Postprint of: Plant and Soil (2020) https://doi.org/10.1007/s11104-020-04485-5

Variation in morphological and chemical traits of Mediterranean tree roots: linkage with leaf traits and soil conditions

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Author contribution statement

TM conceived the study, TM, CMNF and MTD conducted fieldwork, CMNF and PM measured morphological and chemical traits, TM, CMNF and MGM analysed the data, TM wrote the first draft, TM, CMNF, MGM, MTD, PM and RV participated in the interpretation, discussion and preparation of the final version.

Abstract

Aims: Root functions are multiple and essential for the growth and survival of terrestrial plants. The aim of this work was to analyse the main trends in the variation of root traits, their coordination with leaf traits and their relationships with soil conditions.

Methods: We measured the variation of 27 fine root traits (five morphological, 20 chemical and two isotopic signatures) in trees of seven species of a mixed plantation in a metal-contaminated and remediated site of Southern Spain.

Results: We found evidences supporting the existence of a root economics spectrum (RES). However, other dimensions were identified as being independent of the main RES: mainly the variation in the carbon concentration, the accumulation of trace elements associated with tolerance of metal-rich soils, and the fractionation of $\delta^{15}N$ as a time-integrated trait of mycorrhizal-mediated nutrition. In general, roots and leaves were functionally coordinated, although most of the trace elements showed strong root-leaf discordance. The soil conditions interacted with the fine root traits in feedback processes. The ability of tree roots to accumulate trace elements and to reduce their translocation to leaves is a desirable trait for the phytoremediation of metal-contaminated soils.

Conclusions: Roots are multifunctional. Understanding the variations in the root traits of trees will help us to predict both the responses of forests to global changes, including soil contamination, and the provision of soil-based ecosystem services.

Keywords

Root traits, Root economics spectrum, Root-leaf coordination, Trace-element contamination, Root multifunctionality

Introduction

Advances in trait-based plant ecology are focused on the analysis of functional traits across individuals and species, to predict emergent properties of communities and ecosystems (Garnier et al. 2016; Laliberté 2017). There are evolutionary and biophysical constraints limiting the existing spectrum of plant traits (Reich 2014). In a global view of the functional diversity of vascular plants on Earth (analysis of key traits for more than 46,000 plant species), most of the trait variation was concentrated in a two-dimensional spectrum of plant form and function: plant size and leaf economics spectrum (LES) (Díaz et al. 2016). The LES reflects the trade-off between resource acquisition and resource conservation. At one end of the spectrum, there are species with high photosynthetic and respiration rates, high nitrogen (N) and phosphorus (P) concentrations, low leaf mass per area (LMA) and low leaf longevity. At the other end of the spectrum, there are species with the opposite traits (Wright et al. 2004).

Although roots are essential organs for terrestrial plants, no root traits were considered in the global study of Díaz et al. (2016). Root functions are multiple and essential for plant growth and survival: they include nutrient and water acquisition, anchorage, resource storage and support of symbiotic soil microbes. At the ecosystem level, they contribute to soil structure and to the carbon and nutrient cycles (Erktan et al. 2018). Therefore, an understanding of how root traits vary is fundamental to the comprehension of plant functional ecology.

Some studies support the existence of a “root economics spectrum” (RES), analogous to the LES, with a trade-off between resource acquisition and conservation (Reich 2014; Roumet et al. 2016; de la Riva et al. 2018a). Thus, plants growing in favourable environments would develop lighter roots with a lower dry matter content and higher specific root length to maximize resource acquisition. By contrast, plants growing in adverse or limiting environments would exhibit a resource conservation strategy, developing denser roots with a higher dry matter content and lower specific root length. However, the RES hypothesis was challenged by Weemstra et al. (2016) who argued that root traits are constrained not only by resource uptake, but also by other drivers (like soil texture and chemistry), and that the RES hypothesis does not incorporate soil heterogeneity and mycorrhizal symbiosis. Moreover, several studies (Kramer-Walter et al. 2016; Kong et al. 2019) have found that high SRL roots can be constructed with any density, indicating exceptions in the RES.

Resource acquisition is coupled and linked among plant organs. Thus, fast acquisition and processing of water and nutrients by roots would require fast acquisition and processing of carbon (C) by leaves (Reich 2014). A strong coordination between root morphology and aboveground traits was found for a set of 80 woody species (de la Riva et al. 2018a). Root traits that achieve nutrient conservation favour tissue longevity and slower growth rates, and in consequence diminish nutrient requirements and amortization of the construction costs (Poorter and Villar 1997; Villar et al. 2006; de la Riva et al. 2016b, 2018a). However, other studies did not find correlations between leaf and root traits, suggesting that trade-offs in different organs operate independently and that the leaf-root coordination may depend on specific limiting factors in each habitat (Tjoelker et al. 2005; Kembel and Cahill 2011; Fortunel et al. 2012).

Contrasting leaf habits in trees - that is, evergreen versus deciduous - are usually associated with different functional traits. For example, deciduous species are characterised by acquisitive traits such as lower LMA, higher rates of photosynthesis and respiration, and higher nutrient concentrations, in comparison to evergreen species, which tend to exhibit more-conservative traits (Wright et al. 2004; Villar et al. 2006; de la Riva et al. 2018b). However, few studies have investigated the differences in root traits between evergreen and deciduous trees; for example, Martinez et al. (2002) did not find differences in root C or N concentrations between deciduous and evergreen species of *Quercus*.

Most tree roots are intimately associated with mycorrhizal fungi in a symbiosis that is crucial for nutrient acquisition and tolerance of diverse stresses (drought, heavy metals or pathogens), while the fungus obtains carbon compounds from the plant (Smith and Read 2008). In fact, that combination of root and fungus (mycorrhiza) can be considered as the functional absorptive trait, in which fungal tissues may represent up to 54% of the “root” N concentration (Ouimette et al. 2013). The degree of root colonisation by mycorrhizal fungi has proved to be a useful plant trait to understand ecosystem processes (Soudzilovskaia et al. 2015; Navarro-Fernández et al. 2016; Laliberté 2017). Fungal traits, like the type of hyphal exploration, add more complexity to the soil-fungus-plant relationships and resource acquisition strategies (Chagnon et al. 2013; Gil-Martínez et al. 2018; López-García et al. 2018).

Besides unravelling the RES, another research challenge is to understand how different drivers of global change impact a suite of root traits, and to predict their cascading effects on soil-based ecosystem processes (Bardgett et al. 2014). Root traits are plastic and respond to physical soil limitations, the heterogeneous distribution of soil water and nutrients and biotic interactions (Bardgett et al. 2014). For example, soil compaction limits the formation and penetration of thin roots, inducing a lower specific root length (SRL) in tree seedlings (Alameda and Villar 2012). Under dry soil conditions, plants tend to develop thinner roots, with greater SRL and increased root hair density, to improve water acquisition (Comas et al. 2013; Olmo et al. 2014). Nitrogen deposition decreases fine root biomass, C:N ratio and fungal colonization, while increasing root respiration (Li et al. 2015).

As a global change driver, soil pollution may also promote the adjustment of root traits in plants. For instance, a high concentration of trace elements in soil often reduces root elongation and alters root architecture (Kahle 1993). Although, at a global scale, soil pollution is one of the main threats to soils and the ecosystems services provided by them (Rodríguez-

Eugenio et al. 2018), its effects on plant functional traits have not been fully addressed. Among the different soil pollutants, heavy metals are relevant stressors, altering the plant-soil interactions (Krumins et al. 2015).

In this study we analysed the variation in morphological and chemical root traits in seven Mediterranean tree species, and explored the linkages with aboveground traits and soil conditions in a heterogeneously-polluted environment (Guadiamar Green Corridor, in SW Spain). This area is a large-scale example of the phytoremediation of land contaminated by a mine-spill, with high concentrations of metals. A mixed plantation of native trees and shrubs was set up after cleaning and remediating the soil (Domínguez et al. 2008; Madejón et al. 2018a, b). This large-scale experiment is an opportunity to explore how the soil conditions (in this case, the concentration of heavy metals) affect the root traits of different tree species coexisting in a similar environment. We addressed the following hypotheses:

1) Roots of different tree species differ in their functional traits in accordance with the root economics spectrum (RES). However, there are other root dimensions (independent of RES) that reflect root multifunctionality (Weemstra et al. 2016).
2) Root and leaf traits are coordinated in accordance with the plant economics spectrum. Fast plant growth depends on the coordination of roots and leaves, with roots ensuring a water and nutrient supply sufficient to maintain acquisitive leaves with high photosynthetic rates and high evaporative demand (Reich 2014).
3) Soil conditions and metal contamination affect root traits. Root traits are plastic and respond to physical soil limitations, the heterogeneous distribution of soil water and nutrients, biotic interactions (Bardgett et al. 2014) and soil pollution (Kahle 1993).

Material and methods

Study area

The study area is the Guadiamar Green Corridor (Seville, Spain). The climate is Mediterranean with mild, rainy winters and hot, dry summers. The average annual rainfall is 450 mm and the mean annual temperature is 17 ºC, with a maximum of 33 ºC (in July) and a minimum of 5 ºC (in January). For more details, see the area description in Domínguez et al. (2008) and Madejón et al. (2018a).

The study area was affected by a mine-spill (in April 1998) that polluted the soil with trace elements. After the spill, the soil was cleaned up, remediated and afforested with native species of shrubs and trees in mixed patterns to simulate a diverse forest (Madejón et al. 2018b). In a plot of about 14 ha (37º 23.165´ N, 6º 13.668´ W) within the remediated area we randomly selected five replicates of seven tree species (35 tree samples in total), with an average distance of more than 100 metres between replicates of the same species, resembling a “common-garden experiment”. The area was afforested in 2000, using seedlings (1-2 years old) grown in a nearby nursery. The tree species were selected for this study according to their contrasting leaf habits: deciduous species (*Populus alba* L., *Celtis australis* L. and *Fraxinus angustifolia* Vahl) and evergreen species (*Quercus ilex* subsp. *ballota* (Desf.) Samp., *Olea europaea* subsp. *europaea* var. *sylvestris* (Mill.) Lehr, *Ceratonia siliqua* L. and *Pinus pinea* L.); hereafter we use only the genus name for simplicity.

The soil in the plot is of the Fluvisol type, being acidic (pH below 5) and nutrient-poor, with a loamy texture (about 20% sand). In the spill-affected and remediated soils the residual contamination by trace elements such as As, Cd, Cu, Pb and Zn was still high during the study (16 years after the spill). However, there was a low transfer rate of trace elements to the aboveground parts of the woody plants (Domínguez et al. 2008; Madejón et al. 2018a, 2018b).

Trait measurements

Roots were sampled (in late autumn, December 2014) at the individual tree level, by excavating the first 20–30 cm of the soil adjacent to the tree trunk base. We selected the fine roots (< 2 mm in diameter) for the trait analysis. Although fine roots are composed by absorptive and transport roots (McCormack et al. 2015), we assume that, given the small diameter of the selected roots (range from 0.35 to 0.53 mm), most of them should be absorptive.

In the selected fine roots we measured the following morphological traits: specific root length (SRL, root length per unit of root dry mass, m g^{-1}), root mass per area (RMA, root mass per unit of root area, g m^{-2}), root mean diameter (RDI, mm), root dry matter content (RDMC, root dry mass per unit of water-saturated fresh mass, mg g^{-1}) and root tissue mass density (RTD, root dry mass per unit of root volume, mg cm^{-3}). We followed methods in Pérez-Harguindeguy et al. (2013) and de la Riva et al. (2016a) to characterise these variables. However, in this study, we adopted the trait RMA as an analogue of leaf LMA, being the key functional trait for roots (see the arguments in favour of using LMA in Poorter et al. 2009 and de la Riva et al. 2018b). The roots were scanned with an EPSON Perfection V700 photo scanner at 1200 dpi. The length, diameter, area and volume of the roots were obtained by analysing the scanned root samples with WinRHIZO 2009 software (Regent Instruments Inc., Quebec, Canada). The mycorrhizal type associated with each tree species was assigned according to several sources (Maremmani et al. 2003; Manaut et al. 2015; Navarro-Fernández et al. 2016). We assigned the "ectomycorrhizal type" (ECM) to those tree species (*Pinus*, *Populus* and *Quercus*) that form symbiotic associations predominantly with ECM fungi, although they can also associate with arbuscular mycorrhizal (AM) fungi. In contrast, trees of the "arbuscular mycorrhizal type" (AM) are exclusively associated with AM fungi (see Table S1).

