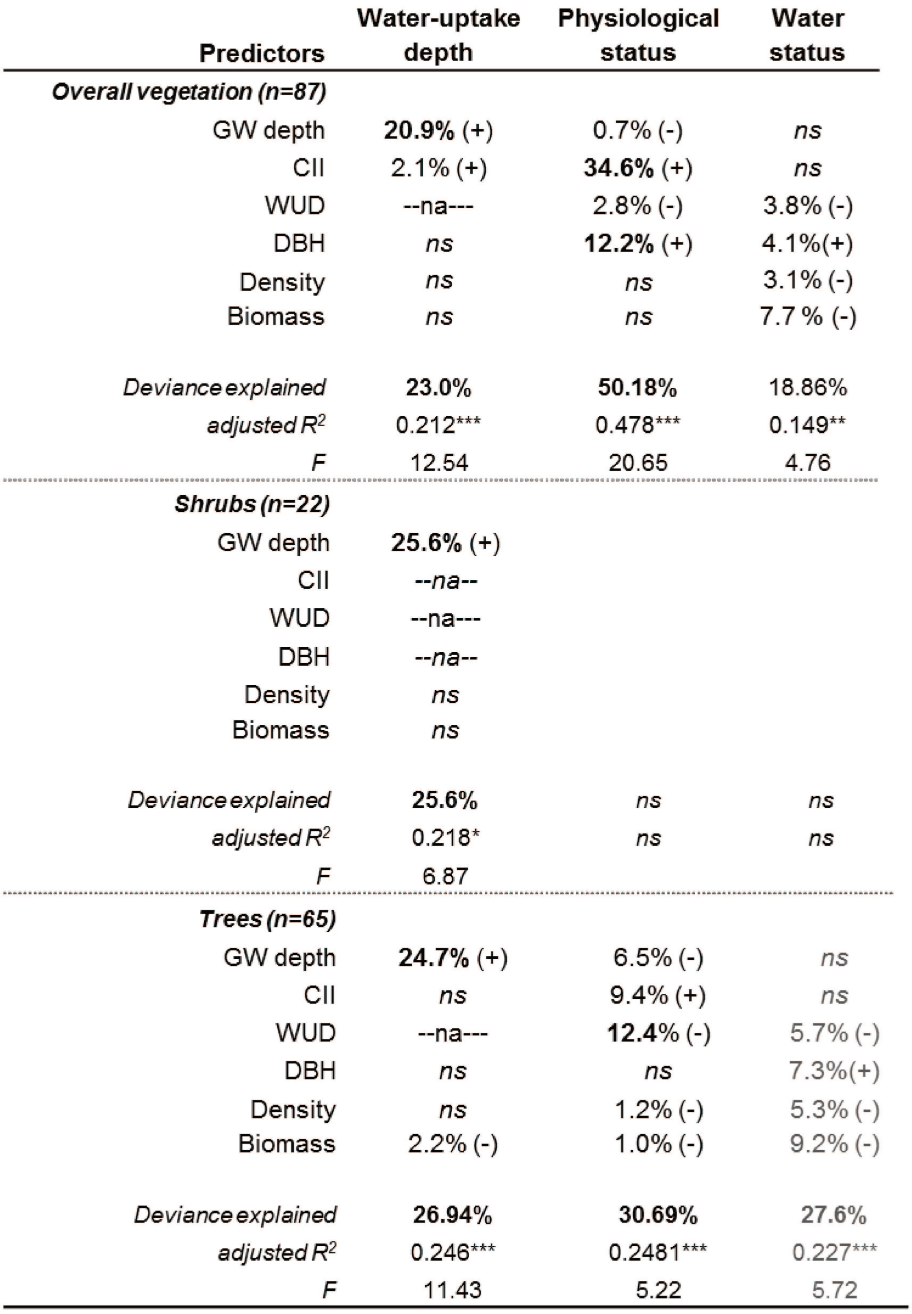
Table S1. Individuals of the 33 woody species sampled per sampling plot. Species names described in Table 1. Columns in grey are the sub-set of species selected for water-sources-use calculation and multiple regression analysis (see methods section for details).

plot	**Afra**	**Blig**	**Ccon**	**Cven**	**Eedu**	**Epan**	**Esch**	**Eumb**	**Fpac**	**Ggar**	**Gmac**	**Gopp**	**Gsp.**	**Isp**	**Jpub**	**Kpet**	**Lpub**	**Mbra**	**Mlit**	**Mmul**	**Mnob**	**Mrac**	**Mspl**	**Mtom**	**Nopp**	**Oarb**	**Pgla**	**Psp1**	**Psp2**	**Rfer**	**Sgui**	**Tgui**	**Tsp1**	
L1					1			1				1		1														3						7
L100															3				2				1		1			3			1			11
L13															1			1		2								3				1		8
L20					3						1																	3	2					9
L25			1		1				2	1		1			1					1								3						11
L31											1		1		2											1	1	3	3					12
L33																			1			1					1	3	4					10
L38																						5		1				3						9
L46							1			1		1					1		1			1		1				3						10
L53					2				1									1									2	2						8
L6					1																							4	3				1	9
L60	1						1	1	1			1										4		1				2						12
L67							1						1		1				2			2			1		1	3		1				13
L74											2		2						1					2				3						10
L78						1	1						3						2									3						10
L8				1							3					1		1		1		1						3						11
L86													1					1										3						5
L94		1											1								1							3						6
Total	1	1	1	1	8	1	4	2	4	2	7	4	9	1	8	1	1	4	9	4	1	14	1	5	2	1	5	53	12	1	1	1	1	**171**

Table S2. Spearman correlations (ρ coefficients) between the PCA axes (PC1 and PC2) and the leaf-level physiological variables. *** p<0.001; ** p<0.01; *p<0.05.

variable	**PC1 (37%)**		**PC2 (18%)**	
$\delta^{13}C$	**0.59**	***	**-0.53**	***
$\delta^{15}N$	**-0.55**	***	**0.58**	***
C(%)	**0.67**	***	-0.17	**
N(%)	ns		0.31	**
NDVI	**0.81**	***	0.26	**
PRI	**0.69**	***	0.39	***
CHL	**0.85**	***	0.35	***
WI	ns		**0.58**	***

Table S3 Results of the multiple regression between ecophysiological response variables and the six predictors considered in this study, considering the dominant woody community (n=87), the shrubs (n=22) and the trees (n=65). See methods and Fig. S2 for further statistical details. Deviance explained (%) by each abiotic predictor, deviance explained by the model, adjusted R^2 and F value are represented in the table. The ecophysiological response variables considered were estimated potential water uptake depth (Water-uptake depth), the scores of first axis of PCA (as a proxy of physiological status related with photosynthetic activity), and the Water Index (WI, a proxy of plant water status). Predictor variables not selected (p>0.05), and not included in the model, are denoted as '*ns*'. Predictors that explain more than 10% of the deviance are in bold. *** p<0.001; ** p<0.01; *p<0.05. Positive coefficients are denoted with (+), and negative with (-).



