

Evolutionary changes in leaf and root traits predates changes in mycorrhizal associations in seed plants.

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Abstract

Changes in root morphological traits of seed plants has been typically associated with transitions from the ancestral arbuscular mycorrhizal (AM) to the alternative ectomycorrhizal (ECM) or non-mycorrhizal (NM) associations. However, changes in root morphology also coincide with changes in leaf physiology and growth habit during the diversification of Angiosperms. To explore the evolution of root systems and their role in the diversification in seed plants, we assembled a 600+ species database to reconstruct historical changes in root, leaf and growth form in seed plants. Our findings show important shifts in diameter, specific root length and tissue density as Angiosperms diversified. For most plants, changes in morphology occurred before the acquisition of novel mycorrhizal affiliations, but along with changes in leaf hydraulics and growth form. These findings suggest that adaptation in root systems was crucial during plant diversification and defined important ecological divergences among phylogenetic clades.

Introduction

The symbiotic association between roots and mycorrhizal fungi has fascinated botanists, mycologists and ecologists for centuries (Cairney 2000). In this symbiosis, plants host fungi inside or around cortical tissue in the root tips, providing habitat and C assimilates to the fungi, whereas fungi improve plant defense and nutrient and water acquisition (Fitter 2005, Selosse *et al.* 2015). Approximately 90% of all extant plant species engage in some type of mycorrhizal association, making this symbiosis arguably the most widespread and ecologically important mutualism in nature (Brundrett 2009, Maherali *et al.* 2016). The origin of mycorrhizal associations predates plant colonization of terrestrial ecosystems. The ancestral association started when the Glomeromycota formed the first arbuscular mycorrhizal (AM) association with the primitive subterranean tissues of ancestral plants approximately 500 million years ago (Redecker *et al.* 2000, Field & Pressel 2018 but see Orchard *et al.* 2017).

Root morphological traits have evolved continuously since the initial colonization of terrestrial habitats. Currently, plants can show 20-fold differences in root diameter, root length per unit dry mass (specific root length; SRL) and root dry mass to root volume (root tissue density; RTD) (Guo *et al.* 2008, Comas & Eissenstat 2009, Comas *et al.* 2014). These traits are frequently used as proxies for strategies in resource acquisition and conservation in root systems, and the way they relate varies by ancestry and habit form, suggesting different evolutionary pressures shaping the interaction between traits in different groups of plant independently (Ma *et al.* 2018, Kong *et al.* 2019). In addition, many species evolved particular structures such as rhizomorphs, beaded roots, root clusters, root hairs, dauciform roots and root nodules (Dohoux *et*

al. 2000, Markmann & Paniske 2009, Datta *et al.* 2011), usually associated with the adaptation of plants to limiting environmental conditions (Lambers *et al.* 2006, Oliviera *et al.* 2015).

Historically, botanists considered switches to novel fungal symbiosis intrinsically associated with morphological changes in roots (Brundrett 2002). For instance, Pinales and Gnetales within the Gymnosperms, were the first to depart from the ancestral AM association, developing ectomycorrhizal (ECM) associations with Dikarya fungi during the Early Cretaceous (Wang *et al.* 2000), which corresponded with thinner roots and higher SRL than AM plants (Brundrett & Tedersoo 2018). In Angiosperms, there was greater diversification in mycorrhizal associations, with possibly 22 independent transitions to form associations with Dikarya fungi (ECM widespread in Eudicots and rarely Monocots, Brundrett 2002; Ericoid restricted to Ericales, Setaro *et al.* 2006). Many of the species in these groups too are characterized for having roots with high SRL, thin diameters and denser tissues (see Valverde-Barrantes *et al.* 2017 for a review on root traits by phylogenetic groups). Other angiosperm clades evolved to exclude the mycorrhizal association altogether (non-mycorrhizal, NM), notably within the Caryophyllales, but also in the Proteales, Poales, Brassicales, Dipsacales and Lamiales (Lambers *et al.* 2009). The evolution of the NM habit also appears to be associated with thinner roots, lower density and higher SRL (Laliberté 2016, Freschet *et al.* 2017).

