Root economics spectrum and construction costs in Mediterranean woody plants: The role of symbiotic associations and the environment

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Abstract
1. Many studies have quantified the functional variation of fine root traits to understand the overarching trade-off between maximizing resource acquisition or conservation (root economics spectrum [RES]). However, we know remarkably less on how plant strategies along the RES are actually constrained by the amount of photosynthates required to construct roots (i.e. construction costs, CC) or how below-ground interactions with symbiotic organisms modify root trait patterns and their relationships with CC.

2. Our main aim was to quantify CC of fine roots (<2 mm) and their underlying components (carbon, minerals and organic nitrogen concentrations) in 60 Mediterranean woody species with contrasting symbiotic association types (ectomycorrhizas, arbuscular and ericoid mycorrhizas and N-fixing bacteria). We examined (a) whether the covariation among fine root traits along the RES was related to the intrinsic cost of producing roots and whether this relationship was dependent on the type of root symbiosis; (b) whether the relationship of each CC component with the RES was dependent on the type of root symbiosis and (c) whether soil water and nutrient availability determined differences in CC across sites.

3. According to the RES hypothesis, fine root traits showed a main covariation trend (acquisition vs. conservation), defined by the first PCA axis, which also segregated species by their two main contrasting symbiotic types (arbuscular and ectomycorrhizal). We found a positive relationship between root CC and the RES (i.e. PCA axis 1) and, interestingly, slopes differed among symbiotic types, in response to the different role of each specific CC component. In addition, independently of symbiotic type, root CC decreased linearly with soil nutrient availability and quadratically with plant water availability.

4. Synthesis. Our study demonstrates that woody plants display different functional strategies in their root CC, related with their position on the RES, and that CC differ among symbiotic groups. The influence of the root CC components across species varied among symbiotic associations, pointing to a trade-off between...
The functional variation of fine root traits and their interactions with symbiotic organisms is an uprising research topic to better understand the large diversity of plant below-ground uptake strategies across the world (Bergmann et al., 2020; Kong et al., 2019; Shi et al., 2020). It is well known that leaf traits covary across plant species following an overarching trade-off between maximizing resource acquisition and productivity or maximizing resource conservation at a productivity cost, the so-called leaf economics spectrum (LES; Wright et al., 2004). In analogy, seeking trait patterns related with a resource uptake trade-off on fine roots (<2 mm diameter), several studies have suggested that the economics spectrum theory (Reich, 2014) can also be applied to roots, that is the root economics spectrum (RES; de la Riva, Marañón, et al., 2018; Marañón et al., 2020; Prieto et al., 2015; Roumet et al., 2016).

The ‘economic spectrum theory’ is based on the main idea that tissue construction is constrained by a fundamental trade-off between fast growth rate (or resource uptake) and long life span (or resource conservation), which is well represented along environmental resource gradients for leaves (Wright et al., 2004, 2005). Assuming a functional similarity between leaves and roots, it would be expected that plant species growing in more productive environments have thinner roots with lower tissue density as well as higher specific root length (SRL) or specific root area (SRA) along with shorter life span, favouring strategies with a faster return of investments. By contrast, in environments with lower resource availability, opposite traits would be expected, that is denser roots with a higher root dry matter content (RDMC) and lower SRL, resulting in roots with longer life span and slower return of investment (de la Riva, Marañón, et al., 2018; Roumet et al., 2016). As for leaves (Reich, 2014), the currency of root economics is the carbon required to construct fine roots that explore the soil for resource acquisition. For example, SRA (i.e. the root area per unit dry biomass) reflects the available area for root uptake at a given biomass cost. Thus, root construction costs (CC) are defined as the total amount of metabolic compounds required to construct the chemical constituents needed to build one gram of root biomass (Villar et al., 2006). This cost of producing roots includes both the chemical compounds consumed in the biosynthesis of the tissue constituents (i.e. respiration processes that supply reductant and ATP for energy-requiring processes), as well as structural resources (e.g. carbon, and organic and inorganic compounds) required to build the root (Penning de Vries et al., 1974; Poorter & Villar, 1997).

Nonetheless, inferences of morphological root traits as proxies of resource economics strategies based on the costs of building roots remain rather speculative, mainly because most of the assumptions regarding a relationship between root traits and resource economics have been based on trait correlations and/or their covariation along resource gradients. Thus, the actual cost and energy involved in developing the structure and function of these fine roots have generally been overlooked. Moreover, although root construction costs are frequently mentioned in the literature as a factor explaining the variation in root trait values along species and resource gradients, few studies have actually addressed this question experimentally (e.g. Martínez et al., 2002; Roumet et al., 2016; Villar et al., 2006).

One key strategy to enhance plant nutrient acquisition is the association of roots with symbiotic micro-organisms (Wang et al., 2011). These symbiotic associations imply root morphological changes (i.e. changes in root trait values) that could potentially decouple the relationships among root traits commonly defined by resource economics (e.g. Bergmann et al., 2020; McCormack et al., 2017; Weemstra et al., 2016). In this regard, Bergmann et al., (2020) have recently proposed two orthogonal gradients of root specialization, which reflect a main plane of variation that the authors called ‘root economics space’. One represents the classical trade-off between fast and slow resource strategy determined by the variation of root nitrogen and root tissue density. However, the authors argue that the main dimension of trait variation is the ‘collaboration’ gradient, ranging from roots with traits associated with a fast resource acquisition for an efficient soil exploration (e.g. high SRL) and short life span, that is, the ‘do-it-yourself’ strategy, to roots with a higher inversion of carbon to outsource resource uptake via symbiotic associations that enhance nutrient acquisition. As the authors highlight, such an ‘outsourcing’ strategy has morphological consequences for the roots. For example, some plant species develop thick roots (i.e. high diameter) with a thick cortex, with higher dependence on mycorrhizal fungi for a more efficient resource acquisition. Other species from the Fabaceae family, known to have leaf traits associated with fast-growing strategies (Powers & Tiffin, 2010), develop root nodules with rhizobial N₂-fixing bacteria, enhancing the resource uptake needed to compensate for the fast rates of respiration required to rapid root growth (Li et al., 2016; Shane et al., 2006). These types of symbioses may allow plants with thick, light roots to have an acquisitive strategy despite growing in resource-limiting environments because symbionts increase the soil exploration capacity and enable them to have a faster acquisition of nutrients and water (Kramer-Walter & Laughlin, 2017). These types of strategies allow roots to increase their resource uptake with low values of tissue density, enhancing a faster pay back of the construction cost when environmental conditions...
become favourable; for example, in Mediterranean environments, the development of proteoid roots has been observed to fluctuate seasonally in relation to nutrient availability (Shane et al., 2006). However, there is not unanimous agreement about the mechanisms underlying the costs of these root modifications, and thus, little progress has been made in understanding the underlying role of the symbiotic associations in the root morphological remodelling in relation to resource-use strategies (McCormack et al., 2017).

