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REGULAR ARTICLE

# Root traits across environmental gradients in Mediterranean woody communities: are they aligned along the root economics spectrum?

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**Abstract**

*Background and aims* Root traits play a critical role in plant resource-use strategies and ecosystem functioning, but there is great controversy regarding their identity and functionality in different dimensions of belowground functional variation. Here, we explored the level of covariation among a suite of key root traits (i.e. specific root length, root dry matter content, diameter and density) as well as between them and two aboveground traits related with plant function (leaf nutrient concentration and specific leaf area). We also evaluated whether these patterns of trait covariation were consistent at different spatial scales and organisational levels.

*Methods* We collected fine root (< 2 mm) samples of 534 individuals (of 80 woody species) along a wide regional range of environmental conditions in southern Spain.

*Results* In general, strong correlations among most of the root morphological traits were found, supporting the existence of a 'root economics spectrum', as well as between root traits and the aboveground traits. However, root diameter was not completely aligned along this ecological axis, supporting the idea of a multidimensional spectrum of root traits. The main syndrome of root trait covariation was consistent at the different spatial scales and organisational levels. Soil nutrients and water availability were the main drivers of root trait variation.

*Conclusions* Our results indicate that root trait variation is primarily aligned along a leading dimension related to resource economics. However, the distinct pattern of root diameter may indicate a multidimensionality of belowground traits that needs to be explored in greater depth.

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**Keywords** Growth rate · LMA · Nutrients · Root diameter · Root tissue density · SLA · Specific root length · Water availability

## Abbreviations

| | |
|---|---|
| SRL | Specific root length (root length per unit of root dry mass) |
| SRA | Specific root area (root area per unit of root dry mass) |
| RDI | Root diameter |
| RDMC | Root dry matter content (root dry mass per unit of water-saturated fresh mass) |
| RTD | Root tissue mass density (root dry mass per root volume) |

## Introduction

Functional plant traits are commonly used as indicators of species ecological functions to better understand resource-use strategies and species distribution along the landscape (Violle et al. 2007; Reich 2014). In particular, belowground components (specifically root traits) play a critical role in plant and ecosystem functions, including resource storage (i.e. water and nutrients) and net primary productivity (Caldwell and Richards 1986; Eissenstat et al. 2000; Lambers et al. 2006). Roots are mainly involved in the uptake of nutrients and their transport to leaves, with root activity and morphology frequently being correlated with leaf traits associated with nutrient supply (Brouwer 1962; Lambers et al. 2006). Despite their recognised importance in plant resource-use strategies and functioning, studies considering root traits are still poorly represented in comparison with those including aboveground traits; thus, root trait data represent only 7% of the TRY database with respect to leaf trait data (Laliberté 2017). This under-representation of root traits is partly due to the difficulty in estimating and measuring them (Holdaway et al. 2011). Thus, more insights into plant–soil interactions and plant functional strategies will be gained from approaches that include the belowground fraction.

Variation in functional traits often represents different plant strategies or adaptations to a range of environmental conditions, at local (Pérez-Ramos et al. 2012; de la Riva et al. 2016a), regional (Liu et al. 2010; Chen et al. 2013) and global scales (Díaz et al. 2004; Wright et al. 2004; Díaz et al. 2016). A suite of correlated plant traits - such as low-density tissues, high surface/mass ratios, high metabolic capacity and high nutrient uptake rate - are commonly associated with more-efficient capture of resources and faster growth, whereas slow-growing species have the opposite traits (Lambers and Poorter 1992; Wright et al. 2004). The trade-off between resource conservation and fast growth classifies plants according to a major axis of specialisation, which can be described by a range of trait variation known as the "plant economics spectrum" (Freschet et al. 2010; Reich 2014; Díaz et al. 2016). Although previous studies have evaluated the extent to which this trade-off can be applied to other plant organs, such as stems (Chave et al. 2009) and roots (Prieto et al. 2015; Roumet et al. 2016), information on the belowground fraction is still limited and inconsistent (Eissenstat 1991; Ryser and Lambers 1995; Tjoelker et al. 2005; Barkaoui et al. 2016). Therefore, the "root economics spectrum" concept remains under debate (Chen et al. 2013; Kramer-Walter et al. 2016).

In spite of some studies demonstrated that root traits matched the parallel leaf traits along the broadly-known acquisition-conservation axis (e.g. Pérez-Ramos et al. 2012; de la Riva et al. 2016b), recent works suggested that root trait variation is not always one-dimensional and species might segregate along other belowground axes, different from the root economics spectrum (Laliberté 2017). Thus, the multidimensional character of belowground traits seems to be particularly relevant in woody species (Kong et al. 2014; Kramer-Walter et al. 2016), whose trait patterns clearly differ from those of herbaceous species (Tjoelker et al. 2005; Roumet et al. 2016). In fact, a recent meta-analysis supported that tree root traits do not follow the patterns expected from the root economics spectrum (Weemstra et al. 2016). This multidimensional segregation of species along different axes of plant specialisation could be associated with modifications in root tissues as a result of mutualistic associations, specific adaptive solutions to environmental constraints or even competitive interactions (Genney et al. 2002; Fort et al. 2017; Valverde-Barrantes et al. 2017). However, there is great controversy regarding the identity and ecological role of the root traits that represent these additional, potentially-independent dimensions of belowground functional variation (Bardgett et al. 2014; Weemstra et al. 2016).