In the selected trees, morphological traits of fully-expanded leaves were recorded following the methods of Pérez-Harguindeguy et al. (2013). Young, fully expanded leaves still attached to a portion of stem of the previous year were collected from each individual tree. These stems with leaves were stored in plastic bags to prevent water loss and transported to the laboratory, where they were maintained with the basal portion of the stem submerged in water at 10 ºC for 24 h, in darkness, to allow complete re-hydration (de la Riva et al. 2016a). They were sampled in early autumn (October 2014), when we expect them to have their maximum concentrations of chemical elements (Madejón et al. 2004, 2006; Domínguez et al. 2008). We measured the leaf mass per area (LMA, leaf dry mass per unit of area, g m^{-2}) and leaf dry matter content (LDMC, dry mass per unit of water-saturated fresh mass; mg g^{-1}). We also measured the stem dry matter content (SDMC, dry mass per unit of water-saturated fresh mass; mg g^{-1}) and stem wood density (SWD, dry mass divided by the stem fresh volume; mg cm^{-3}; based on the Archimedes principle, measuring the volume of water displaced by immersion of the stem) in the sampled branches and twigs. One of the *Fraxinus* trees suffered summer defoliation and only had young leaves; therefore, it was excluded from the leaf traits dataset (n=34).

A subsample of the roots and leaves collected from each tree was dried and then ground using a stainless steel mill, for chemical analyses. The N and C concentrations and the isotopic ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) were determined, in leaf and root samples combusted at 1020 ºC, using a continuous flow isotope-ratio mass spectrometry system. This involved a Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope-ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany); the analytical measurement errors were ± 0.2‰ for $\delta^{15}N$ and ± 0.1‰ for $\delta^{13}C$. The concentrations of macro- (P, K, Ca, Mg, S) and micronutrients (B, Co, Cu, Fe, Mn, Na, Ni, Zn), as well as non-essential elements (As, Ba, Cd, Pb, Sr), were determined after wet oxidation with concentrated HNO_3 in a Digiprep MS block digestor (SPS Science) equipped with a temperature-time programmable controller and polypropylene digestion tubes. They were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) Varian ICP 720-ES. We assessed the quality of the analyses using the reference sample INCT-OBTL-5 (tobacco leaves), and acceptable recovery rates (between 88 and 105%) were obtained for all the elements discussed here. The exception was Fe, with 77% recovery, but it was kept in the dataset due to its importance as an essential micronutrient for plants, in photosynthetic electron transport and other metabolic functions (Lambers et al. 2008; Tripathi et al. 2018).

We also measured some whole-tree traits: height (HEIG, with a Nikon Forestry Pro hypsometer; m), crown projection area (CRP, estimated from two perpendicular axes of an ellipse; m^2) and accumulated leaf litter (LITT, estimated by averaging the litter biomass collected inside three 25×25 cm quadrats around each tree trunk and extrapolating to the tree crown projection; kg).

Soil conditions

Soil was sampled at 0-10 cm depth using a gouge auger of 2.5 cm diameter. Under each tree canopy, three random points were selected and the cores obtained were mixed to make one composite sample per tree. In addition, soil was sampled in adjacent open sites with grassland (<100 m far from each group of sampled trees; five replicates) to have a treeless reference.

The soil samples were air-dried and sieved (< 2 mm). Soil pH was determined, using a CRISON micro pH 2002 probe and meter, in a 1:2.5 soil-1 M potassium chloride suspension. Organic C was determined following dichromate oxidation of samples and titration with ferrous ammonium sulphate (Walkley and Black method). The total N concentration was measured in a Leco TruSpec CN elemental analyser. The isotopic ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) were measured using the method indicated above for leaves and roots. Available P was estimated by the Bray 1 method. The available potassium (K), calcium (Ca) and magnesium (Mg) were extracted with 1 M ammonium acetate and determined by atomic absorption spectrophotometry. The available concentrations of trace elements were determined, after extraction in 0.01 M $CaCl_2$ at a 1:10 soil-solution ratio, by ICP-OES (Houba et al. 2000).

Data analysis

The relative variation of the root and leaf traits among individual trees was measured as the coefficient of variation (CV, (100×sd)/mean). The variation due to the tree species was tested by analysis of variance (one-way ANOVA) for each morphological and chemical trait in roots and leaves, as well as for the properties of the soil underneath each tree species. Multiple comparisons of tree species means were performed with *post-hoc* Tukey tests. Previously, the data were tested for normality (Shapiro–Wilk test) and homoscedasticity (Levene test), and were Box-Cox transformed when necessary. Alternatively, a non-parametric Kruskal–Wallis test was applied, when the assumptions for the parametric tests were not fulfilled.

We explored the multidimensionality of the root traits by applying principal component analysis (PCA). The first PCA was performed to investigate if the root variation followed the root economics spectrum (RES); therefore, we analysed - for individual trees - the variation in six key traits: the five morphological traits included in de la Riva et al. (2018a) plus the root N concentration, as a key trait associated with the net soil resource acquisition rate. The second PCA was performed to study if the root morphological variation was associated with root chemical traits; therefore, we analysed the variation in all 27 root traits (5 morphological, 20 chemical and 2 isotopes) in the 35 trees. To interpret the covariation trends observed in the second PCA, we analysed the bivariate correlations across the 27 root traits; we produced correlograms using the R *corrplot* package and ordered the traits by hierarchical clustering of correlation coefficients.

The main variation trends in the 29 aboveground traits (4 morphological, 20 chemical, 2 isotopes and 3 whole tree) were analysed separately by another PCA. Then, we selected 24 analogous root-leaf traits to explore the coordination of root and leaf traits; we carried out separate PCAs for the roots and leaves, and explored the correlations between the respective first axes (main variation trends) of each PCA. We also tested the differences in root traits between the evergreen and deciduous tree species.

To evaluate the relationships between root traits and soil variables, we performed Pearson´s correlation tests. We used R software v3.5.1 (R Development Core Team, 2018) for the PCAs, in the *stats* and *ggplot2* packages, and SPSS 20.0 software for Windows (SPSS Inc., USA) for theANOVA and correlation analyses.

Results

Multidimensional root traits

There were significant differences among the tree species for 25 out of the 27 root traits (Table S1 and Fig. S1). In particular, the value of the RMA key trait was lowest for *Fraxinus* and highest for *Quercus*, with differences between deciduous and evergreen trees (Fig. 1).

Root mass per area explained most of the root trait variation, having the highest loading factor (0.97) in the first axis of the six-trait PCA (Table S2 and Fig. S2). The variation trend of this axis, which accounted for 57% of the variance, supports the root economics spectrum hypothesis, with acquisitive traits such as high SRL and N concentration on the negative side and conservative traits such as high RMA and RDMC on the positive side. The second axis (explaining 23% of the variance) contrasted RDI versus RTD (Fig. S2).

In the all-traits PCA, the first axis (accounting for 38% of the variance) was defined by the concentrations of Mg, sulphur (S) and some trace elements (Fe, Pb, As and Cu), which were related to the roots of *Celtis* and *Fraxinus* trees. Moreover, there was a high correlation among almost all the trace elements (except Cd and B) and they grouped together on the left side of PC1. On the positive side of the first axis, the C content and RMA were significantly explicative and were related to the roots of *Quercus* and *Pinus* trees (Fig. 2 and Table S2). A secondary variation trend (second PCA axis, accounting for 18% of the variance) separated root samples with higher RDMC and RMA, associated with *Quercus* and *Pinus* trees, from samples with higher concentrations of K and B, associated with *Fraxinus* and *Populus* roots. Another trend (third PCA axis, accounting for 11% of the variance) discriminated root samples accumulating Cd (positive scores) from those rich in Ca and Sr (negative scores) (Table S2).

Root mass per area was correlated positively with other morphological traits (excepting SRL) and negatively with SRL, and the concentrations of nine nutrient elements and three non-essential elements (Fig. 3). In contrast, SRL was correlated negatively with most morphological traits (excepting RTD) and positively with seven chemical elements. More details about the correlations among root traits can be consulted in Appendix A and Fig. 3.

Coordination between root and leaf traits

The leaf traits varied significantly among the tree species (Table S3) and the main trend in their variation followed the leaf economics spectrum - LES (Fig. S3 and Appendix A).

Differences in leaf habit (deciduous versus evergreen) will be mirrored by root traits variation, if there is coordination between plant organs. Effectively, deciduous trees presented significantly higher values of the acquisitive root trait SRL, and lower values of the conservative RMA, RDMC and RDI, compared to evergreen species. The concentrations of nutrients (K, P, Ca, Mg and S) were higher in the roots of deciduous trees than in those of evergreen trees; although, there was no significant difference for C or N. The accumulation of some essential (Fe, B and Zn) and non-essential (As, Pb and Sr) trace elements was also higher in the roots of deciduous trees (Table S4). By contrast, there was no difference between the leaf habit types in the root concentrations of the other trace elements or for the two isotope ratios.

The relative coordination between the root and leaf traits was also reflected by the positive correlations between some of the 24 analogous traits (Table 1), and by the significant linear correlation between the scores of the first axes of their respective PCAs (Fig. 4). In particular, the analogous morpho-functional traits RMA and LMA were highly correlated ($r=0.55$, $p=0.001$). Among the chemical elements, the root-leaf correlation was significant for C, the nutrients P, Ca and Mg, the trace elements Ba and Sr, and the isotope $\delta^{13}C$.

There was no significant root-leaf correlation for the concentrations of the other 14 elements and $\delta^{15}N$. For N, the root-leaf correlation was significant for samples of *Pinus* and *Populus*, but not for the other species. Some examples of the root-leaf discordance were due to the species-specific patterns of some traits, such as the accumulation of certain elements in leaves but not in roots: Cd in *Populus* and Mn in *Quercus* (Fig. S4).

Relationships between soil conditions and root traits

The spatial variation of soil properties was relatively homogeneous, in comparison with that of plant traits. There were significant differences among the tree species for only seven of the 21 variables tested in the soil underneath the trees: soil pH and available concentrations of S and some trace elements (B, Mn, Ba and Co) (Table S5).

The soil pH was correlated significantly and negatively with RMA, RDMC and RTD, and positively with SRL; no correlation was found with RDI. The soil organic C and available K were correlated significantly and negatively with RDMC and RTD. The soil Mg and $\delta^{15}N$ were correlated negatively with RMA and RTD. A significant and positive correlation with RDMC was also found for soil Mn, Ni and Cd (Table S6).

Of the 17 elements tested, the uptake and accumulation by roots were correlated with the availability in topsoil for only six of them: the macronutrient K, the micronutrients Co, Mn, Na and Zn and the non-essential Sr. A significant correlation between root and soil was also found for $\delta^{15}N$ (Table 1). Those tree-soil correlations would be affected by the difficulty of sampling the whole soil volume explored by the tree roots.

Discussion

Multidimensional root traits

The main trend in variation observed for the root traits of this study supports the root economics spectrum (RES) hypothesis. Trees with lighter roots, lower RMA and richer in N, K and P (like those of the deciduous *Fraxinus* and *Celtis* species) would maximise soil resource acquisition. Contrastingly, trees with denser roots, higher RDMC and lower concentrations of nutrients (like evergreen *Pinus* and *Quercus* species) would exhibit a resource conservation strategy. These results support the existence of a suite of correlated plant (roots and leaves) traits associated with the trade-off between resource conservation and fast growth, known as the “plant economics spectrum” (Freschet et al. 2010; Reich 2014; de la Riva et al. 2018a). However, not always root diameter, root tissue density and specific root length are correlated following the RES. For example, Zadworny et al. (2017) found in *Pinus* *sylvestris* that thicker roots have low densities due to a thicker cortex, thus making them less costly to construct and more suitable to association with mycorrhizal fungi, and enhancing nutrient acquisition. Also, Kong et al. (2019) found nonlinear root trait relationships between RDI, RTD and SRL, which can explain why SRL does not necessarily conform to the RTD-related plant economics spectrum in woody species.

The key morphological traits RMA and SRL, which are indicators of the root uptake potential, correlated with other morphological traits and with some major nutrients (P, K, Ca and Mg), which supports the uptake function of these traits. Phosphorus and K reach the roots mainly by diffusion from the bulk soil to the root surface (Lambers et al. 2008), and therefore a negative correlation between their uptake and RMA (but a positive one with SRL) would be expected. The correlation between RMA and root nutrients (other than N) has been overlooked previously, and this study is a relevant contribution to support the RES. Despite the fact that there are contradictory results concerning the SRL – root N relationship (see reviews in Reich 2014 and Weemstra et al. 2016), based on our data we can concur with Reich (2014) that the RES exists, although not as uniformly and strongly coordinated as the LES.