The main aim of the present study is to quantify root CC and their underlying components (concentration of carbon, minerals and organic nitrogen) in 60 Mediterranean woody species with contrasting symbiotic association types. These species are a subset from a previous study with woody Mediterranean species, where we observed that root morphological traits covaried along an unidimensional main trend of variation (axis 1 in a principal component analysis, PCA) which we interpreted as the RES gradient (de la Riva, Marañón, et al., 2018). Based on current knowledge, we expect that the roots of the acquisitive species differ from those of the conservative ones in their structural characteristics because low resource availability would lead to greater investment in costly structural compounds (Martínez et al., 2002; Villar et al., 2006). Thus, the energetic costs of tissue construction of roots are expected to be higher in species from unproductive habitats than in those inhabiting more favourable environments, showing strong covariation between the RES and the CC. However, in such an approach, the main difficulties for interpretation arise from the potentially large impacts of mycorrhizal fungi—containing rhizobia, that is, bacteria capable to fix atmospheric N—and mycorrhizal species (ErM), defined by the presence of an intracellular surface mycelia colonization with arbuscules and vesicles; ericoid mycorrhizal species (EmM), defined by the presence of an intracellular surface mycelia colonization with interwoven coils (Brundrett & Tedersoo, 2018); and N-fixing species (N-Fix), which develop nodules containing rhizobia, that is, bacteria capable to fix atmospheric N (Li et al., 2016). Despite N-fixing species have also AM symbiosis, they were considered here as a separate category due to their singular nutritional strategy and root economy (Andersen et al., 2017).

We assigned a mycorrhizal category to each species based on direct observations (Andersen et al., 2020): (a) almost all plant species have one type of dominant mycorrhizal association and (b) the mycorrhizal association type is usually well conserved within a monophyletic genus and often within a family. Therefore, these species were assigned with the mycorrhizal association type of the closest phylogenetically related species.

2.2 | Root trait measurements

Fine roots (<2 mm in diameter) were sampled in four individuals per species and site by excavating the top 20–30 cm of soil close to the plant basal stem. Excavated roots were washed using a 0.5-mm sieve, weighed to determine their fresh mass (FM, g) and scanned at 1,200 dpi. Roots were then dried at 60°C during at least 48 hr, and weighed to obtain their dry mass (DM, g). Root length (L, cm), diameter (Rdi, mm), area (A, cm²) and volume (V, cm³) were obtained by analysing the scanned root samples with specific software (WinRHIZO 2009; Regent Instruments Inc.). We selected a subset of morphological traits directly involved in the acquisition,
processing and conservation of resources, which have been considered 'economic traits' from a resource analysis perspective in previous studies (Bergmann et al., 2020; Kramer-Walter et al., 2016; Roumet et al., 2016). Specific root length (SRL, m/g) and specific root area (SRA, m²/kg) were determined as (L/100)/DM and (A/10,000)/ (DM/1000), respectively. Root dry matter content (RDMC, g/g) and root tissue mass density (RTD, g/cm³) were determined as DM/FM and DM/V, respectively. The five root traits—SRL, SRA, RDMC, RTD and Rdi—were selected for statistical analysis (see below).

All root trait measurements were carried out according to the criteria and methodology defined by Pérez-Harguindeguy et al., (2013). For a more detailed protocol of root sample harvesting and trait measurements, see de la Riva, Pérez-Ramos, et al., (2016). Data are available from the Dryad Digital Repository (de la Riva, 2021).

2.3 Construction cost measurements

The root construction cost of each species was determined in a mixture of roots from four individuals. For each species in each site, we measured the concentration of C, minerals and organic N, following the method of Vertregt and Penning de Vries (1987) as modified by Poorter (1994). An exact description of the procedures followed for the chemical analyses and the subsequent calculations is given in Appendix S3 (see Poorter & Villar, 1997 for further details).

2.4 Soil physical and chemical properties

The soil was characterized in each of the seven sampling sites. We collected four soil samples in each site from the top 20 cm, where nutrient uptake mostly occurs (Jobbágy & Jackson, 2001), using a soil auger. In the laboratory, soil samples were air-dried, crushed and sieved at 2 mm, and soil organic matter and nutrients (P, N, Mg, K and Ca) were determined 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 while available Ca, K and Mg were extracted with 1 M ammonium acetate and determined by atomic absorption spectrophotometry. To characterize the soil water availability, we calculated the potential soil water availability of each site (PWA), which represents the soil water storage available for plants. It is based on the soil water retention characteristics and is calculated for each sampling site using the soil structural parameters and the potential evapotranspiration per day (see de la Riva, Marañon, et al., 2018 for more details on the calculation and variables used).