Predictors	Water-uptake depth	Physiological status	Water status
Overall vegetation (n=87)			
GW depth	**20.9%** (+)	0.7% (-)	*ns*
CII	2.1% (+)	**34.6%** (+)	*ns*
WUD	--na---	2.8% (-)	3.8% (-)
DBH	*ns*	**12.2%** (+)	4.1%(+)
Density	*ns*	*ns*	3.1% (-)
Biomass	*ns*	*ns*	7.7 % (-)
Deviance explained	**23.0%**	**50.18%**	18.86%
adjusted R^2	0.212***	0.478***	0.149**
F	12.54	20.65	4.76
Shrubs (n=22)			
GW depth	**25.6%** (+)		
CII	*--na--*		
WUD	--na---		
DBH	*--na--*		
Density	*ns*		
Biomass	*ns*		
Deviance explained	**25.6%**	*ns*	*ns*
adjusted R^2	0.218*	*ns*	*ns*
F	6.87		
Trees (n=65)			
GW depth	**24.7%** (+)	6.5% (-)	*ns*
CII	*ns*	9.4% (+)	*ns*
WUD	--na---	**12.4%** (-)	5.7% (-)
DBH	*ns*	*ns*	7.3%(+)
Density	*ns*	1.2% (-)	5.3% (-)
Biomass	2.2% (-)	1.0% (-)	9.2% (-)
Deviance explained	**26.94%**	**30.69%**	**27.6%**
adjusted R^2	0.246***	0.2481***	0.227***
F	11.43	5.22	5.72

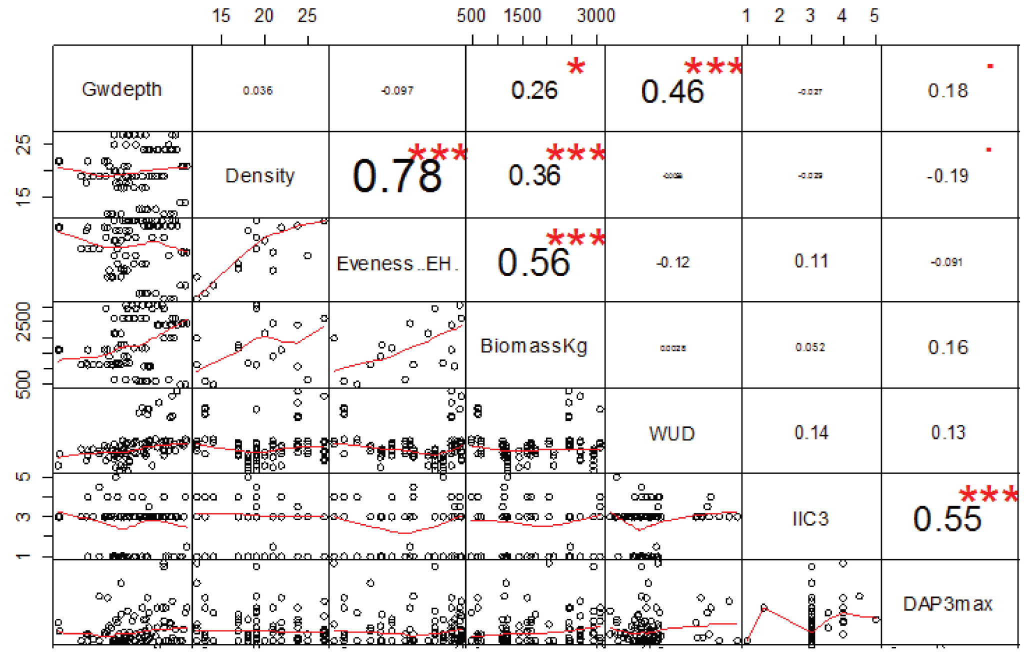


Figure S1. Correlations between the explanatory variables. Pearson |r| and its significance (***p< 0.001, **p< 0.01,*p<0.05), and lowess lines, representing the trend of the relationship, are presented in the figure.

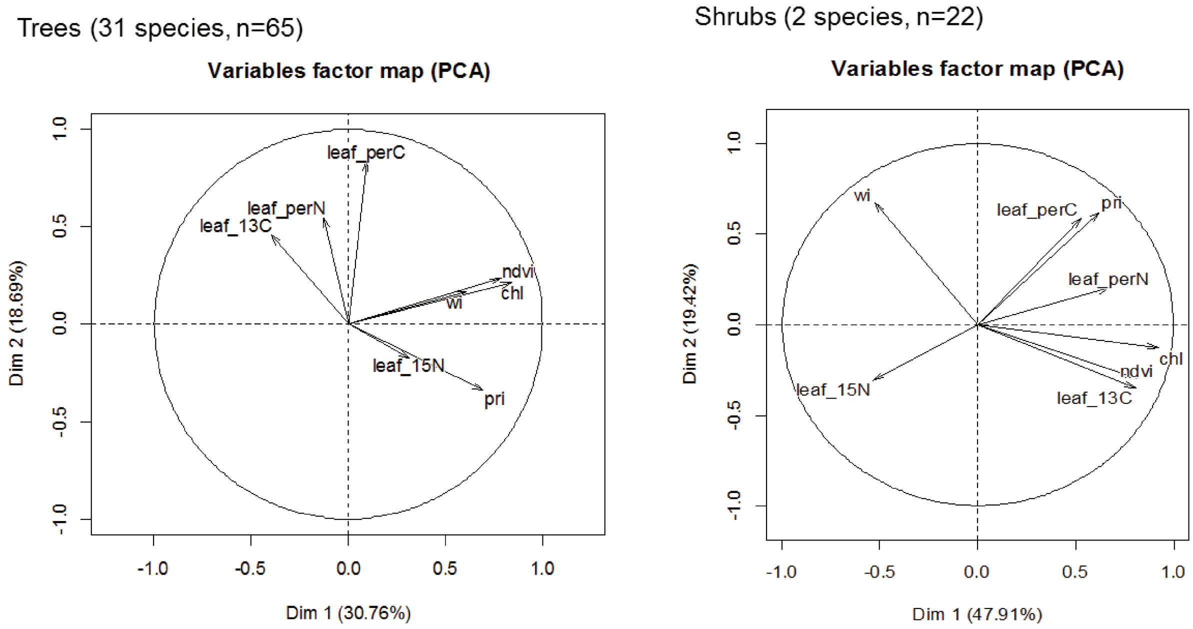


Figure S2. Principal component analysis of physiological traits of each functional type (trees and shrubs). For both growth forms PC1 is an physiological axis related with photosynthetic activity. Trees also incorporate in this axis their water status (WI).