This evidence supporting the RES as a main root dimension does not preclude the existence of other root dimensions representing the multifunctionality of roots (Weemstra et al. 2016; Laliberté 2017).

Firstly, in this study, root C concentration was not correlated with the morphological root traits, but it was negatively correlated with the concentration of root N and 12 other chemical elements. Root C concentration, together with root branching traits (not measured here), defined the second dimension in the PCA of 14 root traits of 96 woody species from subtropical forests in China (Kong et al. 2014).

Secondly, soil resources are multiple (water and nutrients) and plant roots differ in their uptake strategies, such as mycorrhizas, N_2-fixing symbioses, and P-absorbing cluster roots (Lambers et al. 2008). The micronutrients Cu, Mn and Ni and the non-essential element Pb exhibited a trend that was orthogonal of that of the RES (Fig. 2). In particular, the Pb concentration in roots was correlated negatively with RMA and root C, but positively with 13 other elements. Among the root traits which confer improved tolerance to elevated metal concentrations in soils is the ability to bind trace elements to root cell walls and accumulate them belowground; in this way, roots may be barriers impeding the uptake of potentially toxic elements and their translocation to the leaves (Lambers et al. 2008, Zhao et al. 2016). This additional root dimension that confers metal tolerance may be very important for plant fitness in metal-rich environments, like the study site.

Thirdly, the N isotope composition ($\delta^{15}N$) has been used to infer symbiotic uptake of N by mycorrhizal fungi and its transfer to plants, due to the discrimination against heavier ^{15}N in these processes (Hobbie and Hobbie 2008; Hobbie and Högberg 2012). In this study, root $\delta^{15}N$ was a relatively-independent trait; it was correlated negatively with the root C concentration and $\delta^{13}C$ but there was no relationship with morphological or chemical traits (with the exception of root Co and Mn). Recently, Laliberté (2017) has suggested the use of the N isotope composition in plants as a time-integrated trait showing the mycorrhizal influence on N acquisition. Thus, we would expect lower $\delta^{15}N$ values in the roots of trees associated with ECM fungi, which discriminate against ^{15}N and preferentially transfer ^{14}N to their host plants; while no or only slight depletion of ^{15}N is expected for AM plants (Hobbie and Högberg 2012; Craine et al. 2015). However, we found that the $\delta^{15}N$ values in roots of ECM trees (mean=1.61, n=15) were not different from those in AM type trees (mean=1.40, n=20). The natural abundance of ^{15}N in plants is not easy to interpret because it is a single response variable with multiple drivers (i.e. climate, mycorrhizal fungi, and microbial processing; Craine et al. 2015).

Coordination of root and leaf traits

Fast plant growth depends on the coordination of roots and leaves, with the former providing enough water and nutrients supply to maintain acquisitive leaves with high photosynthetic rates and high evaporative demand (Reich 2014). In general, we found that the main root variation trend (PCA axis 1) was significantly correlated with the corresponding leaf variation trend (Fig. 4), supporting the existence of a plant economics spectrum (Pérez-Ramos et al. 2012, de la Riva et al. 2016b, 2018a).

In particular, we found significant correlations between morphological root traits (RMA and RDMC) and the analogous leaf traits (LMA and LMDC), supporting such root-leaf coordination, as reported in other studies (Holdaway et al. 2011; de la Riva et al. 2018a). However, there are exceptions: for instance, *Larix decidua* trees display acquisitive leaf traits, typical of deciduous trees, but conservative root traits, typical of conifers (Withington et al. 2006; Weemstra et al. 2016).

In this study, the C concentrations in roots and leaves were positively correlated. Villar et al. (2006) also found a positive correlation between root and leaf C in 16 woody species. The C concentration is normally high for species with strong structural defences (such as lignin or cellulose) (Poorter and Villar 1997) and, therefore, with a conservative strategy (de la Riva et al. 2016b). On the other hand, there was not a significant relationship between RMA and the root C concentration, although RMA was positively correlated with the C:N ratio (r=0.55, p=0.001). The C:N ratio reflects the relative investments in structure (mainly carbon) respect to cell metabolism (indicated by nitrogen). Thus, plant organs with a higher C:N ratio represent a conservative strategy (Villar et al. 2006; de la Riva et al. 2016c, 2018b).

Our results do not fit the previously-reported global trend of root and leaf N concentrations, which are highly correlated in woody species (n=89, r=0.58, p<0.001; Valverde-Barrantes et al. 2017). Trees with N-rich leaves and high photosynthetic rates are expected to have N-rich and exploitative fine roots (Reich 2014). However, this trend can be influenced by the specific symbiosis (type of mycorrhiza) present. In this study, we found a significant correlation only when analysing the subset of ECM tree species (r=0.72, p=0.002). However, tree species in a symbiosis with AM fungi did not show this root-leaf N relationship. The two types of mycorrhizal trees have different nutrient economies: ECM trees are able to acquire N from the soil organic matter due to the greater enzymatic capabilities of ECM fungi, while AM trees depend mostly on inorganic N (Phillips et al. 2013). In the N-limiting conditions of the study site, we expect ECM trees to be more efficient at taking up soil N, through the root-fungi symbiosis, and translocating it to their leaves. Kong et al. (2019) also found different relationships of root traits depending of mycorrhizal types (ECM versus AM). Thus, for ECM species, thin roots were related with higher root N concentration, but the contrary for AM species. This could explain the positive relationship found in our study between root N and leaf N only for ECM species.

The C isotope composition in leaf tissues is widely used as a functional trait representing the time-integrated measurement of water-use efficiency. It is based on the discrimination by photosynthetic enzymes against the heavier isotope ^{13}C during photosynthesis, and depends on the ratio between the internal and air CO_2 concentrations, in turn regulated by stomatal opening (Seibdt et al. 2008, Pérez-Harguindeguy et al. 2013). The $\delta^{13}C$ values in roots should reflect the isotopic signature of the carbohydrates synthesised in the leaves, although during the leaf-root translocation some ^{13}C enrichment in roots (relative to leaves) has been observed (Cernusak et al. 2009). In this study, the $\delta^{13}C$ values in roots and leaves were positively correlated (Table 1), indicating root-leaf coordination. In general, long-lived tissues are associated with a more-conservative use of resources and a higher efficiency in water-use, usually reflected in their higher $\delta^{13}C$ values (Reich 2014, de la Riva et al. 2016b). However, in this case, the $\delta^{13}C$ values in roots of deciduous and evergreen trees were not different (Table S4).

The plant N isotope composition reflects mainly the soil source of N, and also any isotope fractionation and N pool mixing (Robinson 2001). Although the root values for $\delta^{15}N$ did not show significant differences among species, when analysing the intra-plant fractionation (i.e. $\delta^{15}N_{root} - \delta^{15}N_{leaf}$) there were significant differences among species and mycorrhizal types (Fig. S5). The depletion of ^{15}N in ECM trees may be related to the preferential retention of ^{15}N by the ECM fungal biomass (but not by that of AM fungi) and the consequent transfer of ^{15}N-depleted N to the host trees (Craine et al. 2015).

Nutritional differences among tree species result from the functional diversity in mechanisms of nutrient uptake from soil, nutrient requirements and long-term nutrient use efficiency (Lambers et al. 2008). The coordinated variability in P, Ca and Mg concentrations between roots and leaves indicates that these nutrients are under biological control, due to their importance for plant growth (Newman and Hart 2006; Geng et al. 2014; Zhao et al. 2016).

In contrast, most of the trace elements had a strong discordance between their concentrations in roots and leaves. The excess uptake of non-limiting elements seems poorly regulated by plants, and therefore they exhibit high variability (Ladanai et al. 2010). Plants tend to accumulate trace elements in roots, binding them to cell walls as a detoxification mechanism (Domínguez et al. 2009; Kabata-Pendias 2011; Zhao et al. 2016). However, some tree species have a selective uptake and transport of certain trace elements, accumulating them in leaf tissues. Notable examples are the accumulation of Cd and Zn in *Populus* leaves (Madejón et al. 2004) and the accumulation of Mn in *Quercus* leaves (Madejón et al. 2006), but not in their roots (Fig. S4). In soils contaminated by trace elements, the adequate selection of plant species for phytostabilisation is essential. One of the main criteria is that the selected tree species control the mobility of the trace elements, keeping their root to shoot translocation factors as low as possible, to avoid toxicity risks in the trophic web (Mendez and Maier 2008; Bolan et al. 2011; Madejón et al. 2018b).

Root traits and soil conditions

There are reciprocal interactions and feedbacks between roots and soil. The soil conditions influence root traits and plasticity (Bardgett et al. 2014). In turn, roots modify the rhizospheric soil; for example through root exudates to increase nutrient uptake (Dakora and Phillips 2002). Here, soil pH was significantly related to the root morphological traits. On the one hand, this indicates that roots with traits indicative of lower exploration (higher RMA or lower SRL, as in *Pinus* and *Quercus*) could compensate with higher production of acid exudates to promote nutrient uptake, decreasing the soil pH (Dakora and Phillips 2002). On the other hand, this relationship between soil acidity and a conservative root strategy could be linked to the effects of the litter compounds of these species (with a high C:N ratio and high LMA) on soil. The accumulation of litter with a high C:N ratio, such as that of coniferous species, tends to have an acidifying effect on soil (Augusto et al. 1998; Sariyildiz et al. 2005; Alameda et al. 2012). As a consequence, soil pH usually decreases after the afforestation of grasslands or former agricultural lands with coniferous species (Jug et al. 1999; Sauer et al. 2007; Berthrong et al. 2012). The analysis of the amount and quality of root exudates would be needed to elucidate the causes behind the observed relationship between soil pH and RMA, besides the indirect effects of litter traits on soil chemistry. In any case, as suggested by

Laliberté (2017), it would be worth including root exudation as a physiological trait to advance in trait-based plant ecology.

In metal-rich soils low pH usually leads to a higher solubility of these elements and therefore to a high availability to roots. In this trace-element polluted site, we observed some significant relationships between soil metal content and some root traits; in particular, RDMC was positively correlated to the soil content of Mn, Ni and Cd. One of the first symptoms of plant toxicity to soil metals is the inhibition of root elongation (Kahle 1993; Wisniewski and Dickinson 2003). Other responses to metal toxicity are: collapsing of root hairs, increments of suberification and lignification, decrease of vessel diameter and structural alterations of hypodermis and endodermis (Arduini et al. 1994; Barceló and Poschenrieder 2004). Experimental exposure of *Quercus ilex* roots to Cd resulted in a decline in fine root production and in a reduction in the length of taproots (Domínguez et al. 2009), linked to a high capacity to retain Cd at the root level, likely by binding Cd to cell wall pectins. Thus, the links between pH, soil metal content, and RDMC observed in this study could also indicate a trend towards a more conservative strategy at the root level to promote the immobilization of these metals in the rhizosphere, avoiding their translocation to the aboveground biomass.

The root chemical traits were related to the availability in the soil for some nutrients (K, Mn, Na and Zn), as expected. However, the concentrations of many other elements in the roots were relatively independent of the soil conditions; this weak coupling between the soil and plant concentrations of chemical elements has been found in other studies (Ladanai et al. 2010; Zhao et al. 2016). The uptake and accumulation of nutrients in roots is a complex process which depends on numerous factors - such as the relative allocation within the plant, the developmental stage, the plant species and the environmental conditions (Lambers et al. 2008). More research is needed to understand how those factors affect the transfer of trace elements from the soil to the roots.

A particularly-interesting root physiological trait is the potential to reduce metal availability in soil, by several mechanisms like precipitation of metals, their complexation with organic products, their sorption onto root surfaces or their accumulation inside root tissues (Mendez and Maier 2008). The planting of tree species with higher phytostabilisation potential would improve and remediate metal-contaminated soils (Madejón et al. 2018b). In fact, one of the criteria used to select the best-suited tree species is to have a high bioconcentration factor (root:soil ratio) for different metals, in particular for those with harmful effects (i.e. Cd and Pb) (Madejón et al. 2018b).