2.5 Data analyses

At a preliminary step, to obtain an overview of the dimensional variation of root traits, a principal component analysis (PCA) was performed with the five root traits for the 73 observations (mean values per species and site). As a working hypothesis, we assumed that the main variation trend (PCA axis 1) could represent the root economics spectrum (RES). In fact, root traits were largely intercorrelated (Appendix S4) and all had high absolute loadings in the first axis (PC1, 62.97% of the total variation explained), thus supporting the existence of a below-ground trade-off between resource acquisition and conservation (see de la Riva, Marañon, et al., 2018). Therefore, we used the scores for the PCA axis 1 as a proxy for the RES in further analyses. To assess the segregation of plant species by their symbiotic association type (EcM, AM, ErM and N-Fix) along the main axes of root trait variation (PC1 and PC2), we performed a one-way ANOVA with the PCA scores of the first and second components as the dependent variables and the symbiotic association type as the factor. Post-hoc Tukey tests were performed to check the significance of pairwise differences between symbiotic association types.

Regarding the root construction costs (CC), we tested differences in CC and their components (C: carbon, Min: minerals and OrgN: organic N concentration) among symbiotic association types with a one-way ANOVA. Generalized linear models (GLM) were used to test the relationships between root CC and their components with the RES (i.e. PC1 scores), as well as between root CC and individual morphological root traits. We included the symbiotic association type in interaction with CC into the models to test for differences between the slopes of the relationships across symbiotic types using analyses of covariance ANCOVAs (Kurokawa & Nakashizuka, 2008). Since differences in the slopes between CC and the RES were significant among symbiotic association types, we tested the influence of each individual component of the construction cost (C, Min and OrgN) on the RES within each symbiotic type using multiple regression analyses (except within ErM for which we only had four observations). For this purpose, we conducted a decomposition analysis based on the Sum of Squares (SS) of each component of root CC. The SS can be decomposed into the amount of variability explained by individual terms of the model (SSC, SSMin and SSOrgN) and by the unexplained variability (SSError) given SS = SS + SSMin + SSOrgN + SSError. For example, the percentage of explanation of the root carbon concentration (C) on the variation of root construction cost would be given by (SSC/SSS) × 100. To obtain the variability explained by each component independently of each other and to discard the effect of the covariation (the variance explained by two components) from the analysis, we used the type III sum of squares.

We also used generalized linear models (GLM) to assess the relationship between soil physical and chemical properties, and root construction costs. First, to reduce the number of variables characterizing the resource environmental gradient and their collinearity, a PCA was performed with the soil abiotic variables measured: soil nitrogen (N) and organic matter (OM), availability of nutrients (P, Mg, K and Ca), and potential water availability (PWA; Appendix S5). Second, we conducted a linear regression with quadratic components \( Y = \beta_0 + \beta_1 X_1 + \beta_2 X_1^2 + \varepsilon \), with the CC as the dependent variable and the scores of the first axis of the abiotic PCA representing the gradient of nutrient and water availability as the independent factor.
To control for potential phylogenetic effects on root construction costs and root trait covariation, all the relationships between morphological traits and construction costs were compared with the results obtained by fitting a phylogenetic generalized least squares model (pgls). To this end, we used the pgls function of the Caper package (Orme, 2013), which addresses phylogenetic non-independence among species by incorporating the covariance between taxa into the calculation of the estimated coefficients. The phylogenetic tree (Appendix S6) was obtained with the comprehensive Angiosperm species-level phylogeny from Zanne et al., (2014), as updated by Qian and Yin (2016), which is included into the R package 'S. PhyloMaker' (Qian & Yin, 2016). The distance of the few species that were not found in the PhytoPhylo database were supplanted by the distance of the closest species of the same genus found in the megaphylogeny tree (de la Riva et al., 2019).

All calculations and statistical analyses were performed with the R software (v 2.15.3, R Core Team, 2019).

3 | RESULTS

3.1 | Root construction costs and the root economics spectrum as a function of plant symbiotic type

The principal component analysis showed that covariation among fine root traits was represented by two independent dimensions, encompassing a bi-dimensional plane with a cumulative explanatory power of 94% of all root trait variation (Figure 1). The first principal component (PC1) can be interpreted as a source of variation along the broadly known root economics spectrum (RES), with 63% of the total variation explained (Figure 1). The criterion to define the variation within this first axis as the RES was the same as that the one used by Wright et al., (2004) for the LES: the same directionality of trait loadings and similarly high percentage of variance explained by the principal axis. Within this axis (PC1), RDMC and root tissue density (RTD) were positively associated while SRA and SRL scores were negative. The second principal component explained 31% of the overall variance and was represented mainly by the trade-off between root tissue density (negative scores) and root diameter (positive scores). Interestingly, plant species with contrasting symbiotic association types showed significant differences within the first PCA axis, potentially representing different resource-use strategies ($F_{3,69}$ = 4.84, $p$ = 0.004, Figure 1). Fine roots of AM species had scores associated with acquisitive strategies (i.e. negative scores) in opposition to fine roots from EcM species that had scores associated with more conservative strategies (Figure 1). Fine roots from Nitrogen fixing (N-Fix) and ErM species had intermediate scores and were distributed all along this first axis. When N-fixer species were included within the AM category, the difference in average root traits between AM and EcM species was maintained (Appendix S7).