Chapter 5

5 General Discussion

Groundwater variations greatly altered water balance of coastal sandy forests in different climatic regions. In these ecosystems, interactions between climatic conditions, hydrology and vegetation response capacity determined plant ecophysiological patterns. Under dry conditions, groundwater showed to be an essential contributor to soil water availability. Evaluating water-sources-use and physiological responses to the variations in groundwater depth, we could identify to what extent this hydrological factor is impacting woody vegetation. We found that while groundwater influence on water-uptake depth under dry conditions is globally prevalent, its contribution to overall physiological status of woody species is dependent on the climatic conditions under which plants occur. The physiological impact of increasing groundwater depth showed to be greater with increasing aridity. Our results suggest that in coastal dune forests:

(i) Seasonal shifts of water-sources-use from shallow to deeper soil layers are common across different climates;

(ii) Plant use of groundwater is more likely in rainless periods and in sites with a pronounced dry season;

(iii) Under seasonal dry conditions, groundwater depth is an important driver of water-uptake depth across different climatic regions;

(iv) Increasing groundwater depth (i.e. water-table lowering) is expected to trigger physiological responses of woody vegetation under more arid conditions, constraining the water status and photosynthetic capacity of woody vegetation in semi-arid climates;

(v) Inter and intra-specific variability of both water-uptake depth and photosynthetic traits is greater with dryness (seasonal drought and aridity), reflecting different plant strategies to thrive in particular hydrological conditions.

Water-sources-use under contrasting temporal water availability conditions

By analyzing the mean variation of the relative use of different water-sources from wet to dry conditions across different climates, we found a general shift from shallower to deeper soil layers. This seasonal pattern seems to be quite ubiquitous and has been observed in other arid, temperate, and tropical ecosystems (Jackson et al. 1999; Hasselquist et al. 2010; Esquivias et al. 2014; Ellsworth & Sternberg 2015; Grossiord et al. 2017; Barbeta & Peñuelas 2017b). There was a common use of precipitation during the wet periods in all climatic sites (Fig. 1a), and a mean change to deeper soil layers in the absence of precipitation (Chapter 2).

Precipitation availability regulated the temporal variation of plants' water sources usage, but under dry conditions, groundwater strongly controlled soil water availability and plants' water accessibility. In this kind of ecosystems, where water depletion is rapid and the capacity of the surface soil to retain moisture throughout the dry season is very low, soil water is strongly regulated by hydrological patterns, and a shallow groundwater greatly influences the moisture of upper soil layers. Groundwater is therefore a water source that alleviates water stress during the dry periods and its proportional use decreases in wet seasons.

Interestingly, the seasonal variation of mean water-sources used by the woody vegetation was clearer in the tropical *restinga* (Fig. 1a, and see Chapter 2.2). Although the range of both depth to groundwater and water-uptake depth are lower compared to the other sites, the relative variation of mean water-sources used by the woody community showed to be higher in the tropical ecosystem (Fig. 1a). This implied a low inter-specific variability in both wet and less-wet conditions (Fig. 1b). This means that there was no vertical soil water partitioning among the woody functional types within each water availability condition (Chapter 2.2). *Restinga* forest species do have interspecific variation in physiological traits (Gessler et al. 2007; Rosado & De Mattos 2010; Rosado et al. 2013; Rosado et al. 2016), thus variations in water-use among species could eventually occur. However, this was not the case (Fig. 1c). The restrictive conditions of a very shallow water table tend to limit water partitioning among species and plants may show convergence of water-source-use, overlapping their water-uptake depth, when water resource is not a major limiting factor. Thus, all the woody species were exploring the most favorable layer of soil.

Contrastingly, a higher inter-specific differentiation in water source use was observed in drier climates (Fig. 1b, Chapter 2.1). Moreover, we found an increase of inter-specific variability in water-sources-use with increasing drought (i.e. higher variability in dry season of semi-arid climate) (Fig. 1bc). Thus, under extremely dry conditions, coexisting plant functional types segregated along a wide spectrum of water source use (see Chapter 2.1). Accordingly, relevant seasonal shifts in the water sources used towards deeper soil layers were observed in summer, but not in all functional types (Fig. 1d).

