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

Cristina Antunes [a,b]*, Mari Cruz Díaz Barradas [c], Simone Vieira [d], Maria Zunzunegui [c], Ângela Pereira [e], Andreia Anjos [a], Otília Correia [a], Maria João Pereira [e], Cristina Máguas [a]

(a) Centro de Ecologia, Evolução e Alterações Ambientais, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

(b) PPG - Ecologia, Instituto de Biologia, Universidade Estadual de Campinas, São Paulo, Brazil

(c) Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Spain

(d) Núcleo de Estudos e Pesquisas Ambientais, Universidade Estadual de Campinas, São Paulo, Brazil

(e) Centro de Recursos Naturais e Ambiente, Instituto Superior Técnico, Universidade de Lisboa, Portugal

* Corresponding author: cmaantunes@fc.ul.pt

Running head: plants’ responses to groundwater table lowering

Summary

1. 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.

2. 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.

3. 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 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 changes in water uptake towards deeper 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 δ^{13}C. Unexpectedly, under semi-arid conditions, the shallow-rooted xerophytic shrub *C. album*, associated with an absence of water-use adjustments, showed a decline in WI, CHL, and PRI with groundwater table lowering.

4. 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.

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 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.

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$_{\mathrm{Aug2012\text{-}Aug2013}}$ rainfall = 755.4 mm) comparing to the semi-aridMed site (total annual$_{\mathrm{Aug2012\text{-}Aug2013}}$ rainfall= 566.1 mm) (Fig. S1).

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).

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 δ^{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 δ^{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).

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 $\delta^{18}O$ (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).

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).

In the semi-aridMed site, 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 (Fig. 3a). Comparatively, plants of the humiMed site tended to maintain a shallower water use along the groundwater depth gradient (Fig. 4a).

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 δ^{13}C (Table 3, Fig. S6).

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). 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, δ^{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).

Discussion

As hypothesized, only under drier conditions (summer in semi-arid) 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-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 groups 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 $\delta^{18}O$ 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, and thus rely on superficial unsaturated zone of the soil profile, even in the dry season (water-table capillary fringe) (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. 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 δ^{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 in 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.

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.

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/XXX

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

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 δ^{18}O.

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

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

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

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

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

Fig. 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.

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

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

FIGURES captions

Fig. 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.

Fig. 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).

Fig. 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: δ^{13}C (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).

Fig. 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 stands for significant differences between species.

TABLES

Table1. Species' information and functional type classification considered in the study.

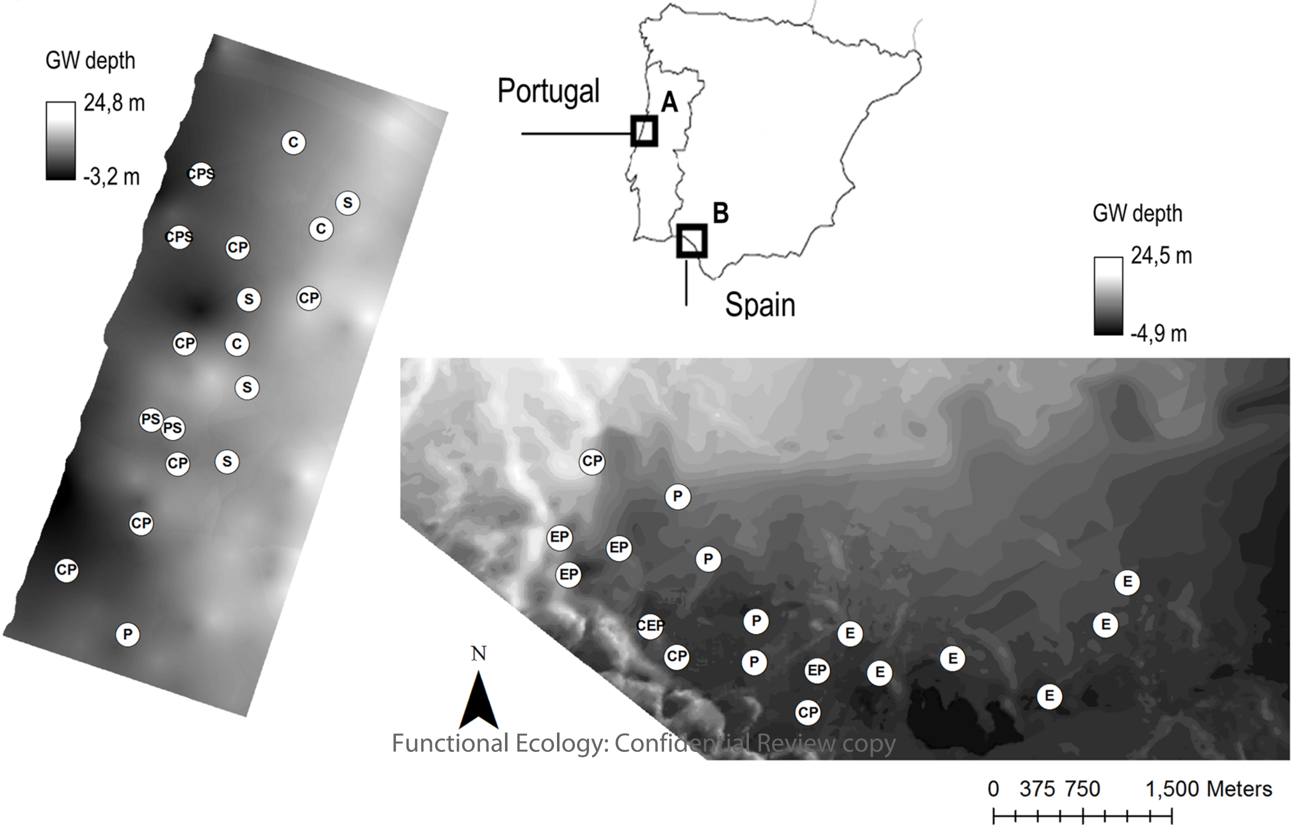
Species' Functional type classification	**Growth form**	**Root system**	**Water requirement**	**Habitat**	**Depth to GW sampling range (summer)**	**Site**
Conifer tree						
Pinus pinaster Ait.	tree	deep/ dimorphic	xerophyte	dunes crests, slopes and border of dune slacks	3.19 – 19.07m	humidMed
Pinus pinea L.	tree	deep/ dimorphic	xerophyte	dunes crests and slopes, and border of temporary ponds	1.59 – 12.28m	semi-aridMed
Hygrophytic shrub						
Erica scoparia L.	shrub	dimorphic	hygrophyte	"Monte Negro" scrub type, inner belt of sandy temporary ponds	0.94 – 6.97m	semi-aridMed
Salix repens L.	shrub	(likely) dimorphic	hygrophyte (phreatophyte)	dune slacks	3.19 – 19.07m	humidMed
Xerophytic shrub						
Corema album (L.) D. Don	shrub	shallow	xerophyte	dunes crests and slopes	3.19 – 19.07m / 2.79 – 12.28m	humid and semi-aridMed