We did not find any apparent effect of the symbiotic association types in root CC ($F_{3,69}$ = 0.44, $p$ = 0.724) or in any of

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**FIGURE 1** Plot of the first and second axes of the principal component analysis (PCA) performed with five root morphological traits (SRL: specific root length; SRA: specific root area; RDMC: root dry matter content; RTD: root tissue density and Rdi: root diameter) and 60 Mediterranean woody species growing along a gradient in resource availability. Each dot represents a mean value for each individual species and site (73 species x site combinations), and different colours represent different symbiotic association types: Arbuscular mycorrhizal species (AM) in red; Ectomycorrhizal species (EcM) in green color; Ericoid mycorrhizal species (ErM) in blue, and $N_2$-fixing species (N-Fix) in purple. Violin plots with median values and 1st and 3rd quartiles (outside box lines) are included in the bottom; different letters indicate significant differences in mean PC1 score values among species with contrasting symbiotic associations (Tukey post-hoc test, $p < 0.05$)
their components (Appendix S8). On the other hand, we found a positive relationship between root CC and the species’ scores along the first PCA axis, associated with the root economics spectrum (RES; $F_{3,65} = 6.50, p < 0.001$; Figure 2), indicating that conservative strategies imply greater root construction costs than do acquisitive strategies. Interestingly, the slopes of these relationships within species differed for some symbiotic association types (ANOVA slope test, $F_{1,31} = 6.50, p < 0.006$; Figure 2), thus supporting indirect effects of symbiosis types on root CC. When they were analysed separately, we found positive relationships between root construction costs and the RES (PC1) for AM ($F_{1,31} = 7.67, p < 0.009$), N-fix species ($F_{1,9} = 16.79, p < 0.002$) and (marginally significant) ErM ($F_{1,2} = 7.45, p < 0.1$), but there was no relationship for EcM species. The second PCA axis was also negatively related with root construction costs ($F_{1,71} = 7.78, p = 0.006$) but no significant interaction with symbiotic status was observed (Appendix S9).

We found that root CC were negatively related with SRA (marginally with SRL) while they were positively related with RTD and RDMC, and that, with exception for Rdi, all the relationships showed significant interactions between construction costs and symbiotic association type (Table 1). The results of these relationships were consistent after accounting for phylogenetic effects (PGLM in Table 1), which suggests that these relationships are phylogenetically independent. Relationships with root traits differed for each component of the CC: SRA was positively related with OrgN and Min concentrations and negatively related with C concentrations (Appendix S10). In contrast, RTD and RDMC showed opposite patterns and were negatively related with OrgN and Min concentrations and positively related with C concentrations (Appendix S10). Again, except for Rdi, all these relationships showed significant interactions between construction costs and symbiotic association type.

**TABLE 1** Results of the relationships (general linear models: GLM, and phylogenetic generalized linear models: PGLM) between morphological root traits (SRA: specific root area; SRL: specific root length; RDMC: root dry matter content; RTD: root tissue density and Rdi: root diameter) and root construction costs (CC), and differences among slopes for symbiotic association types (Arbuscular mycorrhizas, Ectomycorrhizal species (EcM); Ericoid mycorrhizal species (ErM) and N-fixing species (N-Fix)) in 60 Mediterranean woody species growing along a gradient in resource availability. The degrees of freedom ($df$), $F$-values ($F$), significance ($p$-value), $R^2$ of the models and the tendency of the relationship between CC and each trait (Rel) are shown.

| Dependent variable | Effect variable | $df$ | GLM | PGLM |
|--------------------|----------------|------|-----|------|
|                    |                |      | $F_{\text{Rel}}, p, R^2$ | $F_{\text{Rel}}, p, R^2$ |
| SRA                | Symb.type      | 3    | 5.58, 0.001 | 4.20, 0.009 |
|                    |                |      | 15.94, <0.001, 0.42 | 18.15, <0.001, 0.47 |
|                    | Symb.type $\times$ CC | 3 | 4.84, 0.004 | 4.86, 0.004 |
|                    | Symb.type      | 3    | 3.27, 0.026 | 2.32, 0.080 |
|                    |                |      | 2.94, 0.090, 0.26 | 3.24, 0.070, 0.28 |
|                    | Symb.type $\times$ CC | 3 | 3.39, 0.020 | 3.65, 0.010 |
| RDMC               | Symb.type      | 3    | 10.54, <0.001 | 1.42, 0.240 |
|                    |                |      | 19.19, <0.001, 0.50 | 18.97, <0.001, 0.43 |
|                    | Symb.type $\times$ CC | 3 | 4.50, 0.006 | 5.38, 0.002 |
| RTD                | Symb.type      | 3    | 5.98, 0.001 | 5.18, 0.003 |
|                    |                |      | 25.82, <0.001, 0.51 | 26.39, <0.001, 0.54 |
|                    | Symb.type $\times$ CC | 3 | 7.65, <0.001 | 6.59, <0.001 |
| Rdi                | Symb.type      | 3    | 2.23, 0.092 | 2.15, 0.104 |
|                    |                |      | 0.02, 0.888, 0.18 | 0.19, 0.665, 0.27 |
|                    | Symb.type $\times$ CC | 3 | 2.58, 0.061 | 4.25, 0.009 |
3.2 | Components of the root construction costs and symbiotic types

The partitioning analysis of the total variability among the different components of root CC within the RES (PC1) demonstrated that the proportional role of each component in this relationship varied across symbiotic association types (Figure 3; Appendix S11). The relationship between the RES and construction costs was explained by mineral concentrations for EcM species (28%) while organic nitrogen was the most important component for N-fix species (22%). For both EcM and N-Fixing species, the relationship between the RES and construction costs was also explained in a large proportion by the variation in the concentration of root carbon (25% and 23%, respectively). In contrast, for AM species, the relationship between CC and the RES was poorly explained by the root C variation among species (1%; Figure 3), being mostly explained by variations in organic nitrogen (14%) and the concentration of minerals (9%).

3.3 | Root construction costs along the soil resource gradient

The first two axes of the principal component analysis (PCA) for environmental properties explained 82.6% of the total variance (Appendix S5). The first PCA axis explained 55.7% of the total variance and represented a gradient of soil nutrient availability (concentration of soil nitrogen, magnesium, phosphorous, calcium and potassium and organic matter) while the second PCA axis explained 26.9% of the variance and was mainly associated with differences in the potential water availability. A significant negative relationship was observed between the first PCA axis (nutrient availability) and root CC ($p < 0.001$, $R^2 = 0.34$; Figure 4); dissecting by CC components, the relationship was negative with C but positive with OrgN and Min (Appendix S12). Fine root construction costs were higher in species growing in nutrient-poorer areas (i.e. negative scores of the resource gradient PCA, e.g. Doñana National Park) and decreased as we moved to most productive sites (e.g. Alcornocales Natural Park, positive scores of the resource gradient PCA). Contrastingly, the relationship between the second PCA axis and fine root construction costs was quadratic ($p < 0.001$, $R^2 = 0.56$); that is, species from the Cabo de Gata Natural Park and the riparian areas, that is, the most arid and most humid environments, respectively (Appendix S1), showed the lowest root construction cost values (Figure 4).