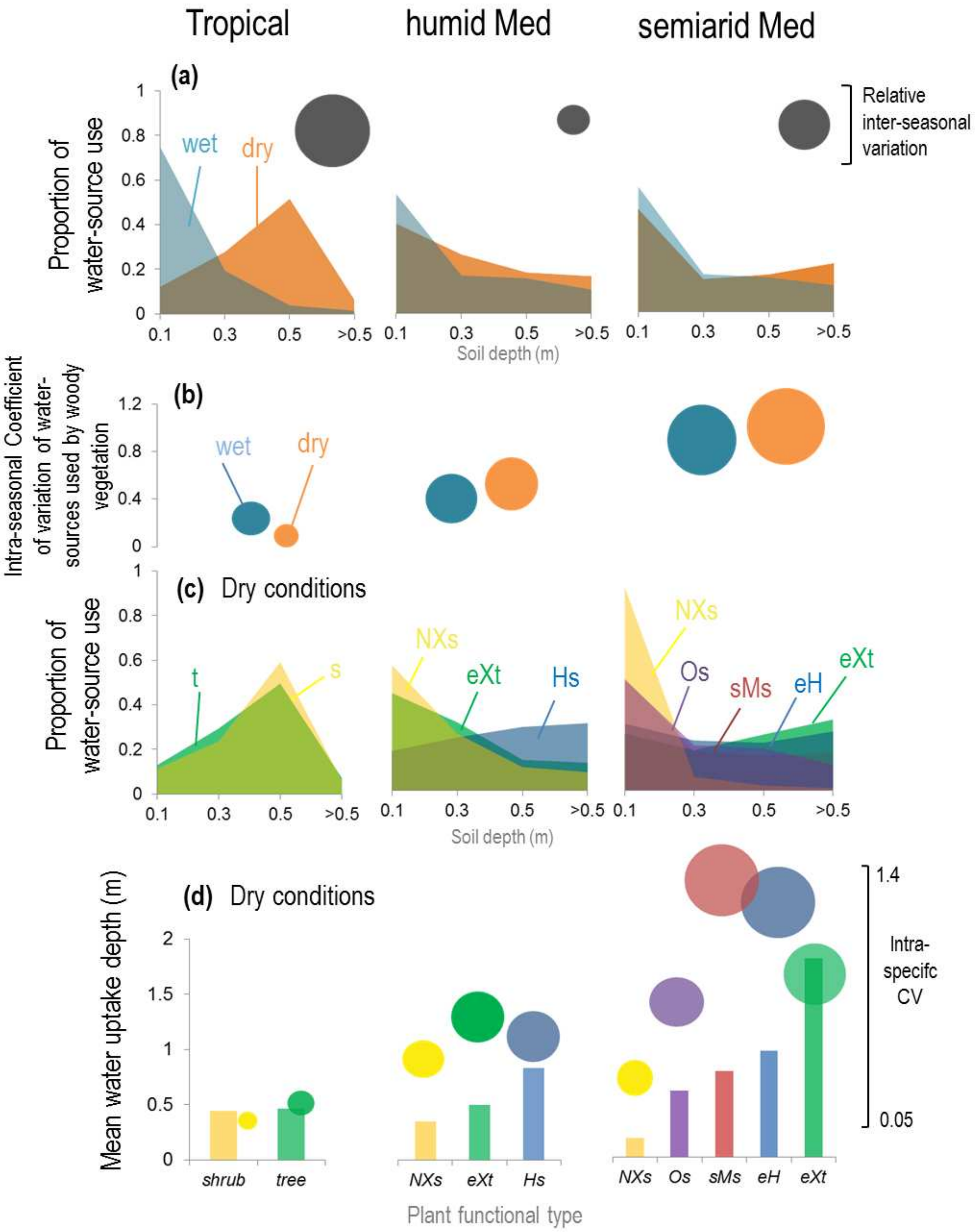


Fig. 1 Seasonal water-sources used by woody vegetation in the studied tropical, humid mediterranean and semiarid mediterranean coastal dune forests. **(a)** Mean proportion of water-source use from different soil depths in wet and dry seasonal conditions (blue: wet, orange: dry) in the three sites. The relative seasonal variation of mean water source used by the woody community [RVs=(|Mean WSU_{dry} – Mean WSU_{wet} |)/Mean WSU_{dry})] is represented as dark grey circles, with their size proportional to the RVs value (0.63, 0.14 and 0.32 for tropical, humidMed and semiarid Med respectively). **(b)** Variation within each water condition (intra-seasonal variation) considering the depth of water uptake of the overall woody vegetation in wet and dry periods. The coefficient of variation was calculated per season and site as: $CVt=SD_{WUD}/Mean_{WUD}$. CVt is represented as blue and orange circles (wet and dry), with their size proportional to the CVt value. **(c)** Mean proportion of water-source use from different soil depths under dry conditions in the three sites. Different colors denote different funtional types (see inner legend): s: shrubs; t: trees, NXs: narrow leaf xerophytic shrubs; eXt: evergreen conifer xerophytic trees; Hs: hygrophytic shrub; Os: aphyllous/broadleaf shrubs; sMs: semi-deciduous mesophytic shrub; eH: evergreen hygrophytes (see details of the species and functional type classification in chapter 2,3 and 4). **(d)** Mean water-uptake depth (bars), and coefficient of variation ($CV=SD_{WUD}/Mean_{WUD,}$ circles) of each functional type in the dry period. Different colors denote a functional type as in (c). Intra-functional type CV ranged from 0.05 to 1.4 (right y-axis), and the size of the representing circles are proportional to the CV value.

The additional water supply provided by groundwater pools was therefore also critically determined by plant functional type. The general differentiation of root depths between trees and shrubs are well documented (Schenk & Jackson 2002), but under very dry conditions not only growth form matters. Remarkably, the increase in deep soil reliance, seen as a fundamental mechanism to cope with top-soil water scarcity (Barbeta & Peñuelas 2017a; Barbeta & Peñuelas 2017b), was not observed in xerophytic narrow-leaf shrubs. We found that, not only important differences in water sources use exist, but that they are linked to broader strategies to cope with drought stress in this semi-arid ecosystem (Chapter 2.1). Our results reinforces the idea that in water-limited systems, water-source partitioning represents an important process of coexistence (Eissenstat & Caldwell 1988; Casper & Jackson 1997; Filella & Peñuelas 2003; Silvertown et al. 2015; Valladares et al. 2015; Palacio et al. 2017).

With greater aridity (and scarcity of soil moisture) it is expected that woody species invest comparatively more resources belowground than aboveground to acquire soil water, as it becomes a more limiting resource for growth and survival (Ledo et al. 2018). Thus, it is not surprising that the higher mean water-uptake depth is found in the semi-arid site (Fig. 1d). However, these belowground investments can be highly variable among functional types (Fig. 1d), along a groundwater depth gradient (Chapter 3.2), and, as previously mentioned, seasonally (Fig. 1a, Chapter 2). The seasonally variations supports the idea that plants water-sources-use is controlled by dynamic shifts in water uptake due to water availability changes. A pre-requisite for such changes is the ability of plant species to rapidly acclimate and explore different soil layers: shallow water-sources in wet and deeper in less-wet conditions. It is noteworthy to mention that this plant water-sources-use adjustments are likely to be linked to fine root production in tropical climate (high turn-over and short term production responses), and linked to a dimorphic root system in semi-arid and humid mediterranean sites.

Under seasonal dry conditions, there was an increase of intra-specific variability in water-sources used by plants from tropical to semi-arid ecosystems (Fig. 1d). Intra-specific plasticity in belowground traits (as water-uptake depth) is an important characteristic for plant resource acquisition and could be a major competitive advantage in acquiring limited soil resources. Moreover, it can be considered an important strategy to allow the co-existence of a diversified plant community in water-limited systems (Kulmatiski & Beard 2013; Skálová et al. 2013; Valladares et al. 2014; Voltas et al. 2015; Wegener et al. 2015; Guderle et al. 2018). This enlightens the importance of intra-specific (or intra-functional group) variability to describe a given species, particularly in water-limited ecosystems, because it can reveal functional variation for the species along environmental gradients (de Bello et al. 2011; Bolnick et al. 2011; Violle et al. 2012; Sébastien et al. 2012; Anderegg et al. 2018). In our case, local hydrological factors, like groundwater depth, played an important role defining the observed belowground intra-specific variability.