The co-varying traits that define the above-mentioned axes of ecological specialisation can be affected by different environmental conditions and evolutionary constraints (Heberling and Fridley 2012; Cordlandwehr et al. 2013). As a consequence, different studies have observed that among-trait relationships are sometimes dependent on the spatial scale (Funk and Cornwell 2013; de la Riva et al. 2016b). At coarser scales (e.g. landscapes), trait covariation usually matches with global patterns (Funk and Cornwell 2013), likely as a result of environmental filters (i.e. climate and geology) and evolutionary constraints. In contrast, at finer scales (e.g. local gradients or micro-habitats), trait selection is more likely to operate through localised abiotic and biotic filters (e.g. resource availability and competition) (Mokany and Roxburgh 2010), and therefore the patterns of trait covariation are weaker. However, the influence of the spatial scale on among-trait relationships remains largely untested, particularly in the belowground fraction.

Root trait variation is primarily driven by soil resources, at both small and large spatial scales (Ostonen et al. 2007; Olmo et al. 2014; Kramer-Walter et al. 2016); however, which root traits are most strongly associated with resource limitation is still unclear (Laliberté 2017). For instance, in Mediterranean ecosystems water availability constitutes the main abiotic constraint that determines habitat suitability, and limits the range of functional traits of the community in accordance with the economics spectrum (Cornwell and Ackerly 2009; de la Riva et al. 2016a). However, other studies in grassland communities have revealed a greater number of functional adaptations to soil nutrient scarcity than to drought (Ordoñez et al. 2009; Pérez-Ramos et al. 2012). Therefore, trait-based studies including the belowground fraction are essential to disentangle the relative importance of different soil resources as drivers of the plant community assembly (Ostonen et al. 2017).

The main objective of the present study was to assess the coordination among root traits and their relations with soil resource availability, to better understand plant ecological strategies and species distribution in Mediterranean forests and shrublands. For these purposes, we quantified five morphological root traits commonly associated to the trade-off among maximizing resource acquisition (low root diameter and high specific root length/area) or increasing resource conservation (high root dry matter and density) (McCormack et al. 2012; de la Riva et al. 2016b; Fort et al. 2017; Valverde-Barrantes et al. 2017). Specifically, we collected fine roots of 534 woody plants (of 80 species) sampled in 18 different plant communities that were distributed along four zones and a broad regional range of environmental conditions in southern Spain. Following a trait-based framework, we tested four overarching hypotheses:

1. The existence of a root economics spectrum. Under Mediterranean conditions, where plant survival and growth are commonly limited by the scarcity of soil water and nutrients, we expected to find a strong level of correlation among root traits. Such a pattern of trait covariation in the belowground fraction would support the existence of a primary one-dimensional root economics spectrum across the resource-use gradient. In addition to the primary dimension of root economics spectrum, we tested the existence of other potentially independent axes of root trait variation and tried to explain their ecological roles.
2. Consistency in patterns of trait covariation at different spatial scales and organizational levels. On the one hand, we expected to find weaker correlations among root traits when considering local scales than when analysing at landscape or regional scales. On the other hand, since dominant species tend to have trait values that are better linked to their environments than those of rare species, relationships among functional root traits are expected to be stronger at the community than at the species level, particularly when they are weighted by the species abundance and not calculated on species presence/absence.
3. Root and leaf traits coordination. We expected to find a strong level of correlation among root traits with leaf traits (leaf nutrient concentration and specific leaf area). Thus, plants possessing roots traits associated with an acquisitive strategy (high specific root area, low root dry matter) will show leaf traits associated with this strategy (higher SLA and higher leaf nutrient concentrations).
4. Environmental filtering effects. Plant functional traits are selectively filtered by environmental conditions. We expected to find a large variation in the root traits across a wide regional range of soil resource availability. The influence of the soil environment on community root traits was expected to be stronger when considering values of species abundances in the calculations.

## Materials and methods

### Study area, species selection and sampling design

The present study was carried out in four zones which covered a large gradient of environmental conditions in southern Spain (SI-Table S1); from subhumid forests to arid shrublands, encompassing a wide range of mean annual precipitation (from 200 to 2000 mm year$^{-1}$). All the plant and soil measurements were carried out in late spring, during the peak of vegetative growth (May–June). In total, 18 sampling sites were selected: nine in the Sierra Morena mountains (local topographical gradient), three in Doñana National Park, three in Alcornocales Natural Park and three in Cabo de Gata Natural Park (SI-Table S1). To analyse whether the pattern of trait covariation found at the regional scale (i.e. covering the whole range of environmental conditions) was consistent at a local scale, the nine sites of the Sierra Morena mountains were distributed along a topographic gradient (from ridges to valley bottoms).

In each site, four 20-m-long transects were set up randomly, with a minimum distance of 10 m relative to one another and avoiding steep slopes. Woody species abundance and composition were determined by measuring the total cover intercepted by the four 20-m lines (total abundance can exceed 80 m due to the overlapping of vegetation layers). The mean relative abundance for each species and site was calculated as the mean value of the four transects. To select the plant species for trait measurements, we ranked them by their relative abundances and chose those comprising at least 92% of the total plant abundance (in each site).