4 | DISCUSSION

4.1 | Relationships between root construction costs and the root economics spectrum as a function of plant symbiotic type

The knowledge of the energy content of the constituents involved in establishing the structure and function of the root system is essential for the analysis of plant performance and ecosystem processes (Laureano et al., 2013; Martinez et al., 2002). In this study, we identified a set of covarying morphological root traits (i.e. SRA, SRL and RDMC) that revealed strong links with the total amount of metabolic compounds required to construct root biomass. We observed here a main trend of variation in the first PCA axis (63% of the explained variance) with a similar contribution of explained variance for all root traits (see Appendix S4) that we interpret as the root economics spectrum (RES), such as main unidimensional strategy within
the multidimensional space of plant performance and functioning at below-ground level composed of both resource and collaboration axes (Bergmann et al., 2020). Thus, in one end, we have species with roots with high density, thickness and dry matter content, whereas in the other end there are species with higher SRA and SRL, according with the RES expectations. RTD also loaded on the second axis of the PCA, but individual correlation analysis aligns with our multivariate interpretation of the RES since RTD correlated positively with RDMC ($r = 0.76$, $p < 0.001$) and negatively with SRA ($r = -0.58$, $p < 0.0001$), both traits strongly loaded on PC1. This, along with the large percentage of variance explained by this first axis, allows the interpretation of RTD within the first axis of variation in our PCA in line with the root economics spectrum (de la Riva, Marañón, et al., 2018; de la Riva, Violle, et al., 2018; Roumet et al., 2016). Furthermore, we interpret the trade-off between RTD and diameter within PC2 axis (33% of the variance) as an anatomical relationship that is also frequently observed in leaves (de la Riva, Olmo, et al., 2016; de la Riva, Tosto, et al., 2016). Plants are able to have similar SLA values (or SRA values in roots by analogy) with different combinations of leaf density/thickness values (or RTD/diameter in roots), which would depend on both the nature of the species and their environment (de la Riva, Olmo, et al., 2016; John et al., 2017; Ryser & Lambers, 1995).

According to the significant relationships found between the RES (first PCA axis) and root CC, higher foraging ability (i.e. higher values of SRA and SRL) implied lower construction costs, probably as a result of proportionally higher investments in organic nitrogen and minerals and lower investment in C, a syndrome that is commonly associated with higher metabolic rates and fast return of investments (Roumet et al., 2016). By contrast, higher construction costs, with a proportionally higher investment in C than in organic N or minerals, were observed in species with more conservative strategies (i.e. with higher RDMC and RTD). This conservative strategy confers a greater abundance of metabolic defence compounds and repair complexes in root tissues, at the expense of higher metabolic machinery and higher maintenance costs (Laureano et al., 2008). Moreover, this relationship between CC and the RES held within groups of species with different symbiotic associations, except for EcM. To our knowledge, this is one of the first studies reporting a relationship between the RES and root construction costs, expanding our knowledge on root functioning in woody species (shrubs, arborescent-shrubs and trees). Our results are in line with a previous study reporting a positive relationship between acquisitive strategies and higher root respiration rates in 62 herbaceous and 12 dwarf shrub species (Roumet et al., 2016). It is also worth noticing that we observed a strong relationship between SRA and root construction costs, but this relationship was weaker for SRL. This supports our previous evidence that SRA is more strongly related to root tissue density than SRL in woody plants (de la Riva, Marañón, et al., 2018), indicating that area rather than length-based measurements could be a better indicator of the rate of investment per unit mass in roots. From our study, Rdi seems not to be a useful trait to differentiate root economic strategies since it was decoupled from other traits in the RES (Figure 1), in agreement with previous studies (McCormack et al., 2015; Roumet et al., 2016). Overall, our results evidence that the trade-off between function and morphology determined by the RES (Roumet et al., 2016) is largely determined by the energy needed in building structural root tissues in woody species. Expanding this knowledge to a larger and more diverse pool of species and ecosystems worldwide would be necessary to determine whether these patterns may be generalized globally.

Our set of 60 woody species covered a wide range of fine root trait values within the root economics spectrum (RES); however, the observed range of root construction costs across species was rather narrow, supporting previous findings both for roots (Martínez et al., 2002; Villar et al., 2006) and leaves (Poorter & Villar, 1997; Villar & Merino, 2001; Villar et al., 2006). A possible explanation for these narrow ranges of CC is that trade-offs between chemical fractions and structural components tend to homogenize the construction costs across species (Escudero et al., 2017; Martínez et al., 2002; Poorter & Villar, 1997). For example, leaves in Mediterranean woody species with contrasting resource-use strategies differed slightly in their C concentration but allocated their C to different anatomical tissues; that is, acquisitive species invested a greater proportion of C in metabolic tissues, whereas conservative species invested a greater proportion of the C in structural tissues, which could also be the case for roots (de la Riva, Olmo, et al., 2016; Escudero et al., 2017). This lower variability in root construction costs could also account for the absence of significant differences across symbiotic association types in this study. In fact, both AM and EcM species showed different biomass stocks with very similar carbon allocation rates, in previous studies (Phillips & Fahey, 2006; Valverde-Barrantes et al., 2007), which supports the lack of differences in construction costs in our set of species, despite the observed differences in their morphological strategies (Figure 1). Another possible explanation could be that roots carry out multiple functions, such as foraging ability, water transport, nutrient uptake and exchanging C and nutrients to support symbiotic associations, functions that may not always be maximized simultaneously (Bergmann et al., 2020; Comas et al., 2012). Whatever the case, the slight variation in construction costs across functional types in our study seems to respond to differences in the morphological traits of the RES.