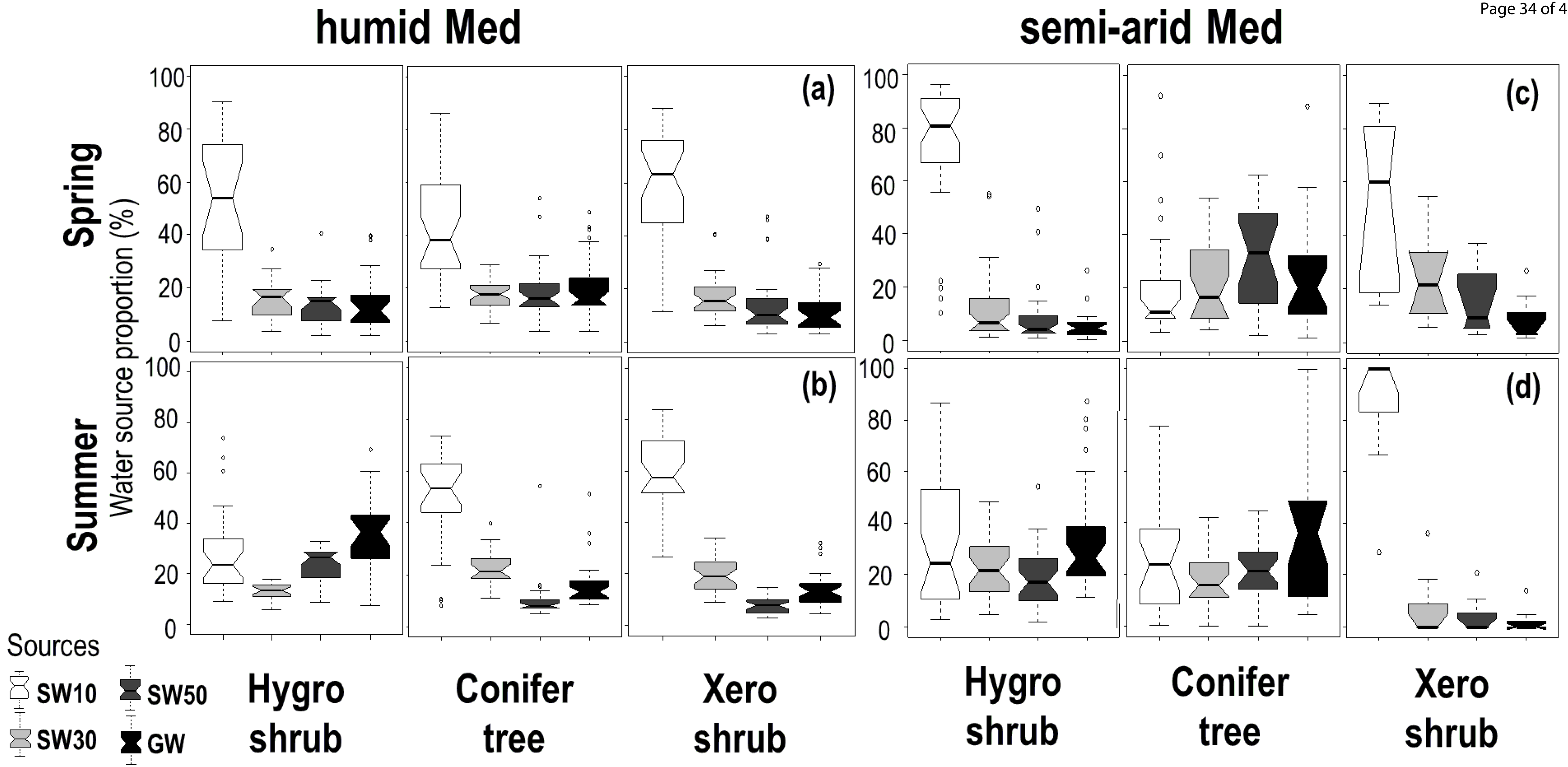
Table 2. Spearman correlations (rho) between water used by plants from each soil layer (in % contribution) and groundwater depths in the humidMed and semi-aridMed sites and in two seasons (spring and summer). Three functional types considered: Hygrophytic shrub (Hygro shrub), Conifer tree and Xerophytic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

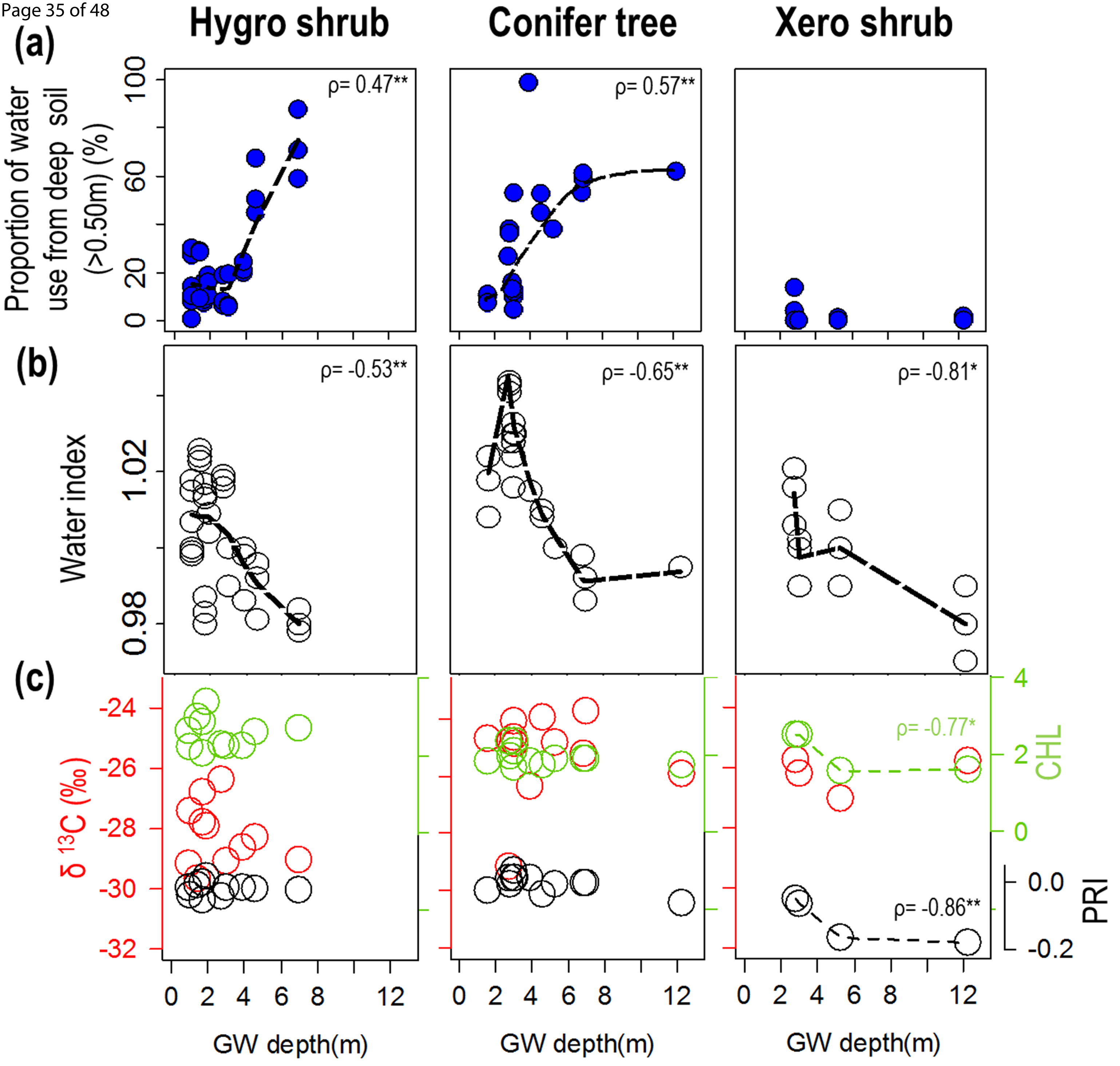
	Spring				**Summer**				
	SW$_{10}$	**SW$_{30}$**	**SW$_{50}$**	**GW**	**SW$_{10}$**	**SW$_{30}$**	**SW$_{50}$**	**GW**	***n***
Humid site									
Hygro shrub (*S. repens*)	-0.30	0.40	0.25	0.19	**-0.57****	0.24	**0.45***	0.27	*27*
Xero shrub (*C. album*)	-0.30	0.32	0.20	0.25	0.19	-0.21	-0.11	0.06	*33*
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*