## Trait measurements

*Root morphological traits* Roots were sampled at the individual level by excavating the first 20–30 cm of the soil depth near the plant basal stem. In the Sierra Morena mountains (local gradient), roots of four individuals per species and sampling site were taken; in the other three zones, four individuals per species and zone were sampled; in total, root traits were measured in 534 individuals from 80 different species (SI-Table S2). The fine roots (< 2 mm in diameter) were selected to measure the following belowground traits: specific root length (SRL, root length per unit of root dry mass), specific root area (SRA, root area per unit of root dry mass), root mean diameter (RDI), root dry matter content (RDMC, root dry mass per unit of water-saturated fresh mass) and root tissue mass density (RTD, root dry mass per root volume). Roots were scanned at 1200 dpi. The length, diameter, area and volume of the roots were obtained by analysing the scanned root samples with WinRHIZO 2009 (Regent Instruments Inc., Quebec, Canada). In the case of plants with rhizomes (like Ericaceae species) we were careful to include only fine roots. Unfortunately, we could not segregate our root samples between both absorptive and transport roots as recommended by McCormack et al. (2012), however the distribution of root length in four root diameter classes (0–0.5, 0.5–1, 1–1.5 and 1.5–2 mm) (SI-Table S2) showed that the percentage of fine roots <1 mm for the 534 observations represents the 94.3% of the total sample (only 5.6% was higher of 1 mm, and only the 1% was higher than 1.5 mm), which suggests that most of the sample belongs to absorptive roots. This database is available as part of the Fine-Root Ecology Database (FRED; http://roots.ornl.gov; Iversen et al. 2017) and Dryad Digital Repository (https://doi.org/10.5061/dryad.dr275).

*Leaf traits* Eight leaf traits have been measured: one morphological trait, SLA (specific leaf area, leaf area per unit leaf dry mass, $m^2\ kg^{-1}$) and seven chemical traits (N, C, P, K, S, Ca and Mg leaf concentrations). Leaves were selected from six individuals per species and sampling site. SLA was calculated as the ratio between the area of the scanned leaves and the dry mass (see methods in Pérez-Harguindeguy et al. 2013). We used here SLA as a surrogate of potential plant growth rate (Reich et al. 2010). SLA has been found to be strongly correlated with potential (maximum) relative growth rate (Poorter and Remkes 1990; Cornelissen et al. 1996; Antúnez et al. 2001; Ruíz-Robleto and Villar 2005). Leaf chemical composition was determined for a mixture of leaves, combining six different individuals per species and zone (six individuals per species and sampling site in the Sierra Morena zone). A total of 120 samples from 72 species were taken for SLA and nutrients. The N and C concentrations were measured using an elemental analyser (Eurovector EA 3000; EuroVector SpA, Milan, Italy), while P, K, S, Ca and Mg were extracted by wet oxidation with concentrated $HNO_3$, under pressure in a microwave digester, and analysed by ICP-OES. See de la Riva et al. (2016a) for methodological details.

## Characterization of the soil environment

The soil was characterised in each of the 18 sampling sites. Two soil samples of the top 20 cm of depth (where nutrient uptake mostly occurs; Jobbágy and Jackson 2001) were collected in each transect and further mixed to give one combined soil sample per transect, to be used for physico-chemical analysis. In the laboratory, the samples were air-dried, crushed and sieved; the fraction of less than 2 mm was analysed using standard soil methods (Sparks 1996). Soil organic matter was determined by the Walkley and Black method, and total N was determined by Kjeldahl digestion. Available P was estimated by the Olsen method; Ca, K and Mg were extracted with 1 M ammonium acetate and determined by atomic absorption spectrophotometry. To describe soil water availability, three variables were calculated (Hénin et al. 1969): (i) potential water availability

(PWA), which is the water potentially extractable by plants. The PWA was calculated for each site as [(pF 2.5 - pF 4.2) × bulk density × soil depth], where pF is the soil water pressure held at 0.1 atm (pF 2.5) or 16 atm (pF 4.2), measured with a Richards Pressure Membrane (Richards 1947). (ii) The number of days per year with a water deficit (WD). (iii) The number of days per year with excess water (WE) (see Appendix S1 for more details). Three of the sampling sites had permanent water availability due to their proximity to a river channel; therefore, in these communities we assumed that WD was nil and WE was 365 days. Mean data of the 9 soil variables for each site can be found at SI-Table S3.

## Data analyses

*Quantification of root trait dimensionality and consistency at different scales* The root traits measured or calculated are not independent. For example, there is a mathematical relationships between SRL and its components RTD (root mass density) and RDI, as SRL = [1/(RTD × RDI$^2$) × 4/π] (Ostonen et al. 2007; Olmo et al. 2014). However, this does not mean that when considering a set of root measurements we will obligatory find strong correlations between SRL and both RTD and RDI. This is not the case because the relationships between the three variables may profoundly change depending of many factors, such as the range of variation of RDI and RTD, and the relationships between them. The relationships among these three root traits is analogue to the variation in leaf mass per area (LMA, g m$^{-2}$) which is the product of leaf thickness (LT, μm) and leaf density (g cm$^{-3}$). Renton and Poorter (2011) discussed the fact that LMA can be related in different ways to its components LT and LD, depending on the intrinsic variation of them and the relationships between these traits.

We analysed the level of correlation among the five root morphological traits (SRL, SRA, RDI, RTD and RDMC) using a principal component analysis (PCA) with the 534 observations of woody plants (belonging to 80 species). The significance of these among-trait relationships was determined by linear mixed models (LMM), considering the species as the random factor. The 'nlme' package in R was used to perform the linear mixed effect model analyses (Pinheiro et al. 2015), and the correlation of these relationships (r) was obtained with the marginal R$^2$, which was calculated with the sem.model.fits function in the 'piecewiseSEM' package (Lefcheck 2015). In order to determine the influence of evolutionary history at the species level (mean values for 80 species), we performed pairwise correlations, including phylogenetically-independent contrasts (PIC) (Webb et al. 2008; Verdú and Pausas 2013). For the specifications related to the phylogenetic tree build, see SI-Appendix S2.