Ecophysiological responses of woody vegetation to groundwater depth

We explored variations in below and above-ground traits along a spatial groundwater depth gradient under dry conditions, in different climatic regions. Although each plant community operates in different ranges (Fig. 2), we could expect them to show similar responses to an important driver in these systems, such as groundwater depth.

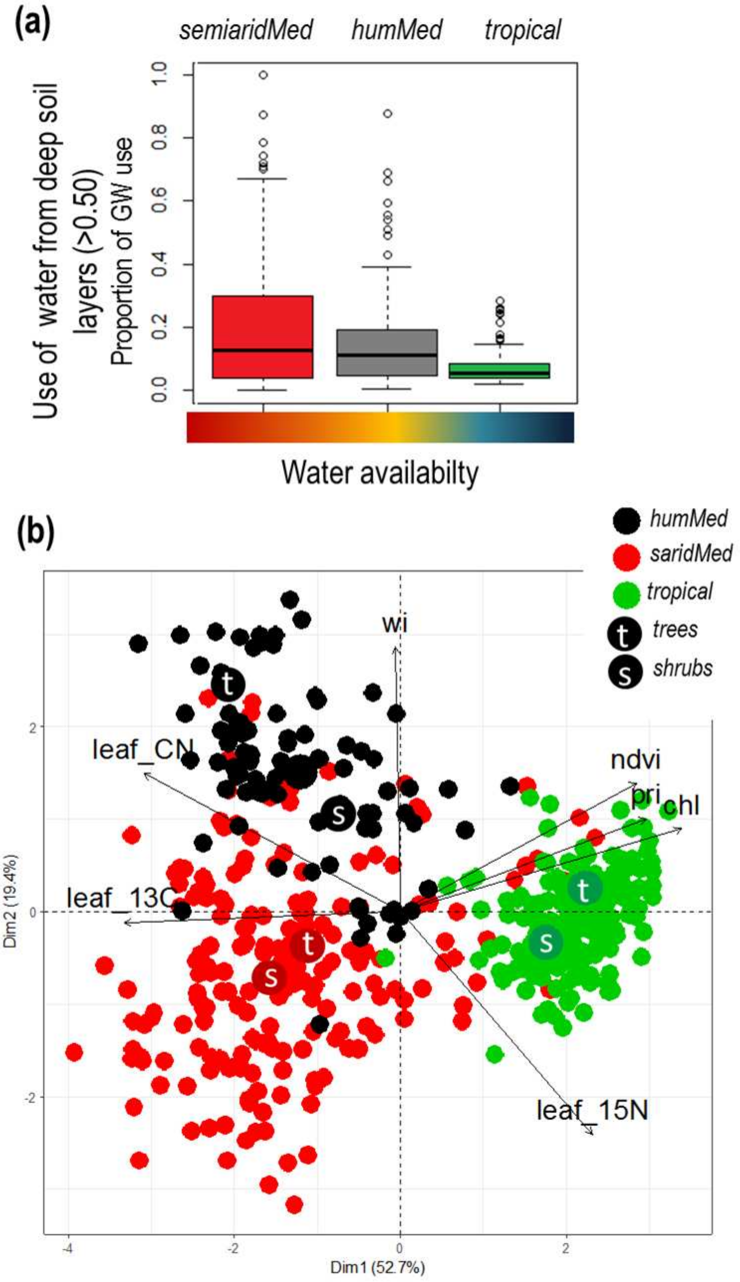


Fig. 2 Groundwater use and physiological characterization of the woody vegetation of the studied tropical, humid mediterranean and semiarid mediterranean coastal dune forests, in the dry period. **(a)** Proportion of use of water from deep soil layers (> 50 cm, i.e. groundwater (GW) use) in the studied three sites differing in water availability (semiarid Mediterranean: red, humid Mediterranean: grey, Tropical: green). **(b)** Principal component analysis of physiological parameters, considering all the studied woody plants. Mean values of trees and shrubs in each site are also represented in the figure. See details of the physiological parameters used and species considered in chapters 2, 3 and 4. Each site is represented by a different color as shown in the inner legend. Variance explained by axis 1 and 2 are shown in the figure.

In fact, groundwater depth was an important driver of water-uptake depth across the different climatic regions, but did not affect plant physiological condition equally (Fig. 3abc).