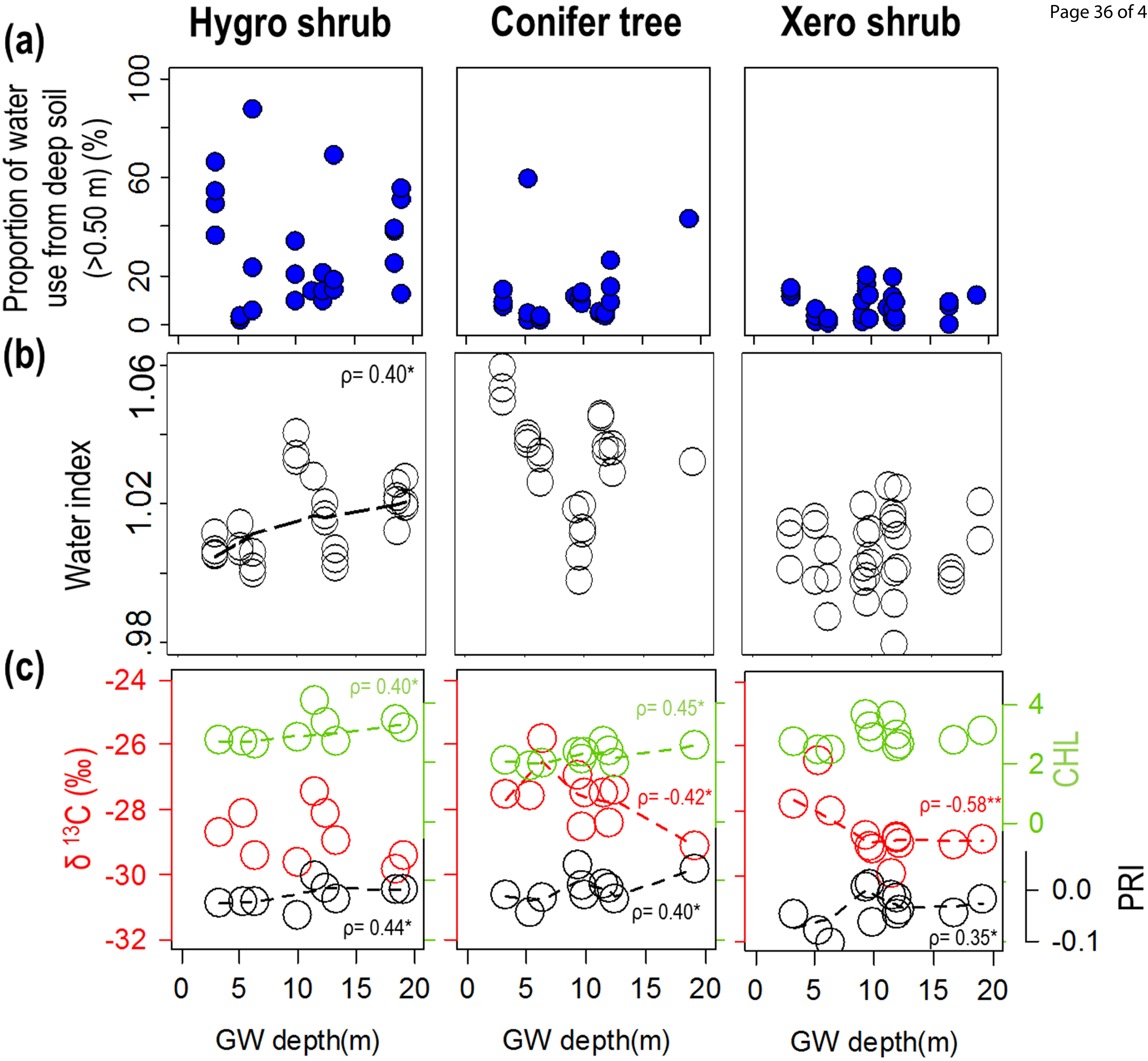
Table 3. Spearman correlations (rho) between groundwater use and physiological variables in the humidMed and semi-aridMed sites, in dry season (summer). Three functional types considered: Hygrophytic shrub (Hygro shrub), Conifer tree and Xerophytic shrub (Xero shrub). Plant reflectance indices: WI -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