Two alternative hypotheses have been formulated to explain this evolutionary decrease in root diameter. The “mycorrhizal” hypothesis suggests that climatic changes during the Cretaceous impeded tissue decay, increasing the proportion of nutrients trapped in organic compounds and forcing plants to acquire novel fungal associations (Comas *et al.* 2012). In particular, the transition from the ancestral AM to novel ECM fungal mutualism might have been caused by the ability of ECM fungi to produce enzymes that directly degrade organic matter (Brzostek & Finzi 2011) and increase net N mineralization (Phillips & Fahey 2006), which is not present in AM fungi. Since ECM fungi do not require cortical tissue and their growth is limited to root tips, the adoption of an ECM affiliation may have accelerated the reduction in root cortical tissue, as well as increased SRL and root tip abundance (Read & Perez-Moreno 2003, Chen *et al.* 2016). Further, in some groups the reduction in cortical area extended to the point that some species abandon mycorrhizal affiliations altogether. Thus, the evolution of high SRL, thin root systems appeared as the results to the acquisition of novel fungal affiliations that required less cortical tissue, with the additional benefit of more efficient soil exploration (Eissenstat *et al.* 2000).

The “integrated” hypothesis proposes that changes in root morphology could be a consequence of changes in physiological leaf demands (Kong *et al.* 2017) and the emergence of new growth habits (Freschet *et al.* 2017). During the radiation of angiosperms in the Cretaceous, leaf venation and foliar area increased exponentially due to the drop in CO₂ levels (Feild *et al.* 2011). Some plant clades evolved leaves with higher vein density and stomatal abundance, allowing a 174 % increase in photosynthetic rates in angiosperms relative to gymnosperms (Brodribb *et al.* 2010). Although increased carbon assimilation rates made Angiosperms more competitive, it also incurred a higher demand for water and nutrients. Kong *et al.* (2017) hypothesized that increases in water expenses selected for a reduction in the relative proportion of cortical to vascular tissue, resulting in thinner roots, higher SRL and higher proportion of vascular tissue, increasing RTD (Li *et al.* 2015). Thinner roots with a reduced cortical area likely have less impedance to water movement (Eissenstat & Achor 1999), thus providing greater hydraulic conductivity (Huang & Eissenstat 2000; Solari *et al.* 2006, Hernandez *et al.* 2009) that could compensate for the increasing demands of higher photosynthetic rates. Moreover, many groups of Angiosperms also lost the ancestral woody habit as a strategy to avoid new climatic extremes during the Late Cretaceous (Zanne *et al.* 2014), which is also associated with thinner roots, greater SRL and lower RTD (Ostonen *et al.* 2007, Prieto *et al.* 2015)

Both hypotheses suggest that climatic changes during the time of Angiosperm diversification influenced the evolution of root systems and mycorrhizal associations in seed plants. Nonetheless, the order of these evolutionary changes is not clear. It remains unknown whether reduced diameter and increased SRL appeared independently of the mycorrhizal state of ancient plants but in coordination with changes at the entire plant level (Valverde-Barrantes *et al.* 2017), or whether mycorrhizal affiliation played an active role in root morphology changes (Baylis 1975, Comas *et al.* 2012, Chen *et al.* 2013, Maherali 2014). In addition,