Conclusion

There is increasing interest in advancing our knowledge about root traits because of their often-overlooked but essential contribution to plant functional ecology. Our results reinforce the existence of a root economics spectrum (RES) as the main determinant of fine root traits in Mediterranean trees, even in soil contaminated by heavy metals. However, this study also supports the idea of root multifunctionality and the importance of fine root dimensions independent of the RES; namely, root carbon concentration, fractionation of nitrogen isotopes as a time-integrated trait of mycorrhizal-mediated nutrition, and the ability to bind trace elements in root cells (associated with tolerance of high levels of metals in soils). We found that roots and leaves were functionally coordinated; however, most of trace elements showed strong root-leaf discordance. We also found links between soil pH, soil metal content, and root traits (RDMC) promoting the immobilization of metals in the rhizosphere. In summary, the rhizosphere is a complex environment where soil, roots and microorganisms interact in feedback processes. An understanding of the multifunctionality of root traits would help us to predict the forest responses to global changes and the provision of soil-based ecosystem services.

Acknowledgements

This work was financially supported by the European Union Seventh Framework Programme (FP7/2007–2013) (Grant No. 603498- RECARE), the Spanish Ministry of Science, Innovation and Universities (Grants No. CGL2014-52858-R-RESTECO, CGL2017-82254-R-INTARSU, and CGL2014-53236-R- ECO-MEDIT), and European FEDER funds. MG-M was supported by the Spanish Ministry of Economy and Competitiveness (Grant No. BES-2015-073882), and MTD by the Universidad de Sevilla (Contrato de Acceso, V Plan Propio de Investigación). We thank J. M. Murillo and J.M. Alegre for their help in the field work, the IRNAS Analytical Service for multielement analyses of plants and soil, and the EBD-CSIC Laboratory of Stable Isotopes for determinations of $\delta^{15}N$ and $\delta^{13}C$.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1. Correlations between root traits and analogous leaf traits (n=34), and between root chemical traits and soil availability of elements (n=35). Soil availability ($CaCl_2$ extracted) of As and Pb were below detectable limits. Pearson´s test coefficient *r* and *p* values are indicated; significant values (*p*<0.05) are in bold. RMA: root mass per area; LMA: leaf mass per area; LDMC: leaf dry matter content; RDMC: root dry matter content.

	Root-leaf		***Root-soil***	
Trait	***r***	***p***	***r***	***p***
RMA/LMA	**0.548**	**0.001**	-	-
LDMC/RDMC	**0.371**	**0.031**	-	-
C	**0.553**	**0.001**	-0.110	0.528
N	0.018	0.920	-0.020	0.910
P	**0.486**	**0.004**	-0.111	0.526
K	0.289	0.098	**0.379**	**0.025**
Ca	**0.826**	**<0.001**	0.166	0.340
Mg	**0.393**	**0.021**	0.163	0.348
B	0.075	0.675	0.025	0.887
Co	-0.250	0.154	**0.380**	**0.024**
Cu	0.087	0.623	-0.002	0.990
Fe	0.131	0.461	-0.073	0.678
Mn	-0.023	0.897	**0.357**	**0.035**
Na	0.051	0.775	**0.596**	**<0.001**
Ni	-0.070	0.695	-0.031	0.861
S	0.023	0.899	0.134	0.441
Zn	-0.093	0.602	**0.346**	**0.042**
As	-0.001	0.998	-	-
Ba	**0.516**	**0.002**	-0.028	0.872
Cd	0.019	0.914	0.071	0.686
Pb	0.331	0.056	-	-
Sr	**0.774**	**<0.001**	**0.381**	**0.024**
$\delta^{13}C$	**0.391**	**0.022**	-0.174	0.317
$\delta^{15}N$	0.165	0.351	**0.437**	**0.009**

Figure legends

Figure 1. Variation among tree species in root mass per area (RMA, g m^{-2}). Mean and SE (n=5) bars are shown; letters indicate significant differences between the tree species (Tukey´s post-hoc test). Deciduous species are marked in grey and evergreen species in black.

Figure 2. Results of the principal component analysis of 27 root traits of seven tree species (n=35). Scores of trait variables and tree samples are represented in the plane defined by first (PC1) and second (PC2) axes. Abbreviations of root traits are: RMA: root mass per area; RDMC: root dry matter content, RDI: root mean diameter; RTD: root tissue density; SRL: specific root length; and for tree species names are: C.a.: *Celtis australis*; C.s.: *Ceratonia siliqua*; F.a.: *Fraxinus angustifolia*; O.e.: *Olea europaea*; P.a.: *Populus alba*; P.p.: *Pinus pinea*; Q.i.: *Quercus ilex*. Symbol fills are in grey for deciduous and in black for evergreen species.

Figure 3. Correlogram across morphological and chemical root traits, ordered according to their correlation coefficients. The strength and direction of the correlations are indicated by the circle size and the colour, shown in the right side scale. RTD: root tissue density; RDMC: root dry matter content; RMA: root mass per area; RDI: root mean diameter; SRL: specific root length.

Figure 4. Correlation analysis between the main variation trends (PCA axis 1 scores) in leaf and root, comparing 24 analogous traits (r=0.59, p=0.0003). Abbreviations of tree species names are: C.a.: *Celtis australis*; C.s.: *Ceratonia siliqua*; F.a.: *Fraxinus angustifolia*; O.e.: *Olea europaea*; P.a.: *Populus alba*; P.p.: *Pinus pinea*; Q.i.: *Quercus ilex*. Symbol fills are in grey for deciduous and in black for evergreen species.

Figure 1

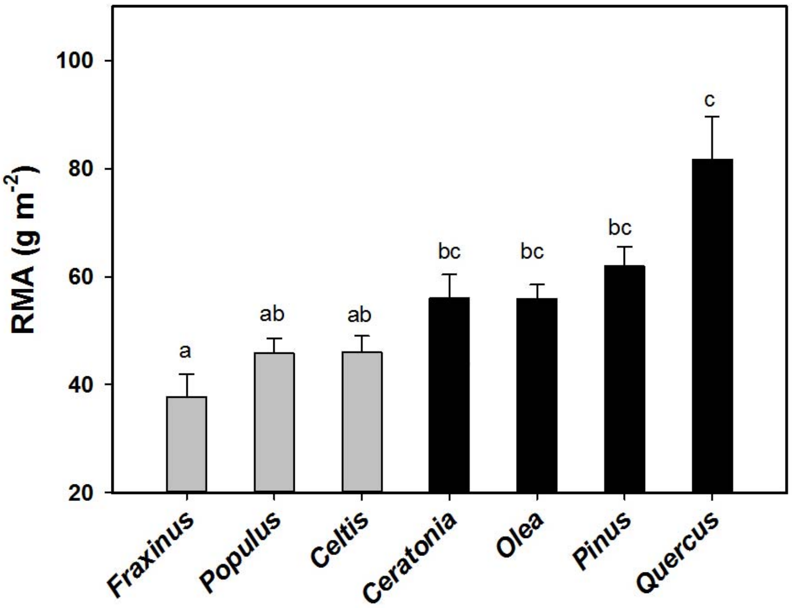
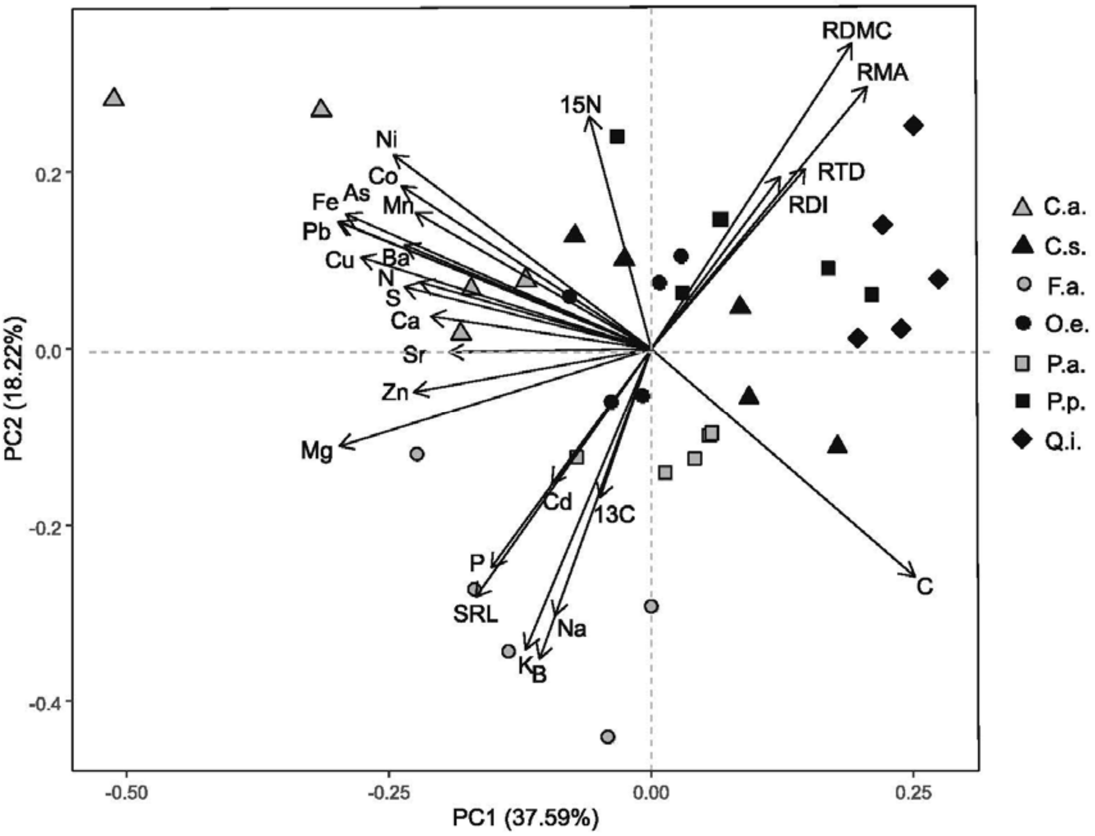

RMA (g m^{-2})
100
80
60
40
20
a
ab
ab
bc
bc
bc
c
Fraxinus
Populus
Celtis
Ceratonia
Olea
Pinus
Quercus

Figure 2



PC2 (18.22%)
PC1 (37.59%)
0.2
0.0
-0.2
-0.4
-0.50
-0.25
0.00
0.25
RDMC
RMA
RTD
RDI
15N
Ni
Co
As
Fe
Mn
Pb
Ba
Cu
N
S
Ca
Sr
Zn
Mg
Cd
13C
P
SRL
Na
K
B
C
C.a.
C.s.
F.a.
O.e.
P.a.
P.p.
Q.i.