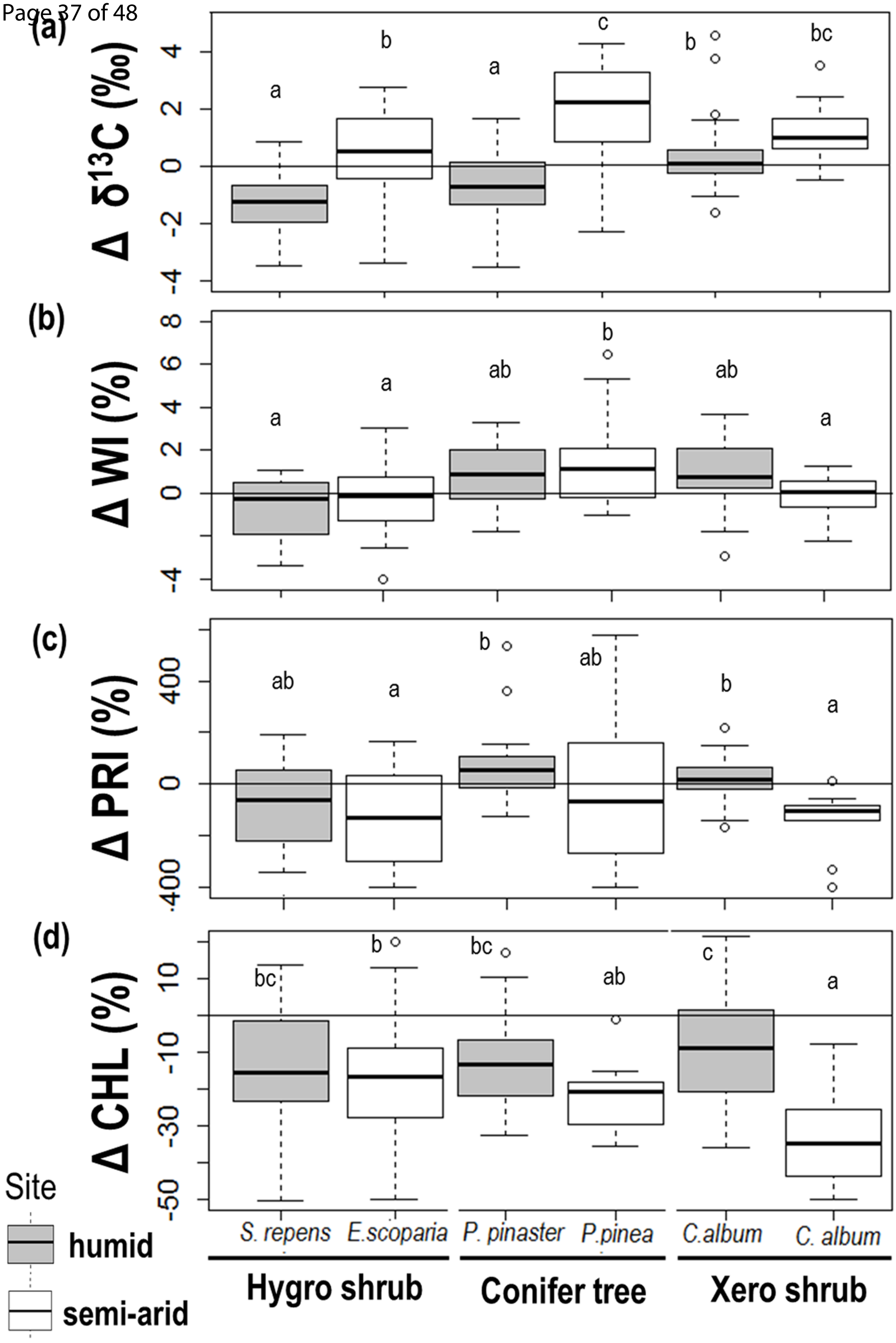
summer	**δ^{13}C**	**WI**	**PRI**	**CHL**	***n***
Humid site					
Hygro shrub (*S. repens*)	-0.04	-0.13	0.06	0.17	*27*
Xero shrub (*C. album*)	-0.29	0.33	0.32	0.23	*33*
Conifer tree (*P. pinaster*)	-0.35	-0.17	0.03	0.07	*23*
Semi-arid site					
Hygro shrub (*E.scoparia*)	-0.18	**-0.37***	0.03	0.16	*33*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


GW depth
24,8 m
-3,2 m
C
CPS
S
C
CPS
CP
S
CP
CP
C
S
PS
PS
CP
S
CP
CP
P
Portugal
A
B
Spain
GW depth
24,5 m
-4,9 m
CP
P
EP
EP
P
EP
E
E
CEP
P
E
CP
P
EP
E
E
E
CP
N
0 375 750 1,500 Meters


humid Med
semi-arid Med
Spring
Summer
Water source proportion (%)
100
80
60
40
20
0
(a)
(b)
(c)
(d)
Sources
SW10
SW30
SW50
GW
Hygro shrub
Conifer tree
Xero shrub
Hygro shrub
Conifer tree
Xero shrub


Hygro shrub
Conifer tree
Xero shrub
(a)
Proportion of water use from deep soil (>0.50m) (%)
100
60
20
0
ρ= 0.47**
ρ= 0.57**
(b)
Water index
1.02
0.98
ρ= -0.53**
ρ= -0.65**
ρ= -0.81*
(c)
δ ^{13}C (‰)
-24
-26
-28
-30
-32
CHL
4
2
0
ρ= -0.77*
PRI
0.0
-0.2
ρ= -0.86**
0
2
4
6
8
12
GW depth(m)
GW depth(m)
GW depth(m)


Hygro shrub
Conifer tree
Xero shrub
(a)
Proportion of water use from deep soil (>0.50 m) (%)
0
20
60
100
(b)
Water index
.98
1.02
1.06
ρ= 0.40*
(c)
δ ¹³C (‰)
-24
-26
-28
-30
-32
ρ= 0.40*
ρ= 0.44*
ρ= 0.45*
ρ= -0.42*
ρ= 0.40*
ρ= -0.58**
ρ= 0.35*
CHL
4
2
0
PRI
0.0
-0.1
0
5
10
15
20
GW depth(m)
GW depth(m)
GW depth(m)


(a)
Δ δ13C (‰)
4
2
0
-2
-4
a
b
a
c
b
bc
(b)
Δ WI (%)
8
6
4
2
0
-4
a
a
ab
b
ab
a
(c)
Δ PRI (%)
400
0
-400
ab
a
b
ab
b
a
(d)
Δ CHL (%)
10
-10
-30
-50
bc
b
bc
ab
c
a
S. repens
E.scoparia
P. pinaster
P.pinea
C.album
C. album
Hygro shrub
Conifer tree
Xero shrub
Site
humid
semi-arid

SUPPORTING INFORMATION: Figures S1, S2, S3, S4, S5, S6 & S7

Contrasting plant water-use and physiological responses to groundwater depth in Mediterranean coastal dune ecosystems