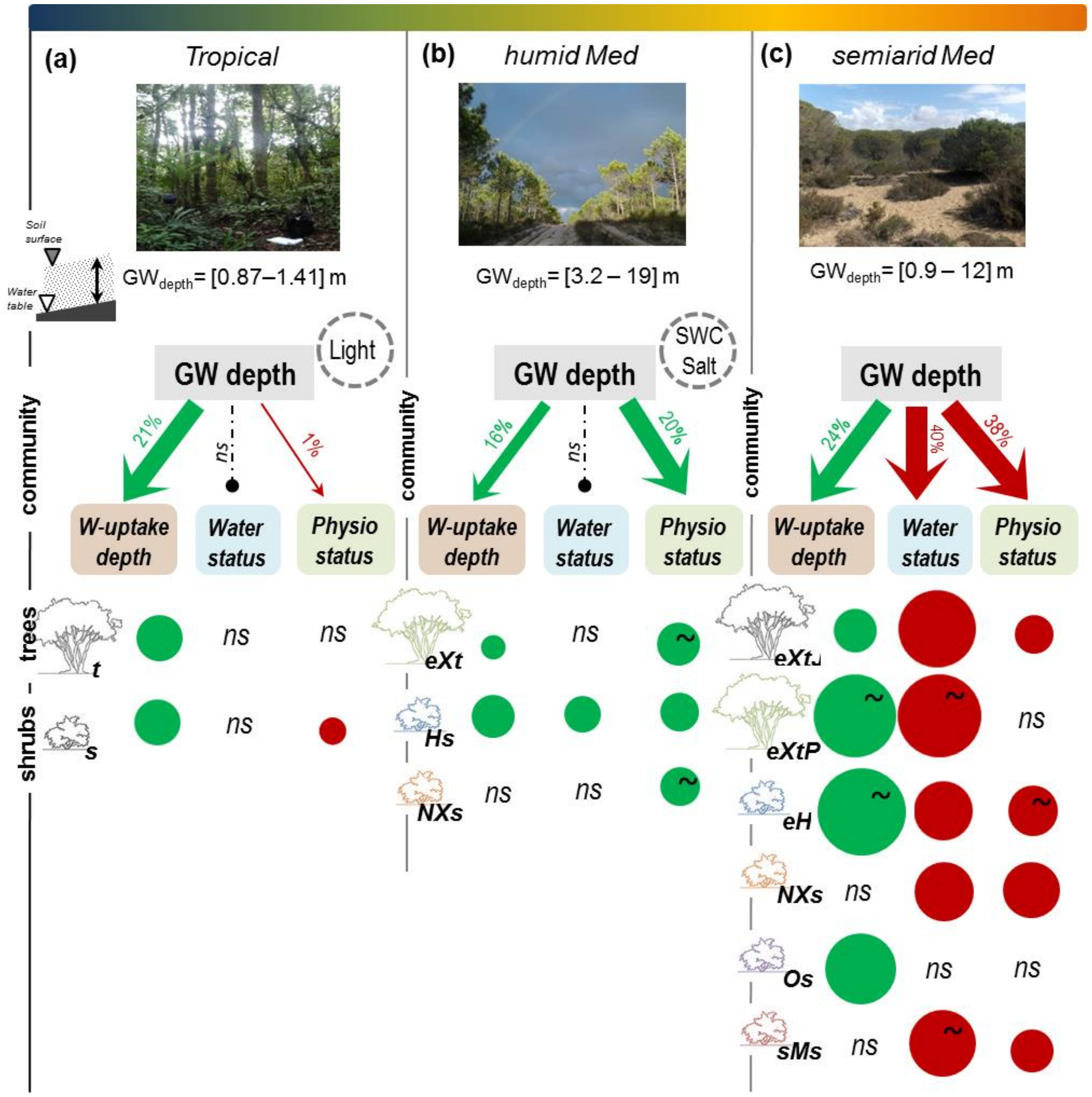


Fig. 3 Effects of groundwater depth on functional traits of woody vegetation in the studied (a) tropical, (b) humid mediterranean and (c) semiarid mediterranean coastal dune forests. The range of groundwater (GW) depth during the dry period is presented in the figure. Effects of GW depth on plants' water-uptake depth, water status and physiological status under dry conditions are shown for the overall woody vegetation and for each functional type. GW depth effects on woody vegetation (community level) are represented by arrows: green if a significant positive effect occurs, red if negative and black dashed line if non-significant. Percentage of the deviance explained by GW depth is also shown (see chapter 3.2 and 4 for details on the traits used and statistical analysis). Further important environmental controls found in the tropical and humid Med sites are also represented in the figure (inside dashed grey circles). GW depth effects on each functional type are represented by circles with size proportional to the contribution of GW depth and color denoting the direction of the effect (green for positve and red for negative). Funtional types considered: s: shrubs; t: trees, NXs: narrow leaf xerophytic shrubs; eXt: evergreen conifer xerophytic trees (J – *Juniperus* sp., P – *Pinus* sp.); Hs: hygrophytic shrub; Os: aphyllous/broadleaf shrubs; sMs: semi-deciduous mesophytic shrub; eH: evergreen hygrophytes (see details of the species and functional type classification in chapter 2,3 and 4). Non-significant effects are denoted with 'ns' and non-linear correlations with a '~' (see chapter 3.2 for further details).

In agreement with Barbeta & Peñuelas (2017b) we found higher mean groundwater depth use in semi-arid conditions (Fig. 2a). Furthermore, different mean values and ranges of physiological conditions were observed for each site (Fig. 2b). Plants' physiological traits were clearly site dependent, underpinning the highly different physiological patterns expected for the different climatic conditions.

Groundwater availability modified water-uptake depth but not physiological conditions of woody vegetation in the tropical sandy forest (Fig. 3a). Variation in light availability was more important as a driver of photosynthetic traits than groundwater depth, acting as the limiting factor in the tropical region. Trees showed higher photosynthetic activity compared with understory shrubs, but did not show a more favorable water status when using deeper soil layers (Chapter 4).

In the mediterranean climate, greater depth to groundwater combined with low soil water availability will likely cause important shifts in plant water-use, including greater proportion of water uptake from deeper soil layers and/or physiological adjustments. However, these responses will depend on the plant functional type and drought intensity (Chapter 3.1). Under a humid Mediterranean climate, the lowering of groundwater table did not negatively influence the studied species physiology (Fig. 3b). Thus, the effects of greater depths to groundwater should be less severe in more mesic conditions. Under higher drought intensity (like in semi-arid summer), dimorphic-rooted species and shallow-rooted species showed different belowground responses to increasing groundwater depth (Fig. 3c) (Chapter 3.1). Interestingly, the interspecific variability of water-uptake depth increased not only with climatic and seasonal drought (Fig. 1), but also with groundwater limitation (at greater groundwater depths a higher soil profile is explored by the different co-existing functional types) (Chapter 3.2). Differential use of water-sources among plant functional types are thus dependent on groundwater availability and an important process in temporarily dry ecosystems (Grossiord et al. 2017; Palacio et al. 2017). This underpins this ecohydrological factor as one of the most general drivers of niche differentiation for plants (Araya et al. 2011; Silvertown et al. 2015). Nevertheless, this hydrological change negatively impacted the overall woody vegetation, affecting traits related to both photosynthetic capacity and plant water status, regardless plants' water-sources-use strategy (Chapter 3.2). Far from responding uniformly in belowground traits, they consistently declined their water content and generally reduced photosynthetic activity with increasing depths to groundwater (Fig. 3c). Thus, plant functional groups can show divergent water-uptake depth responses and distinct operating physiological ranges, but common physiological sensitivity to greater depths to groundwater in semi-arid conditions. These results strongly suggest that hydrological drought has an impact on physiological fundamental processes, constraining the performance of dune vegetation under conditions where water stress is a rule. Interestingly, the ecophysiological shift curves are in agreement with Sommer & Froend (2014) study thresholds of transition of hydrotypes (vegetation states) (Chapter 3.2). These plant composition variations are expected to be primarily based on ecophysiological processes of acclimation, underpinning the great potential of these physiological traits as early indicators of further community changes in this ecosystem.