The pattern of trait covariation was also analysed at the community level (18 cases), with the trait values weighted by the relative abundance of their constitutive species (community weighted means – CWM). The CWM of the root traits were calculated for each site as $\sum P_i \times \text{Trait}_i$, where Pi is the relative abundance of the species "i" in this site and Trait$_i$ is the average trait value obtained for the species "i" in the same site (Garnier et al. 2004). We compared these results with the community unweighted means (UWM) of each trait, considering only the species presence. The significance of these among-root relationships at the community level was also determined by LMM, considering the zone as the random factor.

To assess if the patterns of root trait covariation were consistent within environments located at different positions of the regional range, we tested all the above-explained relationships between root traits within each zone, again using LMM. We also tested the trait relationships at three spatial scales: regional gradient (using the total pool of 534 individuals), local gradient (species from the 9 sites on a topographical gradient of Sierra Morena), and the single zone level (Alcornocales Natural Park, Doñana National Park and Cabo de Gata Natural Park).

We analysed the level of correlation between each of the five root morphological traits and the two groups of leaf traits (leaf nutrient concentration and specific leaf area) using LMM. A general PCA was performed previously with the whole set of leaf nutrient traits (120 observations). The first three PCA axes (PCA scores) of leaf nutrients were used as dependent variables, the five root traits as continuous fixed factors and species as the random factor. The same analyses were carried out to test the relationships between the five root morphological traits and the SLA (a surrogate of potential growth rate; Reich et al. 2010). In addition, to ensure that these results were not affected by phylogenetic constraints, we also determined these relationships with PIC.

*Environmental filtering effects.-* To assess which abiotic factors were associated most closely with root trait variation at the community level, we used LMM with CWM and UWM as dependent variables, soil characteristics as independent factors and zone as the random factor. To reduce the number of variables characterising the soil environment and their colinearity, a PCA was performed with the nine soil variables measured in this study: soil nitrogen (N) and organic matter (OM), availability of phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca), potential soil water availability (PWA), and number of days with a soil water deficit (WD) or with excess soil water (WE). When necessary, variables were previously standardised and log-transformed to fulfil assumptions of normality (based on Kolmogorov-Smirnov) and homoscedasticity. All these analyses were conducted in the R 2.10.0 statistical platform (R Development Core Team 2011) using the packages ‘ape’ (Paradis et al. 2004), ‘car’ ‘FactoMiner’ (Lê et al. 2008), ‘phytools’ (Revell 2012), ‘smatr’ (Warton et al. 2012), ‘nlme’ (Pinheiro et al. 2015) and ‘piecewiseSEM’ (Lefcheck 2015).

## Results

### Dimensionality of root trait variation

There were strong correlations among the five root morphological traits (SRL, SRA, RDI, RTD and RDMC), as indicated by the principal component analysis (PCA) (Fig. 1 and Table 1). Considering the total pool of data, specific root area (SRA) and length (SRL) were positively and significantly correlated ($r = 0.87$; $P < 0.001$), as were root tissue density (RTD) and root dry matter content (RDMC) ($r = 0.81$; $P < 0.001$; Fig. 2). In turn, SRA was negatively correlated with RDMC, RTD and RDI. Interestingly, root diameter (RDI) showed a rather-independent trend (Fig. 1), having negative correlations with SRA, SRL and RTD (Fig. 2, Table 1) but no significant relationship with RDMC (Table 1).

### Consistency of trait covariation patterns at different spatial and organisational levels

In general, the patterns of trait covariation detected along the regional gradient were consistent at the local scale (topographical gradient), as well as when considering only those species present in a single zone (randomly sampled, Fig. 2 and SI-Table S4).

Regarding the potential influence of the organisational level, the above-explained trends in trait covariation detected in the cross-species analyses (even after considering phylogeny; SI-Table S5) were similar to those obtained at the community level (Table 1). The only exception was the correlation between RTD and

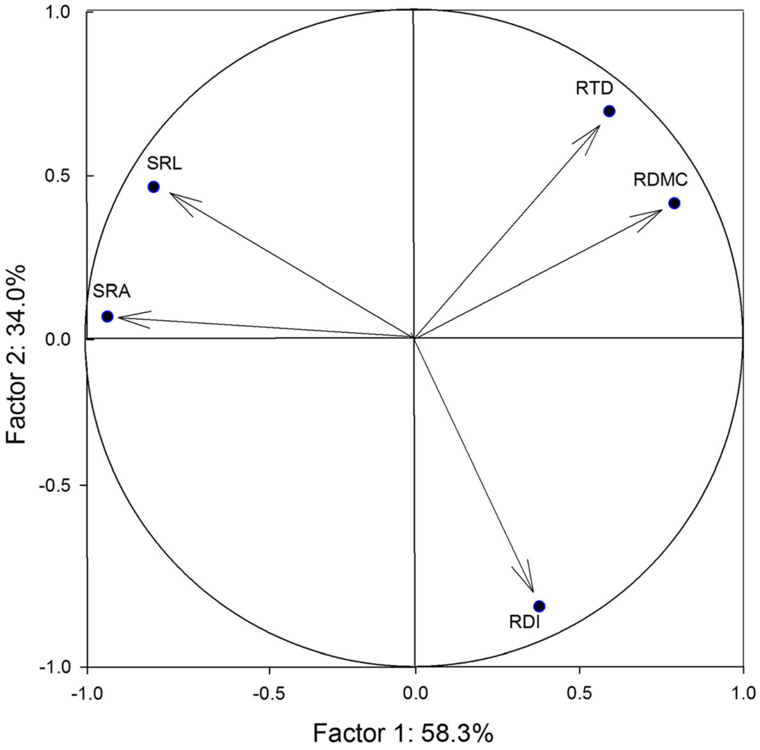

**Fig. 1** Projection of the five measured root traits (n = 534 individuals) on the plane defined by the two main axes resulting from the Principal Component Analysis (PCA). RDI, root diameter; RDMC, root dry matter content; RTD, root tissue density; SRA, specific root area; SRL, specific root length