Functional Ecology

Figure S1

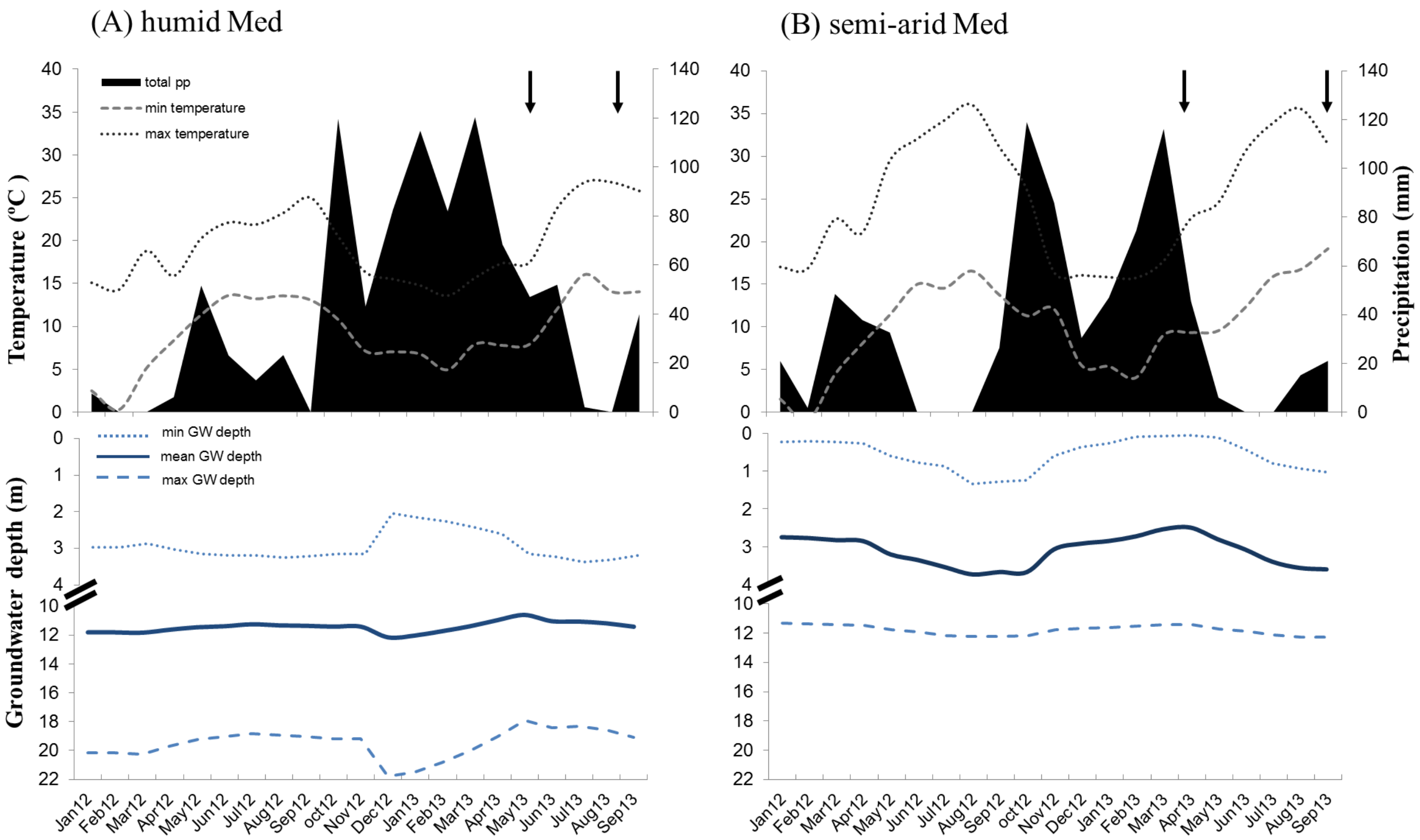


Fig. S1 Climatic and groundwater depth of each study site (from January 2012 to September 2013): (A) humid Mediterranean and (B) semi-arid Mediterranean. Monthly total precipitation (secondary y-axis), maximum and minimum temperature (primary y-axis) are shown in upper panels. Ecophysiological sampling 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 a easier interpretation of GW depth, i.e. 0 m indicates surface soil).

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Contrasting plant water-use and physiological responses to groundwater depth in Mediterranean coastal dune ecosystems

Functional Ecology

Figure S2

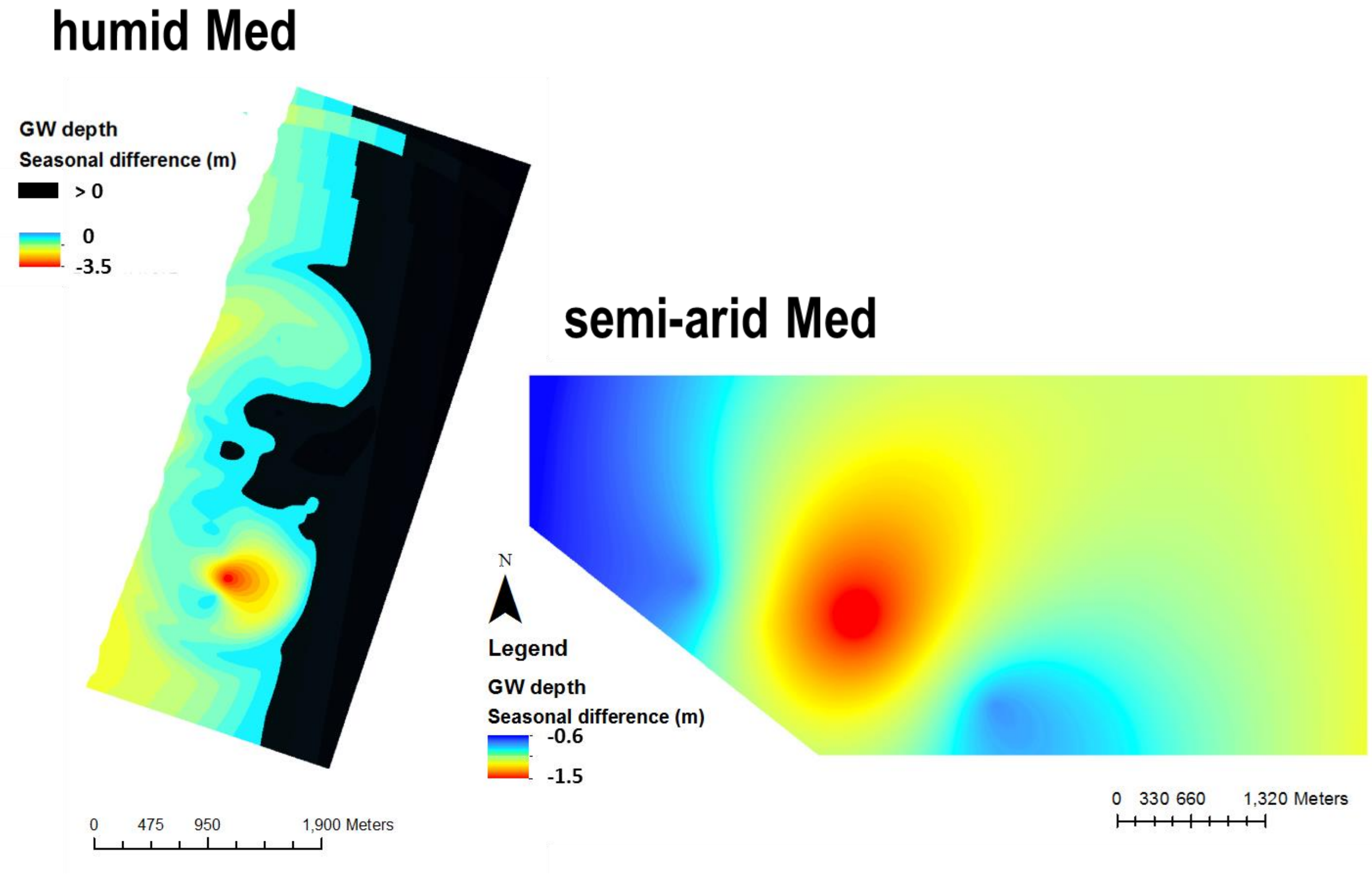

Fig. S2 Groundwater depth seasonal difference (GW depth April – GW depth August) in humid and semi-arid Mediterranean sites. Specific legends in figure. Minus signal represent summer groundwater lowering, seasonal differences higher than zero (no GW lowering, i.e either stable or summer rising water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