higher RTD is usually associated with ECM roots whereas low RTD is linked to NM species, but little evolutionary context have been provided to these observations (Freschet *et al.* 2017, Valverde-Barrates *et al.* 2018). Although it has been suggested that the evolutionary associations between mycorrhizal state, leaf function and root morphology could have occurred simultaneously (Comas *et al.* 2012, 2014, Kong *et al.* 2017), this is unlikely. Fossil calibrations and ancestral reconstruction studies suggest that vein densities in angiosperms increased from 3.3 to ~ 8.6 mm mm⁻² during the Late Cretaceous (90-110 MYA, Hickey & Doyle 1977, Brodribb *et al.* 2010). In contrast, fossil records from ECM roots date back to the Lower Eocene from both Gymnosperm and Angiosperm plants (60-50 MYA, LePage *et al.* 1997, Beimforde *et al.* 2011). Similarly, typical NM families such as Brassicaceae, Caryophyllaceae, Cyperaceae, Dipsacaceae, Proteaceae, and Ranunculaceae evolved in the last 50 MYA (Hill & Brodribb 2006, Magallon *et al.* 2015, Bell *et al.* 2010, Iles *et al.* 2015, Hohmann *et al.* 2015). Unfortunately, equivalent studies comparing changes in root morphology based on fossil records have proven elusive due to the lack of reliable root fossil data, paucity of cortical tissue preservation and the impossibility to identify root fossils to species level in most cases (Heterington *et al.* 2016).

In this study, we test the “mycorrhizal” and “integrated” hypotheses based on the extant patterns of leaf, root and mycorrhizal information of seed plants (Brundrett 2002). If A) morphological root trait evolution was associated with changes in mycorrhizal symbiosis, we predicted A.1) the evolutionary changes in diameter and SRL must correspond with the transition from the ancestral AM to other mycorrhizal groups and A.2) those changes in root traits occurred later than the changes in leaf vein density. Alternatively, B) morphological root trait evolution was driven by changes in aboveground strategies, thus before major switches in mycorrhizal affiliation. In this case, we predicted that a shift to B.1) the ECM state or non-mycorrhizal state appeared later, after the formation of thin, high SRL and denser root systems and B.2) shifts in aboveground traits were concomitant with root changes. To test these predictions, we reconstructed the evolutionary history of root traits, habit, leaf vein density, and mycorrhizal state using a 600 taxon database and tested for associations among these variables using phylogenetically informed statistical analyses. Since changes in mycorrhizal associations vary largely among seed plant clades (Brundrett 2002, 2009), the analysis was done across all seed plants and within their major clades (gymnosperms, magnoliids, monocots, rosids, and asterids).

Methods

Data compilation and mycorrhizae classification

To examine the evolutionary changes in leaf and root morphology among seed plants, we compiled trait data from previously published databases. In the case of leaf vein density, we compiled information from Boyce *et al.* 2009 (257 species); Brodribb & Feild 2010 (272 species); Walls 2011 (87 species) and Li *et al.* 2015 (80 species, see Appendix 1 for a detailed list). Because “vein density” is defined as the average distance between veins (usually secondary and higher orders) within the mesophyll (Brodribb, Feild & Sack 2010), taxa with single-veined leaves like Gymnosperms or parallel veins without interconnections like most Monocots are lacking in this dataset. In the case of root traits, we used data described in Valverde-Barrantes *et al.* (2017) for diameter (D, mm), SRL (g/m), and RTD (g/cm³) deposited in the Fine Root Ecological Database (Iversen *et al.* 2017). Mycorrhizal state was binned into four mycorrhizal groups based on the classification in Maherali *et al.* (2016): AM, ECM, transitional (AMNM) and non-mycorrhizal (NM) based on consensus classification from previous studies. Species reported as AM or NM in different studies were binned in the AMNM transitional stage (Maherali *et al.* 2016). In order to simplify classification, species that evolved to form associations with Dikarya fungi were all classified as ECM (including ericoid mycorrhizal, ectomycorrhizal, and dual AM and ectomycorrhizal species). However, considering the substantial differences in root morphology and anatomical adaptation differences between ECM and Ericoid species, all analysis in this study were repeated with and without Ericoid species to confirm trends.