**Table 1** Correlation coefficients for links among the five root morphological traits considered in this study at different organizational levels: individual (using the total pool of 534 plants), species ($n = 80$), and community (taking into account species abundances - community weighted means, CWM- or considering only values of presence/absence -community unweighted means, UWM)

| Root traits | | Individual level | Species level | Community level | |
|---|---|---|---|---|---|
| | | | | UWM | CWM |
| | | $n = 534$ | $n = 80$ | $n = 18$ | $n = 18$ |
| RDMC | SRL | −0.36*** | −0.47* | −0.69*** | −0.83*** |
| | SRA | −0.64*** | −0.82*** | −0.92*** | −0.85*** |
| | RTD | 0.81*** | 0.84*** | 0.87*** | 0.84*** |
| | RDI | −0.05 | −0.05 | 0.31 | 0.45 |
| SRL | SRA | 0.87*** | 0.84*** | 0.89*** | 0.82*** |
| | RTD | −0.14*** | −0.18 | −0.48* | −0.56* |
| | RDI | −0.86*** | −0.68*** | −0.76*** | −0.69* |
| SRA | RTD | −0.57*** | −0.64*** | −0.80*** | −0.74*** |
| | RDI | −0.58*** | −0.38** | −0.50*** | −0.50* |
| RTD | RDI | −0.33*** | −0.30** | 0.03 | 0.10 |

The significance level of these among-trait relationships was determined using linear mixed models, with "species" and "zone" as random factors for the individual, species and community-level analyses, respectively. See Methods for abbreviation of root traits

The significance level is as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

RDI, which was significant at the individual and species level but not at the community level.

Contrary to what was expected, the correlation coefficients for community unweighted means (UWM, where only species presence was considered) were not lower than for community weighted means (CWM, when species abundances were taken into account).

## Root and leaf traits coordination

The results of the PCA for the leaf nutrient traits are shown in SI-Table S6. The first PCA axis accounted for 52% of the overall variation and was related with the concentration of most of the leaf nutrients. In one extreme (positive values) there were leaf samples with high values of Ca, K, Mg, P, S and N; at the opposite extreme (negative values) there were leaf samples with higher values of C and lower values of the other nutrients. The second axis (14% of the variance) was associated with the leaf P and Ca concentration. The relationships between some root traits, such as SRA, RTD and RDMC, and the first PCA axis of leaf nutrients showed significant results (LMM $P < 0.001$); however, no significant relationships were found for SRL and RDI (SI-Table S7). The specific root area (SRA) showed a positive relationship with leaf nutrient concentration (negative in the case of leaf C) and with SLA (Fig. 3). In contrast, higher values of RDMC and RTD were associated with lower leaf nutrient concentrations (but higher C) and lower SLA (Fig. 3, SI-Table S7). RDI was negatively associated to the second PCA axis of leaf nutrients (SI-Table S7), which was due mainly to the negative relationship of RDI with leaf Ca concentration (data not shown). All the significant relationships observed at the species level were consistent after considering phylogenetically-independent contrasts (PIC) (data not shown).

## Environmental filtering effects

The PCA of soil environmental factors showed a first main axis (38.4% of the variance) associated with soil water availability (PWA and WE) and fertility (concentration of N, P and organic matter), and a second axis of soil variation (31.7% of the variance) defined by the soil concentrations of K and Ca (SI-Fig. S1A). The 18 sites and the four sampling zones were non-randomly distributed along the two soil gradients (SI-Fig. S1B).

Most of the root traits were aligned with the first soil PCA axis of water and nutrients; for example habitats with higher water and nutrient availability had woody communities with low RDMC and high SRA (Fig. 4). All root traits (except RDI) were significantly related to soil resource availability at the community level (for