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Figure S3

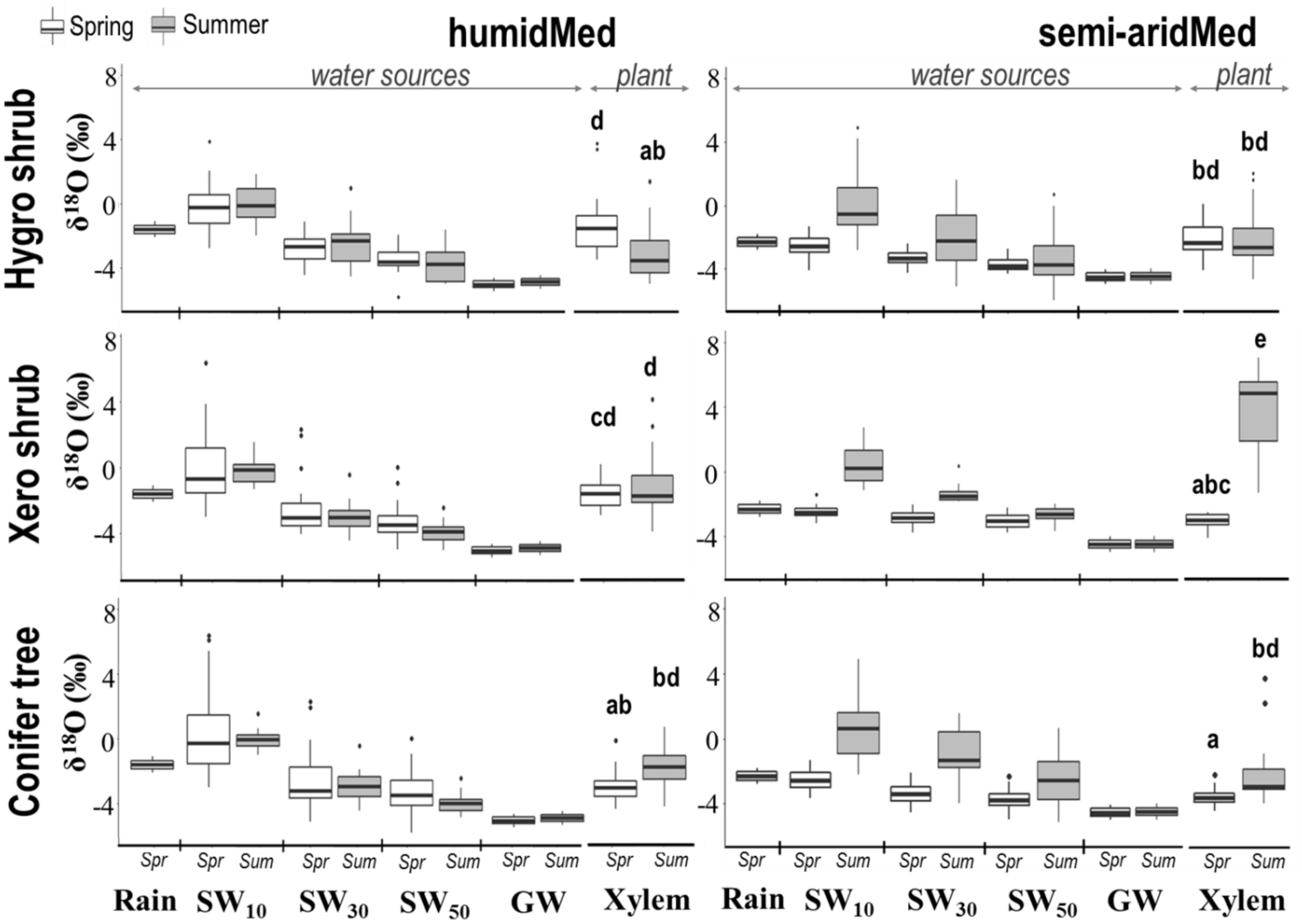

Fig. S3 Boxplots of water sources δ^{18}O (‰) (soil water at three different depths, rain and groundwater) and xylem water of the three plant functional types (xerophytic shrub, hygrophytic shrub and conifer tree). White boxes represent spring and grey represents summer data (*Spr* and *Sum*). Significant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

Figure S4

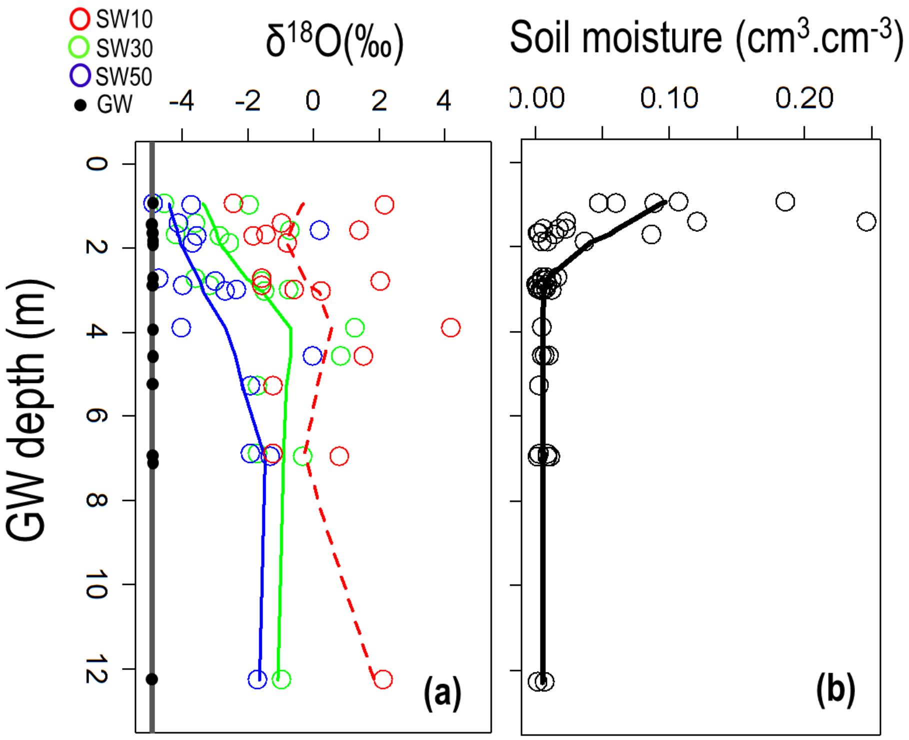

Fig. S4: Summer in semi-arid site: relathionship between groundwater (GW) depth and (a) Mean soil $\delta^{18}O$ at three depths (different water sources are represented by different colors as showed in the inner legend), and (b) Volumetric soil moisture content ($cm^3.cm^{-3}$). (a) Fitted lowess lines: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