We also showed that species segregation along the RES was partly conditioned by their type of symbiotic association, especially for AM and EcM species. Thus, arbuscular mycorrhizal species (AM) tend to have traits associated with more acquisitive strategies while root traits of ectomycorrhizal species (EcM) are associated with more conservative strategies. On the other hand, ErM species occupied more intermediate positions along the RES and N-fixing species were distributed along the whole gradient of resource economics. This segregation between AM and EcM species indicates that these mycorrhizal types tend to show affinity by a particular subset of morphological root traits (Kong et al., 2019). Roots colonized mainly by EcM fungi normally present a higher lignification than those colonized by AM fungi (Brundrett, 2002; Guo et al., 2008), being lignification strongly associated with higher root tissue densities and root dry matter contents (de la Riva, Marañón, et al., 2018; Prieto...
On the other hand, roots from species mainly colonized by AM fungal species tend to exhibit trait values typical of acquisitive and fast-growing strategies, with a higher investment in root area or length per unit root mass (i.e. higher SRA or SRL; Comas et al., 2012; Valverde-Barrantes et al., 2018). One possible reason for these preferences could be that AM fungi species require specific anatomical and architectural adaptations to facilitate the root infection (e.g. higher root cortical areas; Comas et al., 2012). Such anatomical and architectural features increase the contact surface between the root and the AM symbiont (Valverde-Barrantes et al., 2018), and also enhance the root hydraulic conductivity and foraging ability of the host plant species, associated with a faster resource uptake, growth and development (Hernández et al., 2010). Our results suggest that Mediterranean woody species that develop roots with acquisitive traits (e.g. high SRA) also favour the association with AM symbionts, resulting in a positive plant-symbiont feedback loop aimed at maximizing the plant C gain, which will benefit both the plant and the fungi (Allen & Allen, 1991).

### 4.2 Components of the root construction cost and symbiotic types

Despite similar relationships between root construction costs and the RES within groups of species with the same symbiotic association (Figure 2), the influence of each component of the root construction costs on this relationship differed with the type of symbiotic association. These results suggest that the variation of the different components involved in root construction costs across species may be modulated by the specific resource uptake strategy of each symbiotic association type. The variation of root construction costs within roots of EcM species (situated on the conservative extreme of the axis) reflected a shift along their resource uptake strategy as a result of their different C and mineral concentration, which could suggest a different inversion on their structural components. In contrast with the general trend (RES), EcM species showed proportionally lower mineral concentration and slightly lower C concentrations in denser roots with lower root area per dry mass (SRA). In this study, most EcM species belonged to a single family (Cistaceae) that may show stronger phylogenetic conservatism, and thus a more constrained range of variation in CC, than the more diverse AM group (Valverde-Barrantes et al., 2018); this fact could explain the lack of a relationship between the RES and CC in EcM species. Oppositely, the variation of the CC in roots from AM species (situated on the acquisitive side of the axis) seems to be mostly determined by a proportionally different investment in organic nitrogen, which is associated with higher concentrations of active metabolic compounds, such as proteins (Poorter & Villar, 1997) while minerals and especially carbon concentration seems to exert a secondary role. This could be due to the fact that AM species are able to maintain their acquisitive performance either by investing carbon in cheap tissues that facilitate a faster return on investment or by investing carbon in thicker roots that enhance the mycorrhiza association (trade-off from ‘do-it-yourself’ to ‘outsourcing’; sensu Bergmann et al., 2020). In rhizobial N-fixing species, a similar proportion of the investment in construction costs was derived from C concentrations and organic nitrogen, possibly as a result of their wide distribution along the whole resource uptake gradient (including both acquisitive and conservative species), which could lead to an interplay between structural and metabolic compounds according to the economics spectrum theory. We need to acknowledge here that this evidence is based on the known species symbiotic preferences and, although tightly linked to root strategies, may not reflect direct measurements of root mycorrhizal colonization rates. Therefore, our conclusions on the different patterns of investment of root construction constituents in relation with symbiotic roles need to be validated in future studies by direct measurements of mycorrhizal fungal colonization. Nonetheless, we advocate that these findings represent a significant advance in the understanding of the relationship between root morphology, construction costs and symbiotic associations.

### 4.3 Variation of root construction costs along a soil resource gradient

Our prediction that variation of root construction costs across Mediterranean woody species would be related to the resource availability of their respective habitats was only partially supported. We found that root construction costs were influenced by resource availability, and that the intrinsic cost (energy per unit of mass) of root production was higher in species growing in less fertile habitats than in those species inhabiting more favourable areas, thus supporting previous evidences of studies with different woody species (Martínez et al., 2002) or different populations of Quercus ilex (Laureano et al., 2013). It has been frequently observed that soil fertility drives the functional trait composition of Mediterranean woody communities in line with the economics spectrum theory (Cornwell & Ackerly, 2009; de la Riva, Marañón, et al., 2018). In this sense, the shrub species from the two communities in the Doñana National Park (the less fertile sites) showed the highest values of construction costs (averages of 1.76 and 1.69 g glucose/g in Monte Negro and Monte Blanco, respectively). This was likely due to the dominance of conservative resource-use strategies in species inhabiting these sites, which has proven to be advantageous for surviving in these stressful environments (Lloret et al., 2016). Sites from this region are characterized by sand dune soils with very low water retention, which explains their lowest fertility (Appendix S1). In line with these results, Martínez et al., (2002) found that the woody species from Doñana had higher root construction costs than others from more productive environments, due to their higher concentration of costly compounds, for example, cell wall waxes that provide greater protection against water loss (Martínez et al., 2002; Samuels et al., 2008). By contrast, we found that in the most productive areas, such as riparian forests, dominant species were mostly deciduous with fast-growing strategies and low fine root construction costs (1.48 g glucose/g, on average), probably in relation to less costly
chemical compounds such as cellulose and higher concentrations of minerals and organic acids, which allow them to achieve their highest growth rates (Martínez et al., 2002; Poorter & de Jong, 1999). These results support the hypothesis that species from the most stressful environments need to spend comparatively more energy on root construction than do species under favourable conditions (Laureano et al., 2013; Martínez et al., 2002; Villar et al., 2006).