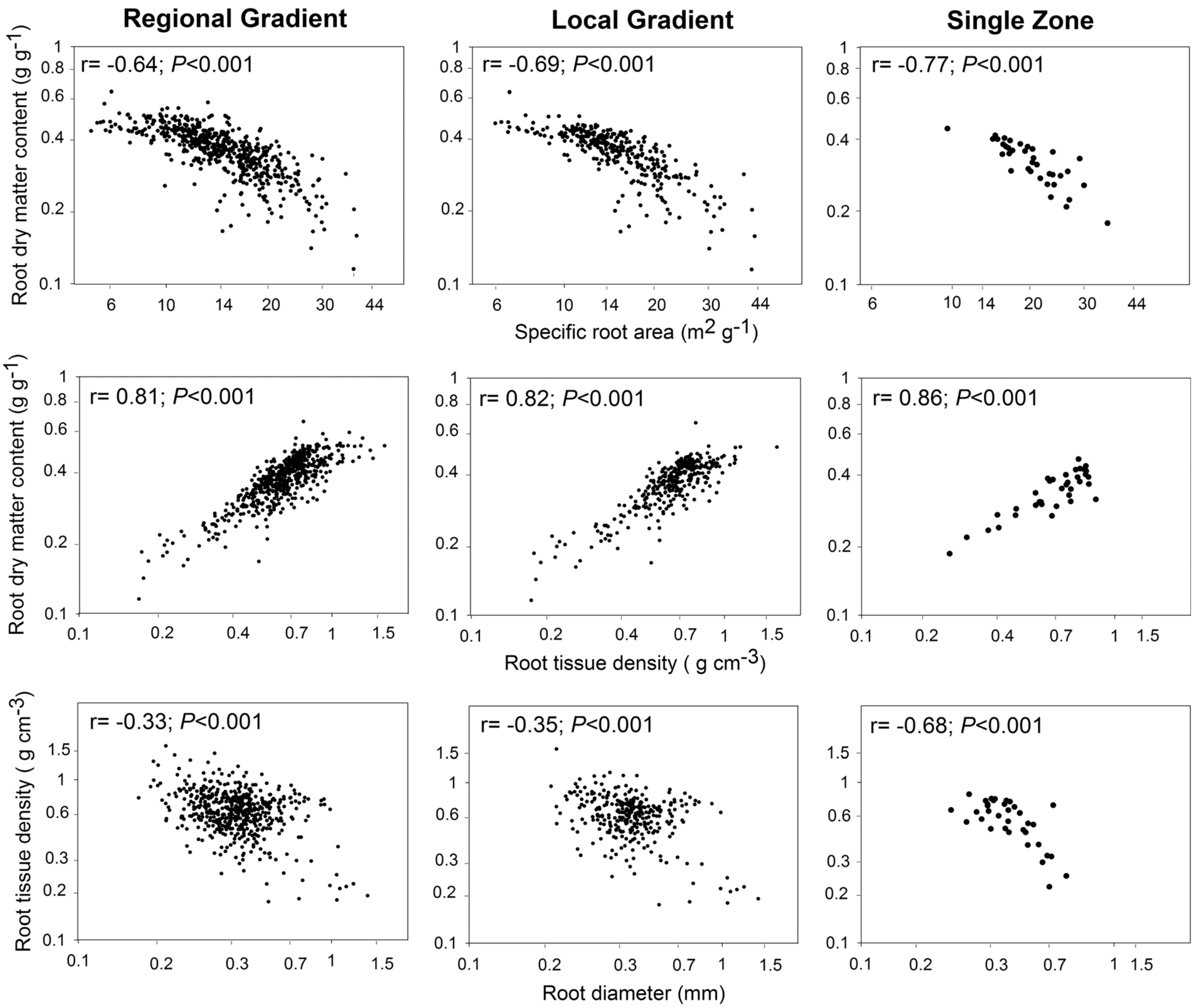

**Fig. 2** Relationships between the different root morphological traits considered in this study, at three different spatial scales: Regional gradient (using the total pool of 534 individuals), local gradient (topographical gradient in Sierra Morena mountains) and a single zone (Los Alcornocales Natural Park)

both CWM and UWM values) (SI-Table S8). Similarly to that found for traits covariation (see above), the correlation coefficients for community unweighted means (UWM) were not lower than those for community weighted means (CWM).

## Discussion

### Dimensionality of root trait variation

Our results confirm that root morphological traits are largely intercorrelated in Mediterranean woody plants, indicating the existence of a belowground trade-off between resource acquisition and conservation. This syndrome was also consistent when phylogenetic relatedness was considered. Therefore, it supports the existence of a "root economics spectrum" (RES), analogous to the "leaf economics spectrum" (Wright et al. 2004), for the set of 80 woody species sampled along a regional aridity gradient in SW Spain. Thus, plants with denser roots had also roots with higher dry matter content and lower specific root area, a syndrome commonly associated with a resource-conservation strategy (e.g. Pérez-Ramos et al. 2012; de la Riva et al. 2016b), whereas the other extreme of specialization corresponded to a resource-acquisitive strategy, with the opposite traits (lower values of root density and dry matter content,