Contrasting plant water-use and physiological responses to groundwater depth in Mediterranean coastal dune ecosystems

Functional Ecology **Figure S5**

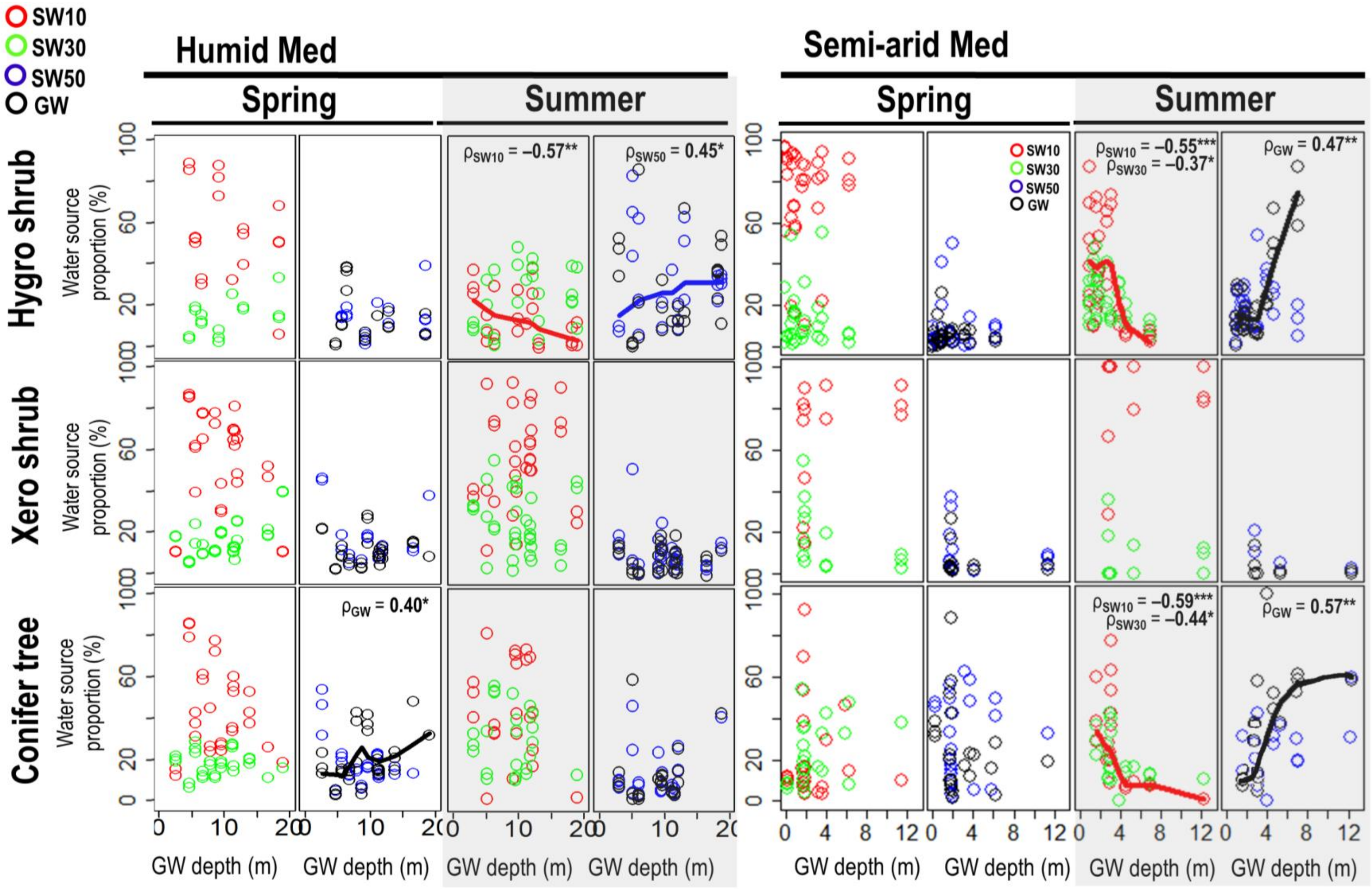

Fig. S5 Water use proportions of the three plant functional types (hygrophytic shrub, xerophytic shrub and conifer tree) along the groundwater depth gradient in spring and summer, at two climatic sites (humid and semi-arid Mediterranean). Different water sources are represented by different colors as 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.