Figure 3

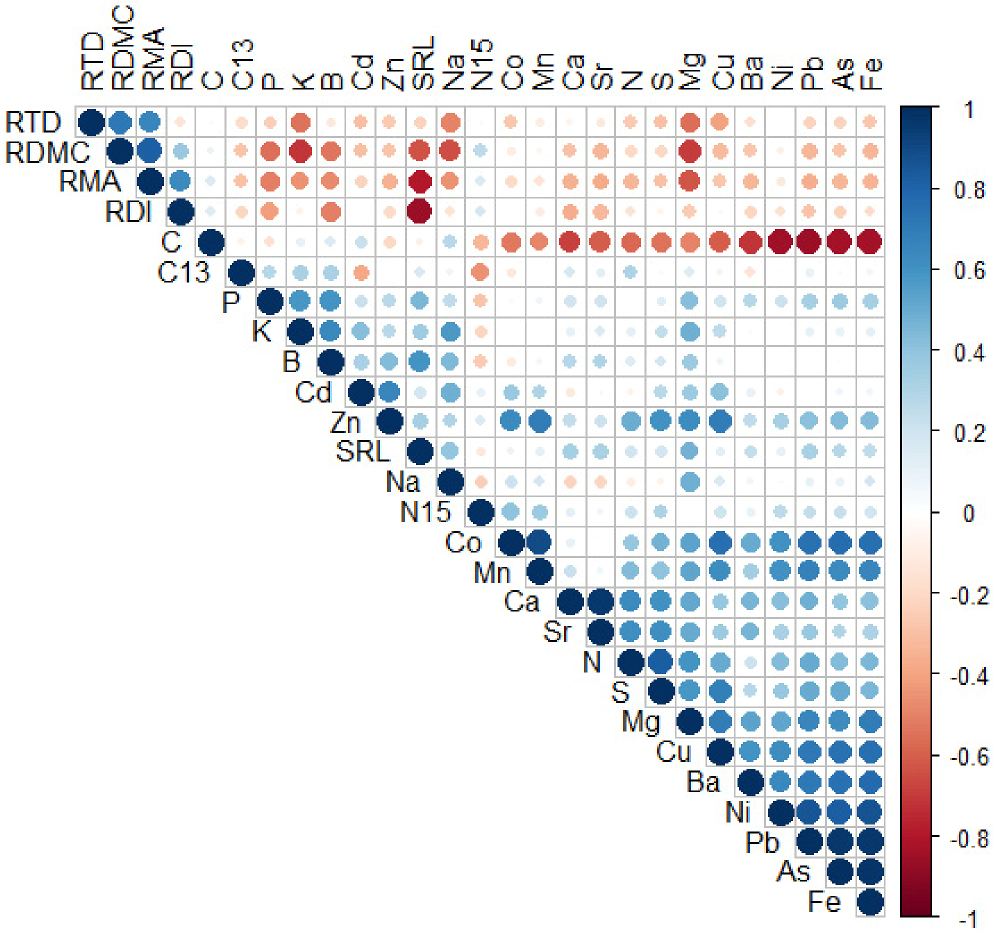
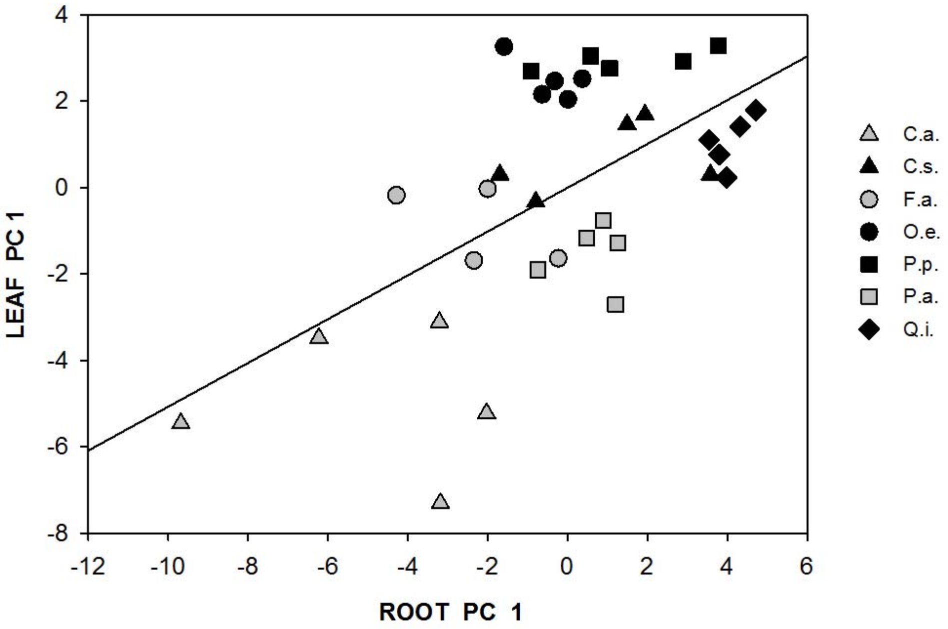

RTD
RDMC
RMA
RDI
C
C13
P
K
B
Cd
Zn
SRL
Na
N15
Co
Mn
Ca
Sr
N
S
Mg
Cu
Ba
Ni
Pb
As
Fe
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Figure 4



Electronic Supplemental Material (ESM)

Variation in morphological and chemical traits of Mediterranean tree roots: linkage with leaf traits and soil conditions

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Appendix A

Descriptive overview of root and leaf traits of seven tree species in the Guadiamar Green Corridor, SW Spain

Variation patterns and correlations among root traits

The variation in morphological root traits among individual trees (measured by CV) ranged from 21.1% (for RTD) up to 51.1% (SRL); whereas the CV of chemical traits ranged from 3.7% (for $\delta^{13}C$) up to 94.2% (As) (Fig. S1). There were significant differences among tree species for 26 out of the 27 root traits, with the exception of $\delta^{15}N$ (Table S1). For example, RMA trait showed extreme values for *Fraxinus* (mean of 38, with minimum of 26 g m^{-2}) and *Quercus* (mean of 82, with maximum of 109 g m^{-2}), and intermediate for the other species.

Root mass per area was a key root trait, positively correlated with other morphological traits (i. e. RDMC, RTD and RDI) but negatively correlated with SRL. There was also a significant and negative correlation between RMA and the concentration of major nutrients (N, Ca, Mg, K and P), some micronutrients (B, Fe, Na and Zn) and even with some non-essential elements (As, Pb and Sr). On the contrary, RMA was not correlated with the root concentration of carbon nor sulphur, neither with other trace elements (Cd, Co, Cu, Mn, Ni and S) nor with $\delta^{13}C$ and $\delta^{15}N$.

Specific root length, another key root trait widely used, was negatively correlated with all morphological traits (except with RTD), and positively correlated with seven elements (B, P, Mg, Na, Pb, Ca and Sr). Interestingly, SRL was correlated neither with root carbon nor with nitrogen.

The concentrations of carbon and nitrogen in roots were negatively correlated between them. Carbon was negatively correlated with most of the chemical elements, in special with Fe, Pb, As and Ni, while nitrogen was positively correlated with Ca, S, Mg and Cu. Magnesium concentration in roots was correlated with all other chemical elements; in contrast, root potassium and phosphorus correlated only with some chemical elements.

Isotopic composition of carbon ($\delta^{13}C$) in roots was negatively correlated with isotopic composition of nitrogen ($\delta^{15}N$) and Cd concentration. Root $\delta^{15}N$ was negatively correlated with carbon and positively with Mn and Co concentrations.

Root traits showed a strong variation among individual trees and differed significantly among tree species (Fig. S1 and Table S1). In general, root macronutrients were less variable than micronutrients; this difference may be related to nutrients limitation and internal regulation. Chemical elements under biological control, like carbon, nitrogen and phosphorus, showed lower variability. In contrast, non-essential trace elements (i.e. As and Pb) were highly variable; the reason may be that plants take up non-limiting elements in concentrations exceeding physiological needs and they tend to be highly variable (Ladanai et al. 2010). According to the hypothesis of stability of limiting elements, those elements most required for plant growth (macronutrients) would be less variable and would present a lower sensitivity to environmental variation (Zhao et al. 2016).

Carbon isotope ratio ($\delta^{13}C$) was the less variable root trait but still presented significant differences among tree species. Root $\delta^{13}C$ is mostly influenced by the isotope signature of photosynthate transported from leaves, and by some fractionation during that transport. Ouimette et al. (2013) found small enrichment in ^{13}C with decreasing root order, expected because their higher distance from foliage, although acknowledging also the importance of stored carbon reserves in the root construction. On the other hand, nitrogen isotope ratio ($\delta^{15}N$) in roots was much more variable among trees than $\delta^{13}C$ (Fig. S1), but the differences among tree species were not significant, probably due to the high individual variability. The variation of root $\delta^{15}N$ is mostly influenced by the isotope signature of surrounding soil and the discrimination of associated mycorrhizal fungi (Hobbie and Högberg 2012, Ouimette et al.

2013). We found a depletion of ^{15}N from root to leaf in ectomycorrhizal trees that could be related to the preferential retention of ^{15}N by the fungal biomass (Craine et al. 2015).

There was a general consistency in the correlations across root traits. The key morphological traits RMA and SRL, which are indicators of the root uptake potential, correlated with other morphological traits (i. e. RDMC, RTD and RDI). A correlation was also found of RMA and SRL with most of the root chemical elements, including major nutrients (N, P, K, Ca and Mg) which support these traits absorption function. In the case of phosphorus and potassium, both nutrients enter the roots mainly by diffusion from the bulk soil to the root surface (although the diffusion coefficient is relatively large for potassium and very low for phosphorus; Lambers et al. 2008); therefore a negative correlation between their uptake and RMA (but positive with SRL) would be expected.

Variations in aboveground traits

There were significant differences among tree species for all aboveground traits, with the exception of $\delta^{13}C$ (Table S3). The main variation trend (first PCA axis, accounting for 27% of variance) ordered the aboveground traits according to the leaf economics spectrum, separating LMA and leaf carbon, from Ca, Fe and other elements (Sr, Ba, Na) (Fig. S3). This first axis of leaf traits clearly separated deciduous and evergreen tree species. The secondary trend (second PCA axis, accounting for 18% of variance) was explained by tree size (HEIG) and leaf Cd and Zn concentrations, clearly separating *Populus* trees (the tallest tree species and the highest Cd and Zn accumulators) from the rest of species. On the opposite side, slower-growth *Quercus* and *Olea* species were associated with higher LMA and LDMC, and higher leaf Mn concentrations.

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Table S1. Mean values ± SE (n=5) of **root traits** of the studied tree species. *F*-statistics from one-way ANOVA test or C*hi-square-value* from Kruskal Wallis test (marked with ^{K}superscript) are indicated, depending on data normality and homoscedasticity. Significant level is $p < 0.05$ (in bold). The type of colonization with ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi that the different species can form is also indicated. RDI: root mean diameter; RDMC: root dry matter content; RTD: root tissue density; SRA: specific root area; SRL: specific root length.

	Celtis australis		***Ceratonia siliqua***		***Fraxinus angustifolia***		***Olea europaea***		***Pinus pinea***		***Populus alba***		***Quercus ilex***		**Effect of tree species**	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Statistic*	*p*
Structural																
RDI (mm)	0.35	0.01	0.53	0.04	0.40	0.05	0.50	0.01	0.49	0.02	0.36	0.04	0.49	0.03	15.9^K	**0.01**
RDMC (mg g^{-1})	306.4	26.3	314.1	12.7	198.1	16.6	304.4	17.5	368.0	10.2	297.3	11.4	431.8	12.4	25.6^K	**<0.01**
RMA (g m^{-2})	46.0	3.0	56.0	4.5	37.8	4.2	55.9	2.6	61.9	3.7	45.8	2.9	81.7	7.9	10.4	**<0.01**
RTD (mg cm^{-3})	520.2	23.9	426.8	22.0	383.6	36.1	446.7	18.5	513.5	35.0	519.3	41.7	659.0	31.0	8.4	**<0.01**
SRL (m g^{-1})	20.1	1.7	11.6	1.8	24.2	4.9	11.5	0.8	10.9	0.9	21.0	3.9	8.5	1.2	7.5	**<0.01**
Root chemistry																
C (%)	37.8	2.0	43.9	0.8	46.2	0.6	43.6	0.5	45.3	0.8	44.6	0.3	45.8	0.1	19.9^K	**<0.01**
N (%)	1.83	0.23	1.35	0.19	0.99	0.05	1.18	0.05	0.59	0.04	0.78	0.03	0.53	0.04	29.6	**<0.01**
P (mg kg^{-1})	900.7	109.4	633.0	63.1	1142.0	76.2	808.4	45.6	560.7	44.9	1120.2	54.7	720.1	54.5	11.4	**<0.01**
K (mg kg^{-1})	6326.2	689.1	7490.7	542.5	12784.5	619.7	6156.2	906.3	4374.8	219.5	6640.5	619.5	5911.5	653.4	12.8	**<0.01**
Ca (mg kg^{-1})	29278.5	4358.8	15495.0	3665.3	9448.2	434.5	7138.8	614.9	3278.3	130.4	16260.8	1453.0	4542.9	554.3	28.8^K	**<0.01**
Mg (mg kg^{-1})	2060.1	132.7	1373.5	150.2	2005.5	109.9	1798.9	215.0	1193.5	61.2	1440.7	126.0	532.2	19.7	42.5	**<0.01**
S (mg kg^{-1})	3180.1	510.5	2646.5	762.8	2097.9	232.9	1718.6	173.7	1342.3	160.3	1981.7	241.8	904.2	121.0	6.4	**<0.01**
As (mg kg^{-1})	22.2	7.2	5.2	0.9	10.7	2.2	11.6	1.4	7.8	2.8	6.5	1.6	1.9	0.4	8.8	**<0.01**
B (mg kg^{-1})	16.8	1.5	11.7	1.1	24.4	2.5	12.3	1.3	9.2	0.3	19.4	1.2	13.9	0.8	26.5^K	**<0.01**
Ba (mg kg^{-1})	16.7	3.8	10.1	1.5	10.6	1.1	13.0	1.4	11.8	1.6	13.4	1.8	5.9	1.5	2.9	**0.03**
Cd (mg kg^{-1})	0.80	0.17	1.13	0.36	2.36	0.46	0.95	0.15	1.90	0.16	1.49	0.17	0.67	0.10	6.5	**<0.01**
Co (mg kg^{-1})	3.54	0.83	1.25	0.36	2.45	0.55	2.57	0.18	3.07	0.59	1.05	0.16	1.07	0.23	4.6	**<0.01**
Cu (mg kg^{-1})	138.7	26.1	98.1	34.9	120.2	18.5	114.8	7.6	85.2	25.8	77.6	10.5	45.0	10.1	12.0^K	**0.06**
Fe (mg kg^{-1})	6070.2	1530.1	1729.7	270.8	3324.1	585.3	3924.8	277.2	2527.4	775.1	1825.0	292.6	652.3	133.0	10.9	**<0.01**
Mn (mg kg^{-1})	157.6	14.5	42.8	9.1	105.4	25.3	96.4	9.5	107.0	20.3	45.5	4.2	67.0	10.8	7.3	**<0.01**
Na (mg kg^{-1})	350.0	43.8	241.0	23.4	1644.9	170.6	912.1	208.4	809.3	36.5	364.6	72.0	119.6	14.0	30.3^K	**<0.01**
Ni (mg kg^{-1})	17.2	4.6	4.1	0.9	6.1	1.3	7.3	0.6	6.2	1.7	4.0	0.5	4.3	0.5	16.9^K	**0.01**
Pb (mg kg^{-1})	74.9	17.0	15.3	2.7	35.2	7.8	36.0	5.4	22.7	7.6	16.7	2.8	6.4	1.4	11.5	**<0.01**
Sr (mg kg^{-1})	48.3	6.8	34.4	7.6	18.8	1.2	13.8	1.6	6.1	0.3	35.3	4.4	6.9	0.6	32.9	**<0.01**
Zn (mg kg^{-1})	172.9	22.7	107.5	30.7	187.0	42.7	138.0	12.4	118.2	9.7	130.3	18.8	76.8	11.9	13.5^K	**0.04**
Root isotopes																
$\delta\ ^{13}C$ (‰)	-25.9	0.2	-26.3	0.6	-26.0	0.4	-25.9	0.3	-27.9	0.3	-26.8	0.3	-26.2	0.3	15.7^K	**0.02**
$\delta\ ^{15}N$ (‰)	2.14	0.31	1.48	0.77	1.06	0.45	0.90	0.42	2.13	0.30	1.01	0.19	1.69	0.08	1.6	0.19
Mycorrhizal type																
AM	Yes		Yes		Yes		Yes		Yes		Yes		Yes			
ECM	No		No		No		No		Yes		Yes		Yes			