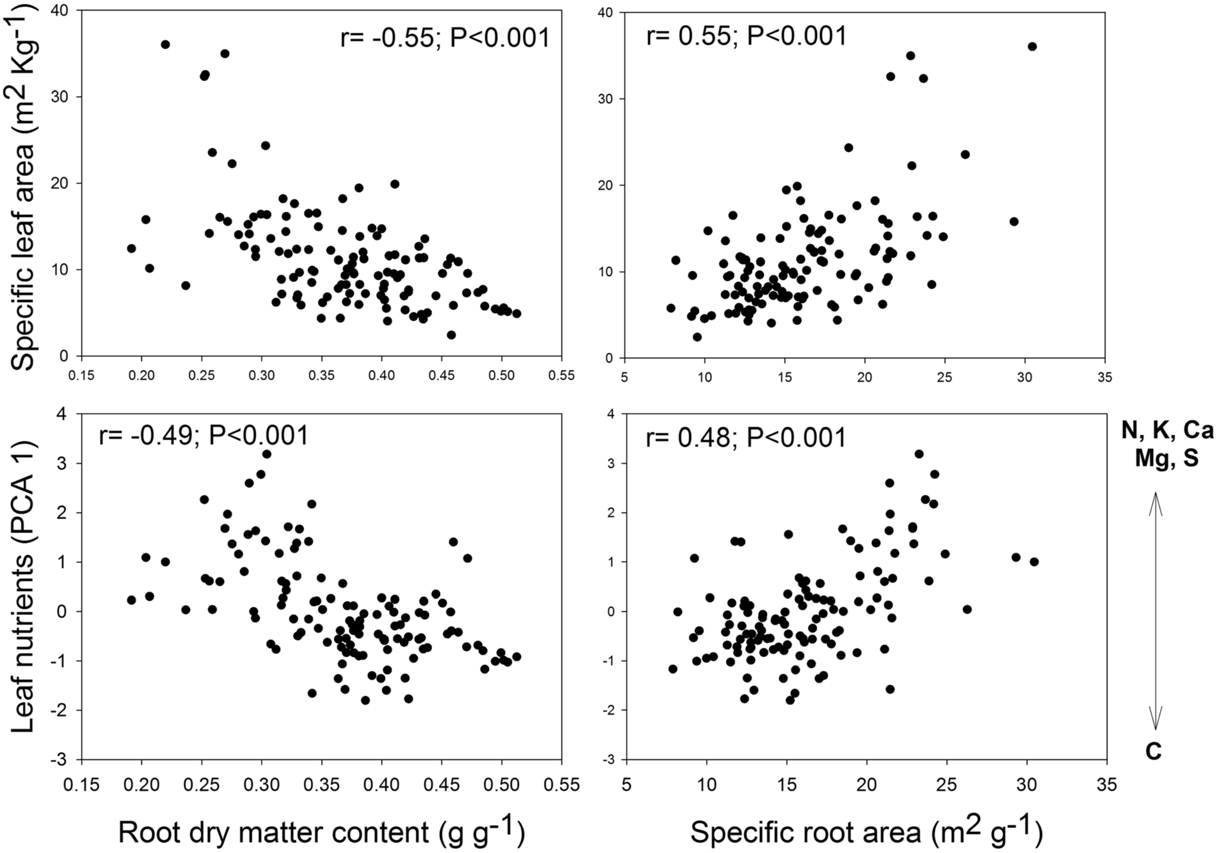

**Fig. 3** Relationships between two key root traits (root dry matter content and specific root area) and the two aboveground variables quantified in this study (first PCA axis from leaf nutrient concentration and specific leaf area, SLA). For better clarity the inverse of the PCA 1 scores have been represented; thus leaves with higher nutrient content have higher, positive scores

and high specific root area). However, our results support only part of the expected RES. Indeed, the RES cannot be restricted to five morphological traits, but should include chemical traits (for example: N and C concentrations), and physiological traits as root respiration and nutrient uptake (Weemstra et al. 2016).

We found that root diameter (RDI) seemed to follow a different pattern compared with the other root morphological traits, being not so clearly aligned along the acquisition-conservation axis. Thus, root diameter was not related significantly to the aboveground traits considered in this study or to the environmental gradient of soil resources (see below). These results suggest that root diameter variation could respond to other independent and ecologically-important dimensions differing from that represented by the trade-off between resource acquisition and conservation (Laliberté 2017). The multidimensional character of the belowground fraction could be due to the multiple pivotal roles that roots play in plant functioning (Kramer-Walter et al. 2016; Weemstra et al. 2016). Structural or chemical soil properties not directly related to soil resources could drive the variability of some specific root traits. For example, the possession of thicker roots could be an efficient strategy to penetrate denser soils with a higher level of compaction (Bejarano et al. 2010; Alameda and Villar 2012). Lower root diameter optimised the acquisition of nitrate by an enhanced SRL, while thicker roots may facilitate P mobilization via mutualistic adaptations (Kong et al. 2014; Weemstra et al. 2016). Thus, a larger root diameter could stimulate mycorrhizal symbiosis (e.g. Comas et al. 2002), with thicker roots being commonly associated with high soil resource acquisition via fungal extraradical hyphae (Smith and Read 2008; Valverde-Barrantes et al. 2017). We found that low RDI was associated to high leaf Ca concentrations and high SLA. It is generally accepted that plants showing calcium deficiency is related to low or non-transpiring tissues, as Ca is not transported in the phloem. Gilliham et al. (2011) showed that tissue Ca supply is often found

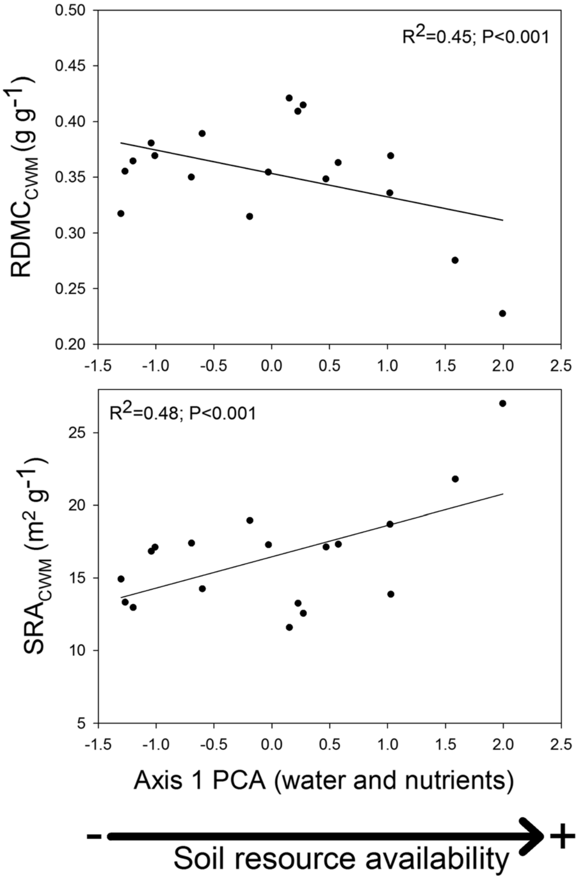

**Fig. 4** Relationships between two root community traits (CWM), specific root area (SRA) and root dry matter content (RMDC) and the first PCA axis that represent soil resource availability. The marginal $R^2$ and *P* values of the linear mixed model are also shown

to be tightly linked to transpiration, which results in highly transpiring organs amassing large concentrations of Ca. Therefore, a low RDI could be related to high capacity to acquire water. The potential existence of multiple resource-uptake strategies in the studied plant communities might explain why some root traits such as RDI were not aligned along the primary acquisition-conservation axis, and suggests that species segregate along multidimensional axes of belowground variation.