Tree configuration and ancestral reconstruction approach

Scientific names were standardized among data sets and brought up to date by query-

ing species names against the International Plant Names Index (<http://www.ipni.org/>), Tropicos (<http://www.tropicos.org/>), The Plant List (<http://www.theplantlist.org/>) and the Angiosperm Phylogeny website (<http://www.mobot.org/MOBOT/research/APweb/>) for a total of 629 species for root traits and 643 species for vein density information, with 117 species shared between datasets. We based phylogenetic trees on a dated molecular phylogeny using seven genome regions compared among >30000 species (Zanne *et al.* 2014). This phylogenetic tree matched 515 species from the root database and 492 species from the vein density database, which were thus used for statistical analysis and ancestral reconstructions. Finally, we assigned mycorrhizal state to all species in each phylogenetic tree for posterior analyses of coevolution between plant traits and mycorrhizal affiliation. Plants were grouped in five different phylogenetic clades, representing major lineages in the seed plant phylogeny: Gymnosperms, Magnoliids (Magnoliids + Basal Angiosperms), Monocots, Rosids (Rosids + Saxifragales) and Asterids (Asterids + Caryophyllales + Santalales).

First, based on trait information from extant species we illustrate the association between root traits and leaf venation in extant species creating a bivariate correlation (Fig. 1) and calculate the variation in root diameter, SRL and mycorrhizal affiliation among major seed plant clades (Fig. 2). To test hypothesis A) shifts in mycorrhizal state associated with root morphology changes were tested using the maximum possible number of species in each category using a logistic regression (OLR) and a phylogenetic logistic regression (PLR), using the package *corHMM* (Beaulieu *et al.* 2013). The PLR incorporates the phylogenetic relatedness among species to maximize the penalized likelihood of the logistic regression (Ives & Garland 2010, Tung, Ho & Ane 2014). Because the AM state is ancestral in seed plants, we compared root morphological traits between AM species to ECM or NM states. Since species classified as AMNM are found either with or without AM fungi based on ecological context (Brundrett 2009; Maherali *et al.* 2016), AMNM plants can be described as engaging in a facultative mutualism, which is distinct from either the AM or NM states (Moora 2014). Therefore, we also tested whether root morphology changed from the AM to the facultative AMNM state. Finally, because plants in the AMNM state can be found in nature without mycorrhizae, we also tested whether root morphology changed with the transition from the AM to the combined AMNM + NM grouping. For all cases, we interpret significant logistic regressions as evidence that a shift in root morphology influenced the likelihood of a switch in mycorrhizal state.

To test hypothesis B, (shifts in root trait correspond with leaf and habit changes but independent from mycorrhizal association), we tested whether the association between leaf and root traits were significant even after accounting for mycorrhizal state and growth habit using phylogenetic generalized least squares models (PGLS) ANOVA models as implemented in the R package *ape* (version 5.0; Paradis *et al.* 2004). First, we tested four different evolutionary models (Brownian, Pagel, Martins and Garland, and Orstein-Uhlenbeck) to determine the best fit for the structural error in the model (Paradis and Claude 2002). Once Pagel's structure was identified as the best fit for the data on the response variables (Diameter, SRL and RTD), we created models with root traits as dependent variables, and leaf vein density, growth habit (herb, shrub and tree) and mycorrhizal state (AM, ECM, AMNM and NM) as independent variables, and the phylogenetic variance-covariance matrix among species as the error structure of the model (101 species for Diameter, 94 species for RTD and SRL). We also calculate ordinary least square (OLS) tests for the same models, in order to determine how the phylogenetic correction influenced the results. For both models (OLS and PGLS), we tested the trend across all seed plants and within Magnoliids, Rosids and Asterids, but not within Monocots or Gymnosperms due to the paucity of leaf venation data for these two groups.