Table S2. Results of the principal component analyses (PCA) for the six key root traits (see ordination in Figure S2) and for all 27 traits (see Figure 2), indicating variance explained by the three main axes and standardized factor loading of each root trait. The highest scores (in absolute value) for each axis are marked in bold. See main text for abbreviations of trait names.

	6 key traits PCA			***All-traits PCA***		
Traits	***Axis 1*** (57.3%)	***Axis 2*** (23.4%)	***Axis 3*** (14.7%)	***Axis 1*** (37.6%)	***Axis 2*** (18.2%)	***Axis 3*** (11.0%)
RMA	**0.97**	-0.06	0.05	**0.61**	**0.61**	0.13
SRL	**-0.87**	-0.42	-0.04	-0.49	-0.58	-0.23
RDMC	**0.87**	-0.28	0.23	0.56	**0.71**	-0.01
RTD	0.60	**-0.76**	0.16	0.43	0.42	-0.31
RDI	0.68	**0.71**	-0.06	0.36	0.40	0.45
N	-0.38	0.25	**0.89**	**-0.65**	0.15	-0.26
C				**0.74**	-0.53	0.34
Ca				**-0.62**	0.07	**-0.62**
Mg				**-0.88**	-0.23	0.09
K				-0.35	**-0.70**	0.17
S				**-0.70**	0.14	-0.05
P				-0.45	-0.51	-0.11
As				**-0.86**	0.31	0.04
B				-0.31	**-0.72**	-0.12
Ba				**-0.69**	0.24	-0.09
Cd				-0.28	-0.31	**0.69**
Co				**-0.70**	0.38	0.50
Cu				**-0.82**	0.21	0.30
Fe				**-0.88**	0.29	0.06
Mn				**-0.66**	0.32	0.37
Na				-0.27	**-0.62**	0.51
Ni				**-0.73**	0.45	-0.08
Pb				**-0.88**	0.29	-0.04
Sr				-0.57	-0.01	**-0.61**
Zn				**-0.67**	-0.10	0.40
$\delta^{13}C$				-0.14	-0.35	-0.41
$\delta^{15}N$				-0.17	0.54	0.21

Table S3. Mean values and SE (n=5) of **aboveground traits** of the studied tree species (except n=4 for leaf traits of *Fraxinus*). *F*-statistics from one-way ANOVA test or C*hi-square-value* from Kruskal Wallis test (marked with ^{K}supersript) are shown, depending on data normality and homoscedasticity. Significant level is $p < 0.05$ (in bold). LDMC: leaf dry matter content; LMA: leaf mass per area; RDI: root mean diameter; SRL: specific root length; SDMC: stem dry matter content; SWD: stem wood density; HEI: tree height; CRP: crown projection area; LITT: litter accumulation on soil surface.

	Celtis australis		***Ceratonia siliqua***		***Fraxinus angustifolia***		***Olea europaea***		***Pinus pinea***		***Populus alba***		***Quercus ilex***		**Effect of tree species**	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Statistic*	*p*
Structural																
LDMC (mg g^{-1})	501.2	8.7	398.3	14.0	409.9	34.1	510.0	15.4	397.1	18.9	466.6	8.0	548.1	12.4	14.0	**<0.01**
LMA (g m^{-2})	153.1	12.1	157.4	13.1	107.8	6.7	237.4	19.2	310.8	17.0	111.4	6.8	229.0	11.0	30.3^{K}	**<0.01**
SDMC (mg g^{-1})	571.4	9.5	381.9	17.5	551.7	21.9	478.2	30.0	357.4	6.4	492.7	13.0	526.7	4.9	26.0	**<0.01**
SWD (mg cm^{-3})	796.3	37.9	829.2	85.7	861.1	30.8	878.6	60.8	520.8	29.4	780.0	57.2	909.6	80.4	5.0	**<0.01**
Leaf chemistry																
C (%)	41.5	0.5	50.3	0.5	46.5	0.8	50.1	0.7	49.8	0.1	46.6	0.4	48.8	0.2	42.8	**<0.01**
N (%)	1.33	0.10	1.53	0.11	1.91	0.22	1.28	0.09	1.28	0.07	1.57	0.08	1.31	0.02	4.6	**<0.01**
P (mg kg^{-1})	671.3	56.6	759.9	81.3	1584.7	275.7	815.5	71.0	630.4	67.8	1103.6	103.4	911.2	55.5	9.9	**<0.01**
K (mg kg^{-1})	6605.2	371.3	7324.1	767.7	8514.2	845.6	9744.1	553.6	5076.9	265.0	7241.5	354.2	5392.1	250.1	11.1	**<0.01**
Ca (mg kg^{-1})	57421.4	5032.2	19745.1	3081.3	27183.0	3898.4	10313.9	723.2	3615.3	51.3	23154.7	1677.0	6755.1	554.5	31.1^{K}	**<0.01**
Mg (mg kg^{-1})	3238.2	242.6	2228.6	162.1	3361.0	684.1	836.2	122.2	2472.4	80.9	3698.3	482.5	1079.2	58.1	14.1	**<0.01**
S (mg kg^{-1})	556.2	52.1	1826.1	202.5	7070.7	1375.6	1633.8	122.3	822.6	38.3	2532.2	521.9	668.1	77.0	51.0	**<0.01**
As (mg kg^{-1})	0.22	0.10	0.07	0.05	0.67	0.03	0.62	0.04	0.71	0.14	0.62	0.11	0.43	0.06	21.9^{K}	**<0.01**
B (mg kg^{-1})	87.8	10.4	53.1	6.3	18.7	1.1	13.3	0.9	35.2	2.1	77.7	8.2	79.0	7.2	81.5	**<0.01**
Ba (mg kg^{-1})	17.9	4.1	5.7	0.8	6.4	1.1	2.3	0.8	0.3	0.0	8.9	1.2	6.1	1.1	51.9	**<0.01**
Cd (mg kg^{-1})	0.18	0.03	0.27	0.07	0.06	0.02	0.05	0.00	0.09	0.01	2.86	0.90	0.14	0.03	24.7^{K}	**<0.01**
Co (mg kg^{-1})	0.19	0.04	0.23	0.03	0.32	0.05	0.35	0.04	0.82	0.05	1.37	0.20	0.41	0.02	32.4	**<0.01**
Cu (mg kg^{-1})	11.5	3.6	3.4	0.4	10.2	1.8	7.1	0.7	2.5	0.2	8.9	0.7	6.5	0.3	25.6^{K}	**<0.01**
Fe (mg kg^{-1})	157.2	17.3	72.9	10.1	111.9	18.4	58.7	2.3	66.6	4.7	102.1	13.3	116.8	16.0	7.9	**<0.01**
Mn (mg kg^{-1})	150.6	27.1	53.3	11.2	27.3	4.9	24.8	1.6	151.1	20.3	87.6	16.6	526.4	93.8	32.2	**<0.01**
Na (mg kg^{-1})	715.9	49.0	227.5	30.5	493.1	91.6	159.1	28.7	89.9	24.5	325.1	36.0	131.3	19.4	31.4	**<0.01**
Ni (mg kg^{-1})	0.52	0.07	0.69	0.09	1.84	0.36	2.19	0.67	0.97	0.18	1.48	0.28	0.63	0.12	6.0	**<0.01**
Pb (mg kg^{-1})	1.54	0.52	0.28	0.11	0.61	0.10	0.64	0.11	0.56	0.09	0.76	0.11	0.88	0.21	14.5^{K}	**0.03**
Sr (mg kg^{-1})	106.4	10.0	38.3	6.4	43.6	8.9	14.3	2.2	2.7	0.2	58.2	5.4	7.4	0.5	117.3	**<0.01**
Zn (mg kg^{-1})	57.0	17.0	36.8	9.2	13.9	2.7	40.2	8.1	52.3	4.8	524.6	66.7	60.1	12.9	32.7	**<0.01**
Leaf isotopes																
$\delta\,^{13}C$ (‰)	-27.2	0.2	-26.9	1.0	-27.2	0.3	-26.5	0.3	-27.4	0.2	-28.3	0.5	-27.8	0.3	1.7	0.17
$\delta\,^{15}N$ (‰)	4.30	0.61	3.95	0.56	1.59	0.42	1.49	0.46	1.09	0.38	0.69	0.22	0.97	0.27	12.1	**<0.01**
Whole tree																
HEIG (m)	6.1	0.5	7.2	0.4	6.6	0.3	4.2	0.3	9.0	0.4	11.7	1.3	3.9	0.2	33.4	**<0.01**
CRP (m^{2})	79.0	9.3	64.3	10.4	53.8	5.3	42.0	6.5	97.3	14.0	176.7	44.5	22.2	4.5	14.1	**<0.01**
LITT (Kg)	35.4	4.6	84.9	21.6	10.9	3.0	14.0	1.9	95.4	13.1	63.7	21.9	12.9	3.8	25.3^{K}	**<0.01**

Table S4. Comparison between root traits of trees, according to their leaf habit (deciduous or evergreen); trait units are like in Table S1. They have been ranked by the significance level (ANOVA´s *F* and *p*) marking the difference between leaf habits. Significant level is $p < 0.05$ (in bold). SRL: specific root length; RDI: root mean diameter; RMA: root mass area; RDMC: root dry matter content; RTD: root tissue density.