### Consistency of trait covariation patterns at different spatial scales and organizational levels

The root functional syndrome shown by the covariation of SRA, SRL, RDMC and RTD for the total species pool was consistent at the different spatial scales considered in this study, suggesting that similar constraints were operating on root construction by woody species across different environments. Thus, these correlations among root traits found at the regional scale were also significant across smaller spatial scales (local gradient and single zone), in contrast to what we had previously observed among plant organs (leaf and stem; de la Riva et al. 2016b, 2017). The close correlation between root morphological traits at different spatial scales suggests that these attributes in Mediterranean woody plants are limited by biophysical constraints, inherent to the compromise between function and structure (Shipley et al. 2006). For instance, it does not seem possible to increase the specific root area at the expense of making roots of higher dry matter content or density.

The patterns of root trait covariation found at the species level were consistent when scaling up to the community level, indicating that similar constraints operate at both levels of organization, and supporting the existence of a “community root economics spectrum” (Prieto et al. 2015). These root traits covary among communities as a result of interspecific or intraspecific variation, or even a combination of both (Leps et al. 2011; Pérez-Ramos et al. 2012).

Contrary to what was expected, the correlations among root traits found at the community level were not stronger when considering information on species abundances rather than merely species presence (Table 1). Also, the community-level root traits (CWM) were not better correlated with soil resource availability than the means traits considering only species presence (UWM, SI-Table S8). Therefore, these results do not support the idea that dominant species have root trait values that are better linked with their environments than those of less-frequent species, as shown in previous studies focused on leaf traits (Cingolani et al. 2007; Domínguez et al. 2012). In the woody communities studied, a common environmental constraint seems to operate among coexisting species, acting as an initial abiotic filter that promotes a particular root morphology syndrome locally adjusted to those environmental limitations, and that this filter excludes from the community those species with unfit trait combinations (Grime 2006).

### Root and leaf traits coordination

Our results provided solid evidence that root morphology is strongly linked to the aboveground traits considered in this study, with plants of higher SLA and higher leaf nutrient concentrations possessing roots associated with an acquisitive strategy. Similar results were found by

Valverde-Barrantes et al. (2017). A high SLA and N concentration are related to high photosynthetic rates and high RGR (Lambers and Poorter 1992; Antúnez et al. 2001; Wright et al. 2004; Reich 2014). This pattern might be explained by the fact that acquisitive leaves with high photosynthetic rates and higher resource requirements need exploitative fine roots (i.e. with high area/mass ratio and low tissue density) to ensure sufficient water and nutrient supply to achieve fast plant growth (Eissenstat 2002; Fort et al. 2013; Reich 2014). In contrast, nutrient conservation can be achieved by increasing root dry matter content and root tissue mass density - which favours tissue longevity and slows down plant growth rates, resulting in lower relative nutrient requirements and slower amortization of construction costs (Ryser 1996; Poorter and Villar 1997; Villar et al. 2006).

### Environmental filtering effects

We have found a non-random variation of functional root traits in woody plant communities over the explored environmental gradient, providing interesting insights into the effects of soil resource availability as a main driver of plant community assembly in Mediterranean environments. Thus, community-level root traits were mainly driven by soil nutrients and water availability, in line with the results found in other studies in Mediterranean environments (e.g. Pekin et al. 2011; Pérez-Ramos et al. 2012; de la Riva et al. 2016a; Kramer-Walter et al. 2016; Navarro-Fernández et al. 2016). Plant communities growing in more-productive environments (i.e. with higher soil nitrogen, organic matter and water availability) were dominated by species having root traits associated with rapid resource acquisition (i.e. high values of SRA and, to a lesser extent, high SRL and low RTD). These functional traits might facilitate a faster growth rate and allow species to better compete for soil nutrients and water (Eissenstat 2002; Kramer-Walter et al. 2016), which usually have been identified as important limiting factors in Mediterranean ecosystems (Terradas 2001; Cornwell and Ackerly 2009; Pérez-Ramos et al. 2012). Contrastingly, plant communities inhabiting dry and nutrient-poor habitats showed root traits related to a soil scarcity-tolerance strategy, such as higher tissue density and dry matter content (Ryser 1996; Holdaway et al. 2011). However, a particular set of root traits can be advantageous under contrasted environmental conditions (Díaz et al. 1998; Cornwell and Ackerly 2009). For example, woody plants might increase their root length in productive environments, to be competitive, but also under soil resource scarcity, thus allowing a greater soil volume to be explored per unit of C invested (Olmo et al. 2014; Laliberté et al. 2015).

In summary, soil resource scarcity could act as an abiotic filter, excluding fast-growing species which are usually less tolerant of these highly-stressing conditions, while more-conservative species would be reduced or even excluded from more-productive sites, probably by strong competition for space and light (de la Riva et al. 2016a, b).

## Conclusions

The high level of covariation reported in this study among root morphological traits as well as between them and several key aboveground traits: leaf nutrient concentration and SLA (a surrogate of potential plant growth rate), indicates that root trait variation is primarily aligned along a leading dimension related to resource economics. In fact, root trait variation was strongly driven by soil resource heterogeneity, according to the acquisition-conservation trade-off. However, the apparent distinct role of the root diameter suggests that belowground traits are not exclusively adjusted to a single axis of ecological variation, likely due to the existence of multiple resource-uptake strategies in the studied plant communities. More studies - including other root traits related to morphology (e.g. rooting depth), architecture (e.g. branching intensity), physiology (root respiration) and other biotic attributes associated with the root system (e.g. mycorrhizal colonisation) - are necessary to discern the potential multidimensional character of the belowground fraction in Mediterranean woody plant species.

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