It is worth noticing that, in contrast with our expectations, we did not find a linear relationship between root construction costs and potential water availability of the site, which may suggest an idiosyncratic pattern of carbon economics inversion along the gradient in water availability. This result is a priori surprising, not only because it contradicts the above-mentioned findings in Mediterranean environments (Laureano et al., 2013; Martínez et al., 2002) but also because we have previously observed that root morphology was strongly associated with soil resource availability in Mediterranean woody species (de la Riva, Marañón, et al., 2018). The main reason for this unexpected finding lies in the fact that the lowest construction costs (mean of 1.39 g glucose/g) were found in plant species from the region with the lowest water availability within the gradient (Cabo de Gata-Nijar Natural Park). This site is extremely arid (de la Riva, Violle, et al., 2018) and, as shown in a study of Atacama Desert, root trait responses with aridity may not follow a linear pattern, but instead they present shifts at the most extreme ends (Carvajal et al., 2019). In the Atacama Desert, plant communities followed a complex pattern, whereby root traits shifted from acquisitive to conservative with increasing aridity, until a certain threshold in aridity was reached and species became more acquisitive again. Our results seem to follow this same pattern whereby fast resource uptake strategies are selected under very low water availability (i.e. Cabo de Gata), maybe because plants can survive under these extreme conditions using the available nutrient resources during short periods of higher water availability, for example, after rain events (Carvajal et al., 2019; Chesson et al., 2004; Querejeta et al., 2018). Soils in Cabo de Gata were very shallow (de la Riva, Violle, et al., 2018) and plant species in this site have acquisitive root traits with very shallow root systems favouring faster water uptake immediately after rain events (Carvajal et al., 2019; Fort et al., 2013). That is, if the nutrient concentration is enough, these species are capable of faster and more profligate water and nutrient use to grow faster during short wet pulses. However, acquisitive plants usually have high nitrogen concentrations in their tissues to maintain high metabolic levels (de la Riva, Olmo, et al., 2016), a nutrient that is frequently inaccessible in soils from arid ecosystems (Noy-Meir, 1973; Ward, 2009). Although specific mechanisms are challenging to infer, our results also suggest that the role played by the type of plant symbiont association could be crucial to overcome this deficit. Associations with AM fungi and N-fixing bacteria often favour higher acquisition of nutrients (especially N and P) in low productive environments (Andrade et al., 2010; Meng et al., 2015; Smith & Smith, 2011; Whiteside et al., 2012), thus supplying the necessary elements to maintain a higher metabolic activity and fast growth during these short periods of water availability. Indeed, the Cabo de Gata site had the highest proportions of AM species across all sites (with 61% of the species being AM) and one of the highest proportion of N-fixing species (22%)—proportions slightly higher than expected (see Appendix S1)—suggesting that symbiotic relationships may indeed be an advantage to maintain acquisitive strategies under resource scarcity, as previously hypothesized (Kramer-Walter & Laughlin, 2017).

5 | CONCLUSIONS

This study supports that the differences in root construction costs in 60 woody Mediterranean species are a good reflection of differences in root morphological traits (mostly SRA and RDMC), in line with expectations from the economics spectrum theory. We observed that different plant species have different resource uptake strategies along the resource gradient (RES), which depend on the trade-offs among metabolic compounds (carbon, organic nitrogen and minerals) that influence root morphology and functioning. The influence of the intrinsic components of the construction cost of roots across species varies among symbiotic associations, which suggests that the trade-off between structural and metabolic compounds may be modulated by the specific resource-use strategy of each type of symbiont. In addition, root construction costs are strongly modulated by soil resource availability (whether nutrients or water) following a nonlinear pattern with water availability shifting from high to low construction costs at the most arid site, which points to an important role of symbiotic associations in this shift. In summary, this study highlights that the root construction cost is determined by the existence of different economic strategies and symbiotic association types, although future efforts should be focused on clarifying the causes driving the different root construction cost values.

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AUTHORS’ CONTRIBUTIONS

E.G.d.I.R., R.V., T.M. and I.M.P.-R. conceived the ideas and designed the study; E.G.d.I.R., M.O., T.M. and I.M.P.-R. conducted the fieldwork; M.O. and R.V. conducted the laboratory work; E.G.d.I.R. and
REFERENCES

Allen, M. F., & Allen, M. F. (1991). The ecology of mycorrhizae. Cambridge University Press.

Andersen, K. M., Mayor, J. R., & Turner, B. L. (2017). Plasticity in nitrogen uptake among plant species with contrasting nutrient acquisition strategies in a tropical forest. Ecology, 98, 1388–1398. https://doi.org/10.1002/眺y1793

Andrade, S. A. L., Silveira, A. P. D., & Mazzafera, P. (2010). Arbuscular mycorrhiza alters metal uptake and the physiological response of Coffea arabica seedlings to increasing Zn and Cu concentrations in soil. Science of the Total Environment, 408(22), 5381–5391. https://doi.org/10.1016/j.scitotenv.2010.07.064

Bergmann, J., Weigelt, A., van der Plas, F., Laughlin, D. C., Kuyper, T. W., Guerrero-Ramirez, N., Valverde-Barrantes, O. J., Brulehde, H., Freschet, G. T., Iversen, C. M., Kattge, J., McCormack, M. L., Meier, I. C., Rillig, M. C., Roumet, C., Semchenko, M., Sweeney, C. J., van Ruijven, J., York, L. M., & Mommer, L. (2020). The fungal collaboration gradient dominates the root economics space in plants. Science. Advances, 6(27), eaba3756. https://doi.org/10.1126/sciadv.aba3756