Root trait	Deciduous (n=15)		Evergreen (n=20)		ANOVA statistics	
	Mean	*SE*	*Mean*	*SE*	*F*	*p*
B	20.21	1.29	11.77	0.58	42.4	**<0.001**
P	1054.3	53.2	680.5	32.2	40.0	**<0.001**
SRL	21.76	2.05	10.62	0.65	33.7	**<0.001**
RDI	0.37	0.02	0.50	0.01	27.9	**<0.001**
RMA	43.20	2.08	63.89	3.34	23.2	**<0.001**
RDMC	267.3	16.6	354.6	13.2	17.4	**<0.001**
Ca	18329.2	2619.7	7613.8	1391.3	14.9	**<0.001**
Mg	1835.4	99.7	1224.5	121.6	13.7	**<0.001**
Sr	34.15	4.10	15.29	3.17	13.7	**<0.001**
K	8583.7	865.9	5983.3	384.9	8.9	**0.005**
Zn	163.4	17.3	110.1	9.80	8.1	**0.008**
Pb	42.28	8.72	20.10	3.36	6.9	**0.013**
As	13.15	2.97	6.62	1.10	5.2	**0.029**
S	2419.9	237.4	1652.9	236.7	5.0	**0.032**
Fe	3739.8	696.0	2208.6	339.8	4.6	**0.040**
Ba	13.57	1.50	10.17	0.92	4.1	0.051
Ni	9.11	2.14	5.49	0.57	3.4	0.073
N	1.20	0.14	0.92	0.09	3.0	0.092
C	42.9	1.17	44.6	0.36	2.7	0.111
Cu	112.2	12.4	85.8	11.9	2.3	0.141
Mn	102.8	15.3	78.3	8.4	2.2	0.143
Cd	1.55	0.23	1.16	0.14	2.2	0.148
Na	786.5	172.5	520.5	93.0	2.1	0.157
RTD	474.3	25.3	511.5	24.4	1.1	0.305
δ^{13}C	-26.2	0.19	-26.6	0.25	1.0	0.314
Co	2.35	0.4	1.99	0.5	0.6	0.444
δ^{15}N	1.40	0.2	1.55	0.2	0.2	0.664

Table S5. Mean values and SE (n=5) of **topsoil parameters** (0-10cm depth) associated to the studied tree species, and adjacent open sites, for comparison; pH, organic C, total N, available concentrations of nutrients and trace elements, and C and N isotope ratios. *F*-statistics from one-way ANOVA test or *Chi-square-value* from Kruskal Wallis test (marked with ^{K}superscript) are indicated, depending on data normality and homoscedasticity (only soil samples under trees were compared). Significant level is $p < 0.05$ (in bold).

	Celtis australis		***Ceratonia siliqua***		***Fraxinus angustifolia***		***Olea europaea***		***Pinus pinea***		***Populus alba***		***Quercus ilex***		**Without tree**		**Effect of tree species**	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Statistic*	*p*
Soil chemistry																		
pH	4.2	0.5	4.7	0.4	4.9	0.4	3.9	0.2	3.2	0.2	4.7	0.4	3.6	0.2	4.0	0.3	3.3	**0.01**
Org. C (%)	1.62	0.13	1.90	0.25	1.79	0.27	1.76	0.15	1.14	0.11	1.57	0.31	1.45	0.18	1.35	0.09	1.6	0.18
N (%)	0.12	0.01	0.14	0.01	0.17	0.03	0.16	0.01	0.12	0.01	0.15	0.02	0.14	0.02	0.11	0.01	7.6^{K}	0.27
P (mg kg^{-1})	14.9	5.0	13.0	1.7	13.4	2.7	9.3	1.1	15.9	2.6	13.3	1.6	10.9	0.8	13.4	3.6	5.3^{K}	0.51
K (mg kg^{-1})	89.8	32.3	223.2	46.2	210.6	36.9	190.4	35.4	124.4	20.5	189.6	28.5	131.0	27.4	151.8	23.7	2.3	0.07
Ca (mg kg^{-1})	2196.0	289.7	1988.0	271.8	1964.0	314.7	1360.2	233.0	1870.0	393.2	1674.0	220.5	1482.8	327.5	1310.0	92.8	1.0	0.44
Mg (mg kg^{-1})	125.5	27.0	138.8	25.2	126.2	14.1	95.1	10.6	99.1	19.3	131.7	24.5	70.3	6.8	92.8	9.4	10.0^{K}	0.12
S (mg kg^{-1})	1228.5	939.0	35.3	17.4	227.7	175.6	187.0	170.7	1318.3	448.3	196.9	153.4	612.9	330.5	337.7	320.5	14.8^{K}	**0.02**
As (mg kg^{-1})	<0.01	-	<0.01	-	<0.01	-	<0.01	-	<0.01	-	<0.01	-	<0.01	-	<0.01	-	-	-
B (mg kg^{-1})	0.83	0.19	0.78	0.16	0.40	0.03	0.32	0.02	0.34	0.08	0.63	0.09	0.42	0.05	0.35	0.03	5.5	**<0.01**
Ba (mg kg^{-1})	0.40	0.15	1.73	0.34	0.88	0.16	1.32	0.23	0.66	0.12	0.68	0.14	1.28	0.62	1.90	0.37	13.5^{K}	**0.04**
Cd (mg kg^{-1})	0.15	0.08	0.09	0.03	0.12	0.06	0.16	0.02	0.19	0.04	0.06	0.04	0.17	0.03	0.12	0.03	1.0	0.44
Co (mg kg^{-1})	0.42	0.34	0.08	0.03	0.17	0.11	0.29	0.09	0.70	0.21	0.08	0.07	0.29	0.10	0.23	0.18	14.5^{K}	**0.03**
Cu (mg kg^{-1})	5.97	5.11	0.56	0.15	0.71	0.26	1.54	0.48	5.83	2.08	1.34	1.15	2.63	0.91	1.68	1.45	11.1^{K}	0.08
Fe (mg kg^{-1})	21.0	19.2	1.6	0.4	1.5	0.3	2.1	0.4	4.6	1.6	1.8	0.3	2.3	0.5	2.4	1.2	1.7	0.15
Mn (mg kg^{-1})	18.6	10.8	9.2	2.7	12.1	6.3	17.8	3.2	31.2	7.6	8.2	3.2	23.6	4.0	20.6	7.4	14.4^{K}	**0.03**
Na (mg kg^{-1})	43.8	2.6	50.3	1.2	54.9	4.9	55.4	2.3	49.7	1.4	41.8	2.5	42.3	1.7	45.3	5.1	4.8	**0.02**
Ni (mg kg^{-1})	0.32	0.22	0.15	0.06	0.19	0.09	0.32	0.06	0.54	0.14	0.15	0.07	0.44	0.09	0.43	0.09	12.2^{K}	0.06
Pb (mg kg^{-1})	<0.01	-	<0.01	-	<0.01	-	<0.01	-	0.02	0.01	0.11	0.02	0.15	0.03	0.09	0.06	-	-
Sr (mg kg^{-1})	3.11	0.20	3.91	0.34	3.26	0.32	2.94	0.31	2.47	0.27	3.25	0.36	2.69	0.22	3.58	0.29	2.5	0.05
Zn (mg kg^{-1})	26.8	20.9	7.1	3.3	10.4	6.7	15.7	3.3	28.1	8.9	5.3	3.8	15.7	2.3	12.5	5.1	10.8^{K}	0.10
Soil isotopes																		
δ^{13}C (‰)	-27.3	0.2	-27.3	0.3	-27.3	0.2	-27.1	0.2	-26.6	0.3	-26.6	0.4	-27.0	0.2	-26.7	0.5	1.2	0.34
δ^{15}N (‰)	2.97	0.29	2.46	0.71	2.23	0.45	2.20	0.26	2.05	0.27	1.80	0.43	1.37	0.64	0.69	0.34	1.2	0.35

Table S6. Correlations of root morphological traits with soil chemical variables (pH, organic C, total N, available concentrations of nutrients and trace elements, and C and N isotope ratios). Significant values ($p<0.05$) are in bold. RMA: root mass per area; SRL: specific root length; RDI: root mean diameter; RDMC: root dry matter content; RTD: root tissue density.

Soil property	**Morphofunctional root trait**									
	RMA		**SRL**		**RDI**		**RDMC**		**RTD**	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
pH	**-0.51**	**0.002**	**0.35**	**0.040**	-0.26	0.130	**-0.57**	**<0.001**	**-0.42**	**0.013**
C	-0.33	0.052	0.22	0.210	-0.07	0.690	**-0.35**	**0.040**	**-0.35**	**0.041**
N	-0.27	0.114	0.22	0.205	-0.07	0.704	-0.32	0.059	-0.30	0.076
P	-0.16	0.361	0.18	0.303	-0.22	0.194	0.09	0.597	0.03	0.856
K	-0.28	0.101	0.12	0.490	0.01	0.954	**-0.41**	**0.014**	**-0.35**	**0.037**
Ca	-0.32	0.061	0.32	0.061	-0.24	0.161	-0.20	0.252	-0.20	0.253
Mg	**-0.40**	**0.016**	0.27	0.123	-0.12	0.494	-0.29	0.095	**-0.37**	**0.027**
S	0.08	0.637	0.05	0.782	-0.14	0.420	0.32	0.058	0.25	0.142
B	-0.21	0.235	0.11	0.537	-0.13	0.473	-0.12	0.484	-0.09	0.600
Ba	0.13	0.451	-0.22	0.197	0.25	0.147	0.12	0.485	-0.04	0.834
Cd	0.26	0.127	-0.11	0.519	0.07	0.691	**0.36**	**0.036**	0.30	0.081
Co	0.13	0.459	-0.04	0.799	0.003	0.985	0.32	0.061	0.17	0.325
Cu	0.04	0.814	0.06	0.727	-0.10	0.552	0.33	0.052	0.16	0.354
Fe	-0.11	0.526	0.15	0.384	-0.22	0.211	0.19	0.261	0.08	0.632
Mn	0.28	0.100	-0.15	0.396	0.09	0.600	**0.43**	**0.009**	0.29	0.090
Na	-0.17	0.337	-0.01	0.987	0.16	0.359	**-0.41**	**0.015**	**-0.38**	**0.026**
Ni	0.32	0.060	-0.15	0.389	0.10	0.587	**0.45**	**0.006**	0.32	0.057
Sr	**-0.38**	**0.023**	0.23	0.178	-0.15	0.379	**-0.36**	**0.036**	-0.33	0.056
Zn	0.08	0.661	0.01	0.934	-0.07	0.683	0.31	0.067	0.19	0.271
δ^{13}C	0.07	0.688	-0.04	0.825	-0.004	0.982	0.21	0.235	0.06	0.749
δ^{15}N	**-0.39**	**0.020**	0.20	0.262	-0.11	0.545	-0.19	0.271	**-0.37**	**0.027**

Figure S1. Rank of relative variation in root traits and their analogue leaf traits, measured as coefficient of variation (CV in %), of seven species (n=35). R/LDMC: root or leaf dry matter content; R/LMA: root or leaf mass per area; 13C: δ^{13}C and 15N: δ^{15}N.

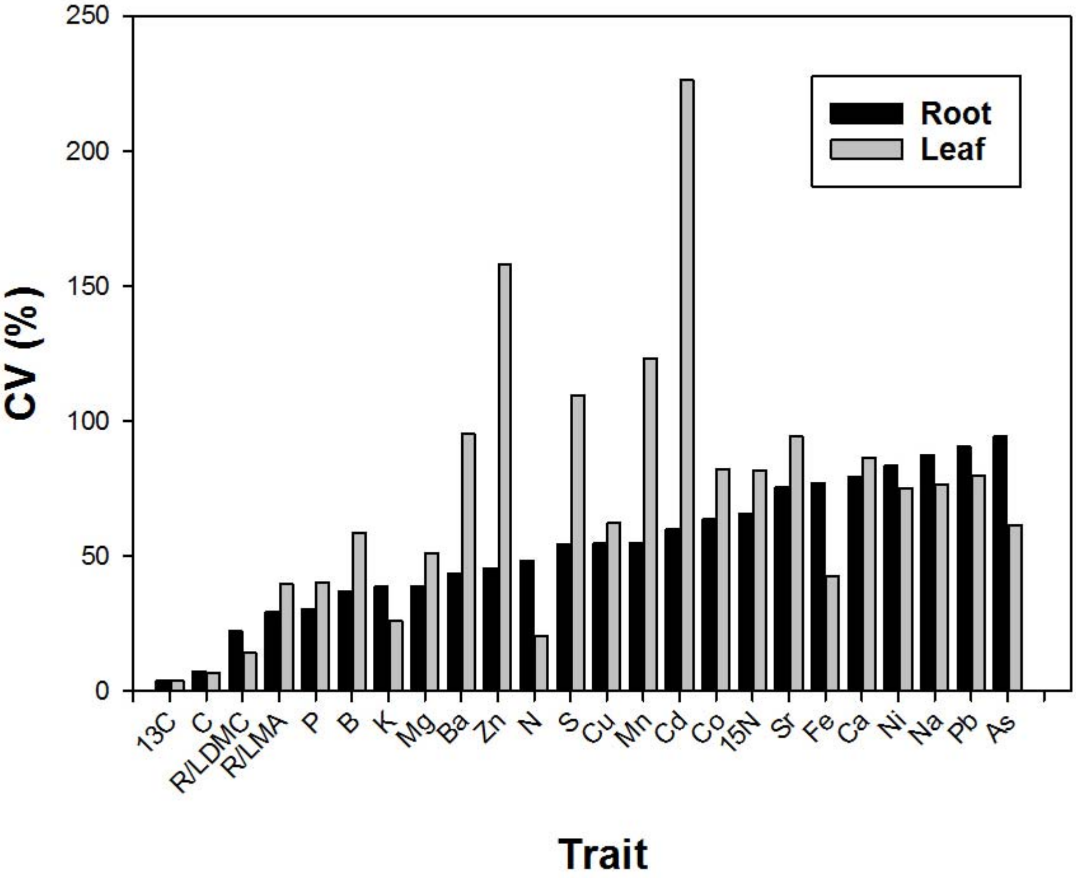

Figure S2. Results of the principal component analysis of six key root traits in trees of seven species (n=35). Abbreviations of root traits are: RDI: root mean diameter; RMA: root mass per area; RDMC: root dry matter content; RTD: root tissue density; and SRL: specific root length; species names are: C.a.: *Celtis australis*; C.s.: *Ceratonia siliqua*; F.a.: *Fraxinus angustifolia*; O.e.: *Olea europaea*; P.a.: *Populus alba*; P.p.: *Pinus pinea*; Q.i.: *Quercus ilex*.