To visualize changes in root and leaf traits over time, we reconstructed ancestral states for each trait using the Zanne *et al.* (2014) phylogeny for seed plants. To reconstruct ancestral node values for vein density and root traits, we used a maximum likelihood (ML) estimation for continuous variables based on the assumption of Brownian dispersion of each character across the phylogeny (O'Meara *et al.* 2006, Revell *et al.* 2012). To do this, we created separate species-level phylogenies for each trait, and then we estimated node means and variances using the Rohlff (2001) recursive re-rooting algorithm as implemented in the function *fastAnc* in the package *phytools* (Revell 2012) for R (ver 3.4.3, R Core Team 2017). For mycorrhizal state, which is a discrete variable, we used a continuous time Markov chain model using the function *ace* in *ape* (Paradis *et al.* 2004). To assign the probability that a particular node was in one of the mycorrhizal states (AM,

ECM, NM), we calculated marginal estimations based on asymmetrical rates (“ARD” mode in the function *ace*) given the phylogeny and tip states. To illustrate whether changes in root morphology occurred before or after mycorrhizal state transitions, we created scatterplots of node values for diameter and color coded accordingly with the estimated probability of mycorrhizal stages at each node, using a threshold of 75% as the minimum to switch mycorrhizal type from the ancestral AM to a new category (see Table S5).

Results

Our results showed a clear shift in root morphology over time in seed plants, with thinner root systems present in more derived lineages, whereas Gymnosperms and Magnoliids showed thicker roots and lower SRL values. RTD was relatively similar across groups (Fig 1), although it decreased from woody to non-woody plants across phylogenetic groups (Fig S1). The transition to ECM state had different effects on root morphology depending on the phylogenetic group (Table 1, Figure S1). Gymnosperms showed a substantial decrease in root diameter after mycorrhizal change (Table S2) but this result is strongly dependent on the sole divergence between the AM Cupressales and the ECM Pinales, so the association between root diameter and mycorrhizal state was not significant after phylogenetic correction (Fig. 2, Table 1). There was evidence of an increase in RTD from AM to ECM species, but only for the analysis on seed plants as a whole. On the other hand, there was no evidence that the transition to associations with Dikarya fungi was associated with root morphology in Rosids, suggesting that this group already had thin roots, high SRL and relatively high RTD by the time they acquired ECM capabilities. (Table 1, Fig 2). The loss of the mycorrhizal symbiosis was significantly associated with root morphology at the level of the seed plants and within some seed plant clades (Table 1). There were significant decreases in root diameter after switching to NM state but mostly when comparing all plants (Table 1, Table S2, Figure S1). SRL increased significantly for non-mycorrhizal groups when compared to basal groups (Table 1) and within Asterids. Non-mycorrhizal species also showed a decrease in RTD at the seed plant level and within Monocots and Asterids. Finally, mycorrhizal changes were not associated with leaf vein density, which corresponds with previous studies reporting similar leaf traits among mycorrhizal groups (Koele *et al.* 2012, Table S3)

We found a significant negative correlation of vein density with both diameter and RTD but a positive relationship with SRL among current species (Fig. 3). Moreover, leaf vein density mycorrhizal state and growth habits were still significant once they were included in the model (Table S4) but the significance of the association was lost after phylogenetic corrections (Table 2), suggesting that the trend is strongly influenced by the morphological difference between plant clades. Habit was the only significantly associated factor with root morphology within clades, suggesting that there are important changes in root morphology associated with ecological strategies and lifespan. In support of the “integrated” scenario, we found no significant effect of mycorrhizal state in root traits after correction for habit and vein density. The same result was obtained after removing Ericoid mycorrhizal plants (data not shown), and after removing ECM species and testing only for the transition from AM to NM ($F = 0.13$, $p = 0.8$, $F = 0.15$, $p = 0.86$ and $F = 0.97$, $p = 0.38$ for Diameter, SRL and RTD respectively). On the other hand, habit remained highly significant ($F = 22.9$, $p < 0.0001$, $F = 10.56$, $p < 0.0001$ and $F = 6.33$, $p = 0.003$, for diameter SRL and RTD respectively). This suggests that the transition to herbaceous habit seems to have meaningful effect on root morphology in Angiosperms (Table 2).