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Figure S6

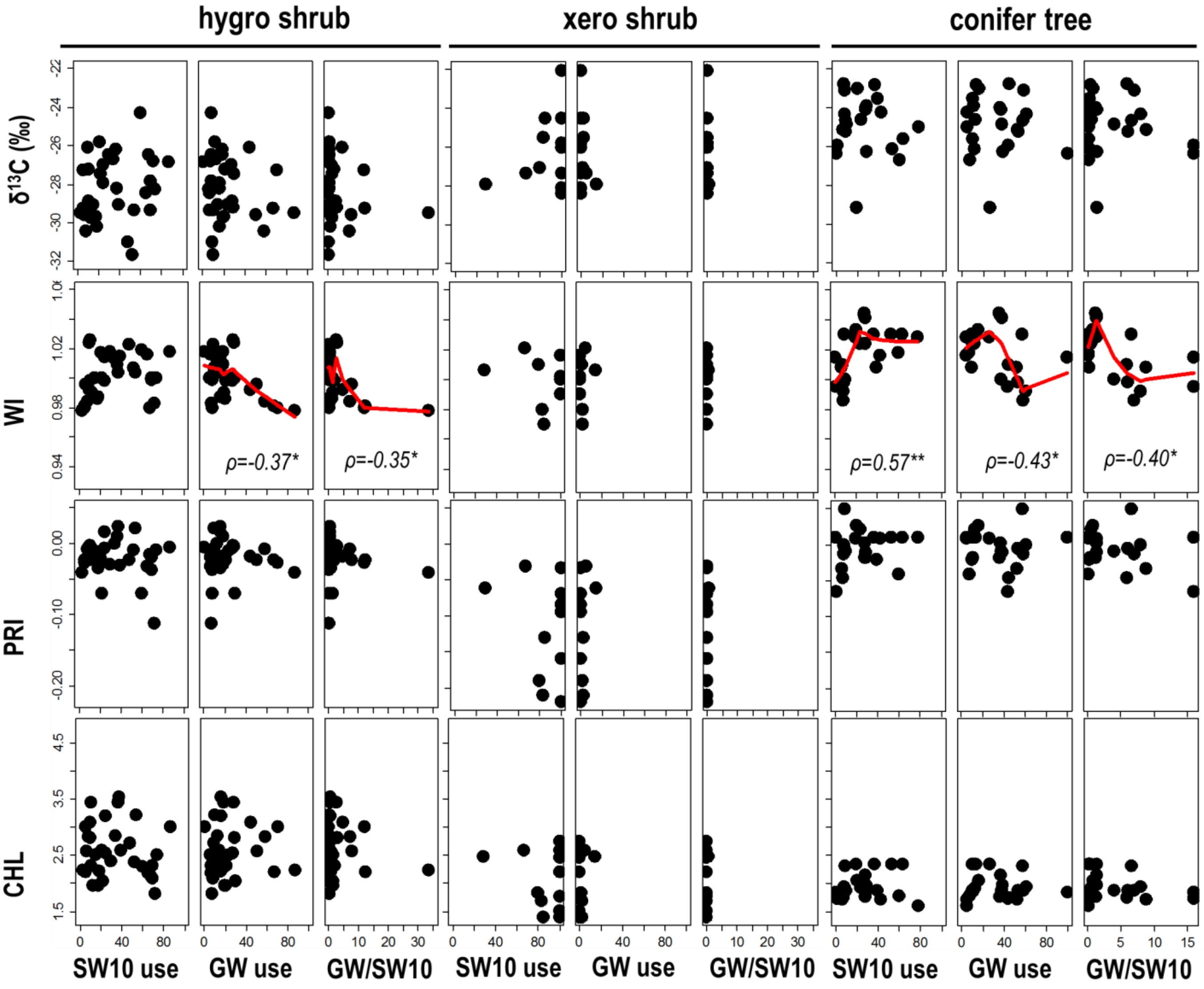

Fig. S6: Relationship between water use proportions and physiological parameters for the three plant functional types (hygrophytic shrub, xerophytic shrub and conifer tree) considering data of summer semi-arid site. Spearman rho (ρ), respective p-value (*$p<0.05$, **$p<0.01$, ***$p<0.001$) and lowess 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.

Contrasting plant water-use and physiological responses to groundwater depth in Mediterranean coastal dune ecosystems

Functional Ecology

Figure S7

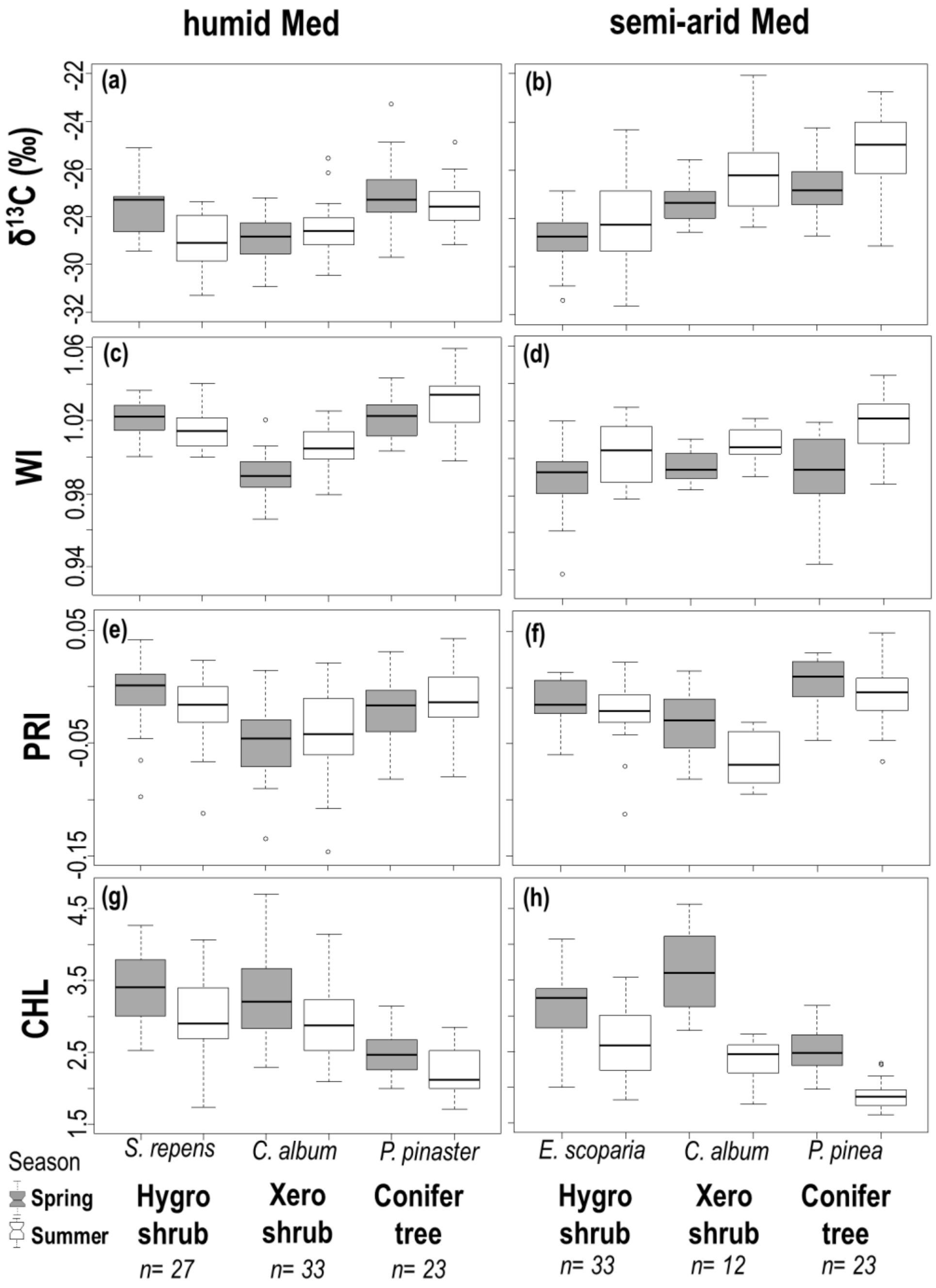

Fig. S7: Spring and summer physiological parameters of the three plant functional types (hygrophytic shrub, xerophytic shrub and conifer tree): (a), (b) $\delta^{13}C$; (c), (d) water index (WI); (e), (f) photochemical reflectance index (PRI); (g), (h) chlorophyll index (CHL); in (a), (c), (e), (g) humid 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.

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SUPPORTING INFORMATION: Tables S1, S2 , S3 & S4

Contrasting plant water-use and physiological responses to groundwater depth in Mediterranean coastal dune ecosystems

Functional Ecology

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 m^3/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).

References of Table S1:

Abrunhosa, M.J. (2002) Projecto de Captações em Osso da Baleia - Pombal. Grundfos 10.

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References of Table S1 (cont.):

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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*****