We have highlighted the ecophysiological responses and vulnerability of coastal sandy woody vegetation to groundwater limitation in different climatic contexts. Our study may contribute for predicting changes in physiological performance and in the state of woody vegetation (and, to the same extent, their vulnerability and survival), that result from ongoing increased groundwater changes. This has, therefore, evident implications for the conservation of plant communities now facing stressful hydrological conditions caused by water extraction and climate change, such as semi-arid coastal dune ecosystems.

Summary

Under groundwater changes, the level of physiological stress is determined by the climatic context, being the extent of groundwater impacts greater with increasing aridity.

Species from more humid sites, where soil water availability is higher, cope well with the observed range of groundwater variations, adjusting the water-uptake depth and maintaining their physiological status.

The combined effect of soil- and ground-water scarcity, under semi-arid conditions, promote physiological stress across functional types. In these circumstances, plant water stress is felt by the overall woody community with increasing groundwater shortage. Thus, woody species under this hydrological context are more susceptible to suffer from water table depletion, particularly species with higher water demand, showing greater root water-uptake adjustments and abrupt changes in physiological status.

Future challenges

Linking plant physiological responses to abundance patterns

To know how woody species physiological performance is related to plants’ fitness and survival is of utmost importance. Disentangling the underlying mechanisms associated with species’ abundance dynamics would allow to define their full vulnerability to groundwater availability. This is particularly applicable in the semi-arid site. To achieve that, correlations between physiological responses (considering the index of physiological performance and reflectance water index) and abundance of woody functional types should be performed. Also, the variations in functional diversity, cover and density along the groundwater gradient should be explored. Variations in composition can be manifested as a shift towards species not dependent on specific hydrological conditions (e.g., relatively shallow groundwater), with the progressive change in hydrology continuing to force a transition in the flora towards an alternative ecohydrological state. If the (physiological and abundance) patterns over the groundwater gradient overlap, and the same shifting points are observed, than groundwater can be considered an important driver of both physiological and fitness variation. Moreover, groundwater depth thresholds that trigger movement to possible alternate stable states can be found. Particularly in the semi-arid Mediterranean site, this would further support the validity of our multi-trait-physiological index and reflectance water-index as possible ‘early warning’ indicators of the groundwater impacts on the vegetation.

Furthermore, the community-weighted mean of the physiological indices (i.e. plot-level index values weighted by species abundances) should be calculated and its variation consider along the groundwater depth. This can have great implications on the extent of the applicability of the defined models. Further spatial extrapolation of community vulnerability to groundwater changes would be also of great interest (see below topics).

Functional types’ hydraulic vulnerability

The extent of woody species hydraulic limits and vulnerability to groundwater depth should be further explored, particularly in the semi-arid site. Observations of high mortality of woody vegetation plants during the last years in this ecosystem seem to support the undergoing hydraulic vulnerability to groundwater limitation, but further confirmation is needed. Because of the complex interactions between water and carbon relations under extreme drought, it is still difficult to disentangle the main cause of woody species mortality. Arguably, however, one of the most important outcomes of drought-induced mortality research it that hydraulic deterioration is ubiquitous under lethal drought, where reductions in carbon reserves are not (Martínez-Vilalta & Garcia-Forner 2017). This emphasizes the importance of plant hydraulics and paves the ground to use water related simple indices as a proxy of woody community vulnerability to groundwater scarcity. Hydraulic safety margins (and P50) are a reasonable proxy of vulnerability to drought. It does not give a complete account of drought resistance strategies in plants, but combined or validated against our physiological multi-trait index and reflectance water-index could help to better define the vulnerability to death due to groundwater changes in this ecosystem. Accordingly, to explored how reflectance water index is related to xylem percentage of conductance loss, using for example the pneumatic method (which provides a good estimate of the degree of xylem embolism) (Pereira et al. 2016; Zhang et al. 2018), would be of great interest.

Spatial explicit maps of vulnerability: scaling-up

Advanced measurement techniques allow the detection of spatiotemporal patterns with greater resolution at both fine (the ones that we used in our study) and broad scales (e.g. satellite imagery, and eddy covariance technologies). The ability to assess plant water use across scales relies not only on the identification and mechanistic understanding of the processes involved but also on capturing the heterogeneity of landscape with leaf- or stand-level approaches.

It is important to contribute for predicting woody vegetation physiological conditions and vulnerability (and, to some extent their fitness and survival), that result from ongoing increased groundwater drawdown in the semi-arid study area. The physiological vulnerability may be examined most effectively by incorporating appropriate plot-specific values of groundwater depth and physiological information of both stand-level community and functional types'. Using the model defined in chapter 3.2 and additionally the one with community weighted mean (see previous topic), we could create spatial explicit maps of physiological vulnerability of the woody dominant community based on groundwater depth maps of the area (at a meso-regional scale). To create a more supported and robust extrapolation maps we should have into account the knowledge driven by the above mentioned ' future challenges' topics.

Besides, further extrapolation is possible providing that an acceptable relationship between actual plant water and a measured variable (e.g. reflectance of foliage in tree canopies) is extractable from a remote sensing data set. If leaf-level data agree well with remote sensing data (e.g. from MODIS satellites), then a better support in mapping will be provided. Threshold responses at large scales are generally not as sharp or dramatic since changes occur more slowly. Nevertheless, dramatic and rapid dieback and mortality of vegetation in response to extreme droughts suggests that strong thresholds and shifts in ecosystem state may operate on larger scales and deserve greater attention.

The phsyiological based-model, explaining the occurrence of physiological/functional shifts in woody community, is a way forward into the ecohydrological vulnerability of these communities. Nevertheless, we will need to take into account parameter uncertainty, and other hydrological and ecological constrains when using models to scale information and make predictions across scales. At a landscape scale, we will have to consider variation in plant species/functional types vegetation cover (see further details on this in the above mentioned topic), as well as possible feedbacks in soil moisture storage capacity and nutrients availability. Nevertheless, with proper validation, vulnerability maps are possible. This can have potentially far-reaching implications for managing, particularly in the semi-arid ecosystem, that are now face changing hydrological conditions caused by human groundwater extraction and climate change.