Brundrett, M. C. (2002). Coevolution of roots and mycorrhizas of land plants. New Phytologist, 154, 275–304. https://doi.org/10.1046/j.1469-8137.2002.00397.x

Brundrett, M. C., & Tedersoo, L. (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytologist, 220(4), 1108–1115. https://doi.org/10.1111/nph.14976

Carvajal, D. E., Loaya, A. P., Rios, R. S., Delpiano, C. A., & Squeo, F. A. (2019). A hyper-arid environment shapes an inverse pattern of the fast-slow plant economics spectrum for above-, but not belowground resource acquisition strategies. Journal of Ecology, 107(3), 1079–1092. https://doi.org/10.1111/1365-2745.13092

Chesson, P., Gebauer, R. L. E., Schwinner, S., Hunty, N., Wiegand, K., Ernest, M. S. K., Sher, A., Novoplansky, A., & Weltzin, J. F. (2004). Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. Oecologia, 141(2), 236–253. https://doi.org/10.1007/s00442-004-1551-1

Comas, L. H., Mueller, K. E., Taylor, L. L., Midford, P. E., Callahan, H. S., & Beerling, D. J. (2012). Evolutionary patterns and biogeochemical significance of angiosperm root traits. International Journal of Plant Sciences, 173(6), 584–595. https://doi.org/10.1086/655823

Cornwell, W. K., & Ackerly, D. D. (2009). Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. Ecological Monographs, 79(1), 109–126. https://doi.org/10.1890/07-1134.1

de la Riva, E. G., Godoy, O., Castro-Diez, P., Gutiérrez-Cánovas, C., & Vilá, M. (2019). Functional and phylogenetic consequences of plant invasion for coastal native communities. Journal of Vegetation Science, 30(3), 510–520. https://doi.org/10.1111/jvs.12748

de la Riva, E. G., Marañón, T., Pérez-Ramos, I. M., Navarro-Fernández, C. M., Olmo, M., & Villar, R. (2018). Root traits across environmental gradients in Mediterranean woody communities: Are they aligned along the root economics spectrum? Plant and Soil, 424, 35–48. https://doi.org/10.1007/s11104-017-3433-4

de la Riva, E. G., Olmo, M., Poorter, H., Uberta, J. L., & Villar, R. (2016). Leaf mass per area (LMA) and its relationship with leaf structure and anatomy in 34 Mediterranean woody species along a water availability gradient. PLoS ONE, 11(2), e0148788. https://doi.org/10.1371/journal.pone.0148788

de la Riva, E. G., Pérez-Ramos, I. M., Tostó, A., Navarro-Fernández, C. M., Olmo, M., Marañón, T., & Villar, R. (2016). Disentangling the relative importance of species occurrence, abundance and intraspecific variability in community assembly: A trait-based approach at the whole-plant level in Mediterranean forests. Oikos, 125(3), 354–363. https://doi.org/10.1111/oik.01875

de la Riva, E. G., Violle, C., Pérez-Ramos, I. M., Marañón, T., Navarro-Fernández, C. M., Olmo, M., & Villar, R. (2018). A multidimensional functional trait approach reveals the imprint of environmental stress in Mediterranean woody communities. Ecosystems, 21, 248–262. https://doi.org/10.1007/s10021-017-0147-7

de la Riva, G. E. (2021). Root and chemical trait. Dryad Digital Repository, https://doi.org/10.5061/dryad.sn1n8prv

de la Riva, G. E., Tostó, A., Pérez-Ramos, I. M., Navarro-Fernández, C. M., Olmo, M., Anten, N. P. R., Marañón, T., & Villar, R. (2016). A plant economics spectrum in Mediterranean forests along environmental gradients: Is there coordination among leaf, stem and root traits? Journal of Vegetation Science, 27(1), 187–199. https://doi.org/10.1111/jvs.12341

Escudero, A., Mediavilla, S., Olmo, M., Villar, R., & Merino, J. (2017). Coexistence of deciduous and evergreen Oak species in Mediterranean environments: Costs associated with the leaf and root traits of both habitats. In E. Gil-Pelegrín, J. J. Peguero-Pina, & D. Sancho-Knapik (Eds.), Oaks physiological ecology. Exploring the functional diversity of genus Quercus L. (pp. 195–237). Springer.

Fort, F., Jouany, C., & Cruz, P. (2013). Root and leaf functional trait relations in Poaceae species: Implications of differing resource-acquisition strategies. Journal of Plant Ecology, 6(3), 211–219. https://doi.org/10.1093/jpe/rts034

Guo, D., Xia, M., Wei, X., Chang, W., Liu, Y., & Wang, Z. (2008). Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. New Phytologist, 180(3), 673–683. https://doi.org/10.1111/j.1469-8137.2008.02573.x

Hernández, E. I., Vilagrosa, A., Pausas, J. G., & Bellot, J. (2010). Morphological traits and water use strategies in seedlings of Mediterranean coexisting species. Plant Ecology, 207(2), 233–244. https://doi.org/10.1007/s11278-009-9468-2

Jobbágy, E. G., & Jackson, R. B. (2001). The distribution of soil nutrients with depth: Global patterns and the imprint of plants. Biogeochemistry, 53, 51–77. https://doi.org/10.1023/A:1010760720215

John, G. P., Scoffoni, C., Buckley, T. N., Villar, R., Poorter, H., & Sack, L. (2017). The anatomical and compositional basis of leaf mass per area. Ecology Letters, 20(4), 412–425. https://doi.org/10.1111/ele.12739

Kong, D., Wang, J., Wu, H., Valverde-Barrantes, O. J., Wang, R., Zeng, H., Kardol, P., Zhang, H., & Feng, Y. (2019). Nonlinearity of root trait relationships and the root economics spectrum. Nature Communications, 10, 2203. https://doi.org/10.1038/s41467-019-10245-6
SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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