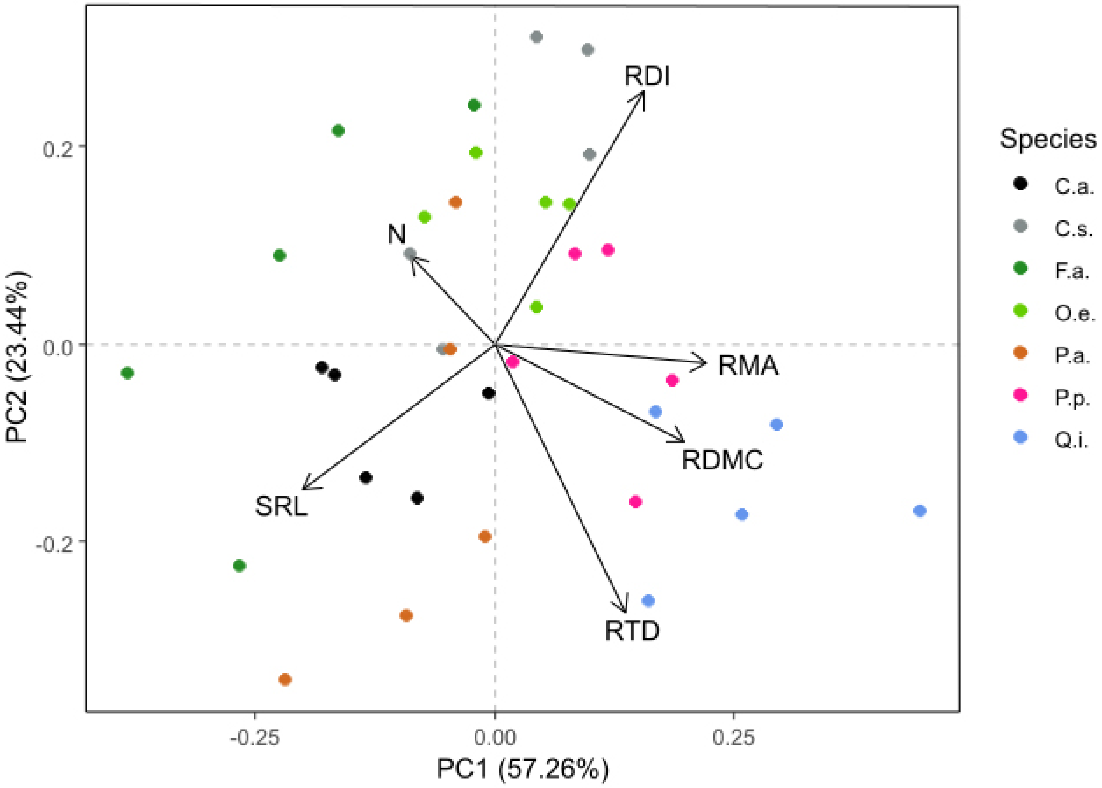

Figure S3. Results of the principal component analysis of 29 aboveground traits in trees of seven species (n=34). Traits and trees are ordered in the plane defined by PCA first and second axes. Abbreviation names for tree species are: C.a.: *Celtis australis*; C.s.: *Ceratonia siliqua*; F.a.: *Fraxinus angustifolia*; O.e.: *Olea europaea*; P.a.: *Populus alba*; P.p.: *Pinus pinea*; Q.i.: *Quercus ilex*.

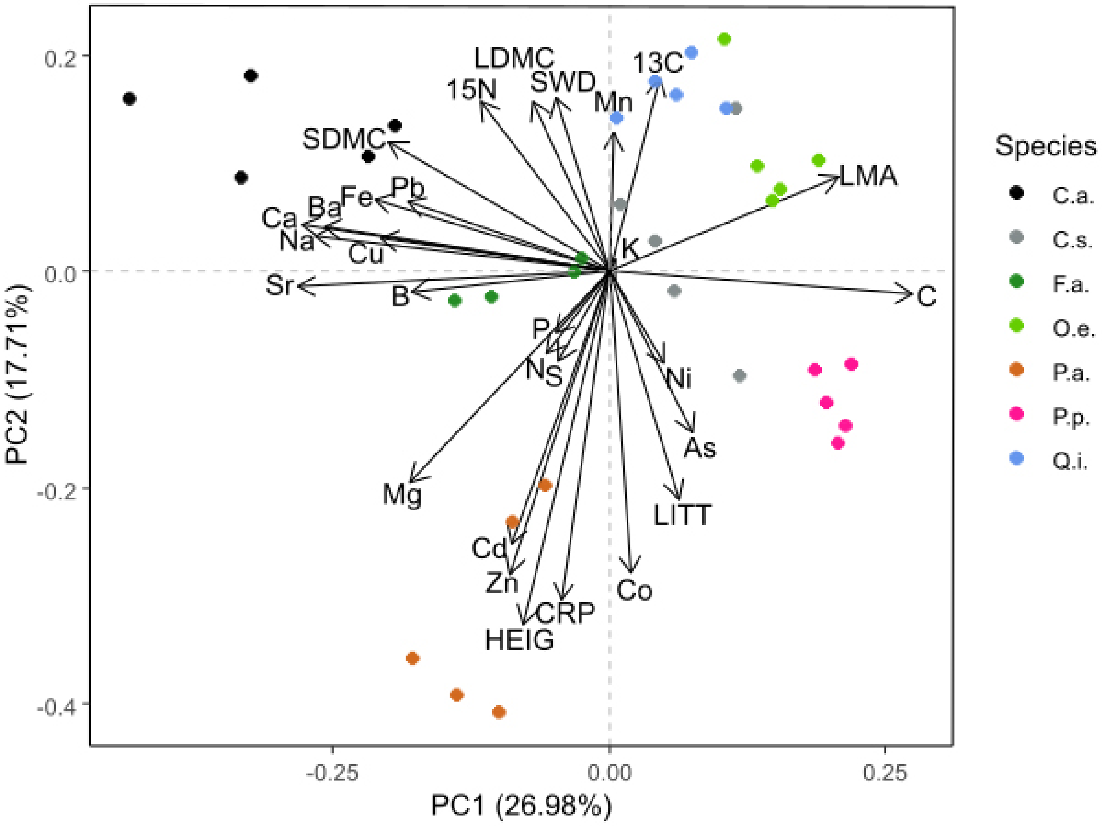

Figure S4. Coordination between root and leaf traits in the three figures of left column, compared with discordant traits in the right column (n=34). Correlations between N in roots and in leaves are significant for *Pinus* (red diamond and solid line, n=5) and *Populus* (blue triangles and dashed line, n=5) but not for the other species and for all data (black circles). Species-specific accumulation of Cd in leaves for *Populus* (blue triangles) and Mn in *Quercus* (green squares) are shown. The values of correlation coefficient and significance are shown in Table 1. LMA: leaf mass per area; RMA: root mass per area.

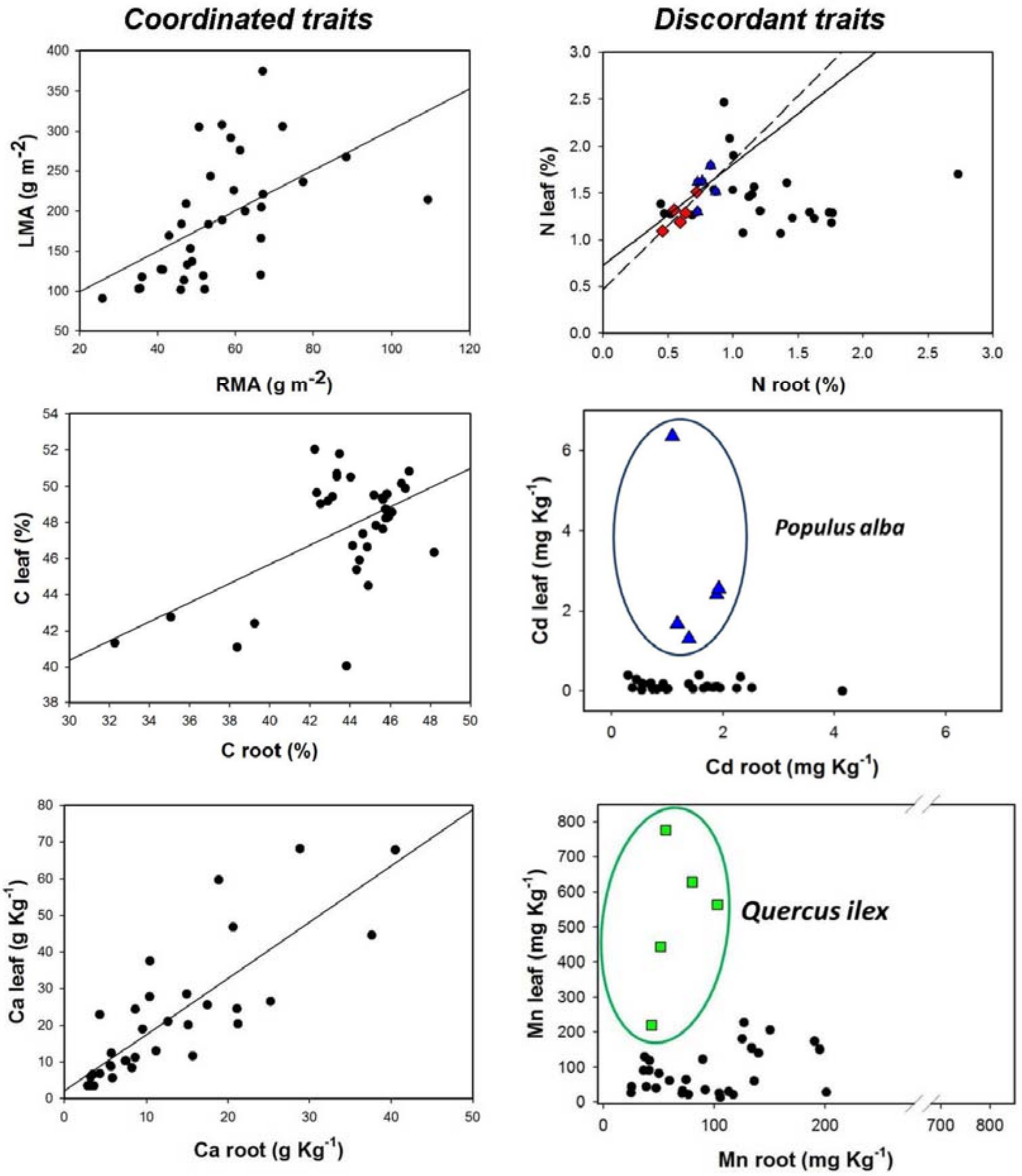

Figure S5. Intra-plant fractionation of N isotope (difference between $\delta^{15}N_{root}$ and $\delta^{15}N_{leaf}$, in ‰), separating ectomycorrhizal (white) and arbuscular mycorrhizal (grey) tree species. Mean and SE (n=5, with the exception of *Fraxinus* n=4) are shown; different letters mean significant difference between species by post-hoc Tukey test. There are significant differences between mycorrhizal types, t=-4.4, p<0.0001.