Assessment of physiological constrains in flooding periods of restinga *forest*

Although *restinga* forest comprises tree species tolerant to flooding, it also contains species sensible to it (de Oliveira 2011). It would be important to see if the physiological conditions differ among species under wet conditions, and if a shallower groundwater would differently affect their physiological status. To do so, the same approaches as in Chapter 2 (but using physiological parameters) and in Chapter 4, applied to data from less-wet period, can be used. If, comparatively, the physiological constrains are higher under wet and generally shallower groundwater conditions, then flooding could be confirmed as a great driver of physiological adjustments.

It would be also interesting to explore the long-term variation of flooding patterns and see what the main tendency in these restinga forest ecosystems is. In other words, if there is an increase in flooding periods or in dry periods. By knowing the main potential groundwater constrain (if flooding or drying soils), than these hydrological trends can be linked to some estimations on woody vegetation future conditions.

Inter-annual variability, recovery trends and long-term responses

There are still many unanswered questions regarding the potential for reversing the physiological transitions observed in the semi-arid community. It is known that, in this particular ecosystem (and considering the same dominant species studied by us), plant cover resilience after an extreme drought event (Lloret et al. 2016) and physiological recovery (Zunzunegui et al. 2011), is dependent on efficiency of water-use. Species with low water-use efficiency are able to maintain green biomass for a longer period of time but are less resilient in the medium term. However, the role that groundwater depth plays in the recovery trends is unknown. Thus, how the inter-annual variability and the physiological reversibility (to spring values) vary along the groundwater gradient considering the overall dominant community is something worth digging. If there is a tendency to lower recovery with increasing groundwater depth, than groundwater depth would be further affecting plants at a medium-term. The decline in recovery may leave the plants more vulnerable to drought the following season and year. In particular, damage that limits a woody species' ability to make use of water or nutrients when they briefly become available again could cause system failure. Further, if a tree accumulates enough physiological damage, particularly over several years, and if the physiological damage crosses some threshold, plant death may occur (Anderegg et al. 2012). One way to assess these long-term patterns in tree species is through tree-ring (width and stable-isotope) analysis. The analysis of tree physiology and growth patterns across a large temporal scale is expected to provide the trees responses to water availability variations and to groundwater limitation. Both contemporary events and historical contingencies shape plants' physiological patterns, so approaches that account for both past events and current constraints are complex but necessary.

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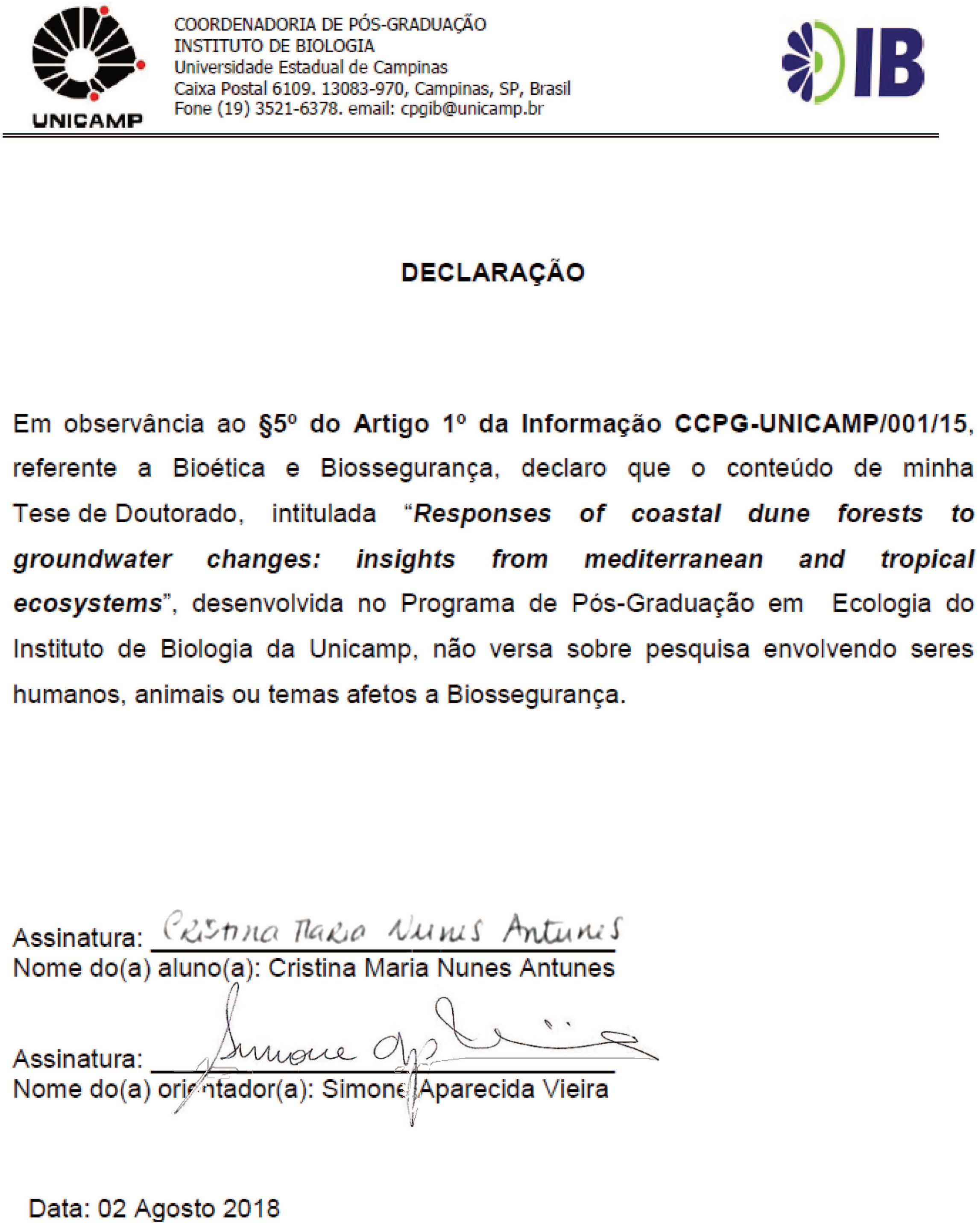
6 Annexes

6.1 Declaração Bioética



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DECLARAÇÃO

Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "***Responses of coastal dune forests to groundwater changes: insights from mediterranean and tropical ecosystems***", desenvolvida no Programa de Pós-Graduação em Ecologia do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

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Data: 02 Agosto 2018

6.2 Declaração direitos autoriais

Declaração

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada **Responses of coastal dune forests to groundwater changes: insights from mediterranean and tropical ecosystems**, não infringem os dispositivos da Lei n.° 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 02 Agosto 2018

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