Finally, the ancestral reconstructions illustrate a shift in leaf venation along with increases in SRL and thinner roots but not with RTD trends (Fig 4). The reconstruction suggests that the ancestral seed plants possessed relatively low SRL, intermediate root diameter and RTD, in addition to low vein density. Root diameter in the Magnoliids, some Eudicots and Monocots increased during time, suggesting that the coarse distal roots represent a specialization rather than the ancestral stage. During the diversification of Angiosperms in the mid-Cretaceous emergent groups of Asterids and Rosids showed a substantial increase in SRL, and reduction in root diameter as vein densities increased, supporting the idea of a coordinated lift in plant hydraulics. In addition, we detected a previously unreported evolutionary divergence in RTD between Rosids and Asterids, with the former evolving to higher values than other plant groups, whereas Asterids showed similar RTD trends to those in the Monocots and Magnoliids clades.

Discussion

Here, we presented the first formal hypothesis test of links among the evolution of root traits, leaf traits, and mycorrhizal state in seed plants and its main clades. Our results indicate that the substantial evolutionary shifts in root diameter and SRL of seed plants were correlated with leaf traits that facilitate enhanced hydraulic capacity and greater photosynthetic performance in the canopy, but also with changes in growth form. The reconstruction of trait values across the phylogeny suggest that switches to ECM associations could have impacted the morphology of Gymnosperm root systems, but had limited influence on Angiosperms. Indeed, Rosids and Asterids apparently evolved thinner, longer root systems before the evolution of ECM or Ericoid associations. Similarly, the evolution of the NM state associated with additional decreases in diameter and RTD, plus increases in SRL in Angiosperms, but those changes were also correlated with transitions from woody to herbaceous habit.

Evolution of mycorrhizal state and root morphology within seed plant clades

Our results contradict the expected close evolutionary relationship between root morphology and mycorrhizal association (Egerton-Warburton & Allen, 2001, Guo *et al.* 2008, Comas & Eissenstat 2009) and suggest that many of the traits previously associated to ECM associations (Brundrett & Tedersoo 2018) were actually achieved by most Angiosperms groups before departures from the ancestral AM stage. In fact, our results are consistent with more recent findings suggesting that switches to ECM association had limited effects on root morphology (Valverde-Barrantes *et al.* 2017), especially when closely related species are compared (Comas & Eissenstat 2009, Comas *et al.* 2014, Kubish *et al.* 2016, Valverde-Barrantes *et al.* 2018).

Our study suggests that the association between root morphology and the loss of the mycorrhizal mutualism (the NM state) is complex. The presence of very fine root systems has been described in several non-mycorrhizal families such as Caryophyllaceae (Magallon & Castillo 2009), the carnivorous Droseraceae and Nepenthaceae (Adlassing *et al.* 2005), Proteaceae and Ranunculaceae (Brundrett 2017) as well as cluster roots in the Fagales (Lambers *et al.* 2008). However, our analyses revealed that the transition to NM state is confounded with habit, even after phylogenetic information was included in the analysis (Table 2, Table S2). Indeed, the herbaceous plants are known to have higher SRL, smaller root diameter and lower RTD than woody plants, which confers lower construction cost, faster turnover rates and faster nutrient acquisition compared to woody plants (McCormack *et al.* 2012). As a result, NM plants likely report on average finer roots than other mycorrhizal groups because they are almost exclusively herbaceous. Moreover, the faster life history of the herbaceous habit, and their tendency to proliferate in disturbed habitats, could further limit associations with mycorrhizal fungi since plants in these environments are unlikely to obtain a net benefit from colonization by AM fungi (Peat & Fitter 1993). This does not mean that all herbaceous plants evolved independence from mycorrhizal associations. Root systems of important groups of herbs, including the entire order of Zingiberales, and Commelinales (Monocots) and several largely herbaceous families like Piperaceae (Magnoliids), Lamiaceae (Asterids) and Urticaceae (Rosids) have been poorly sampled so far but reported as associated largely with AM fungi (Wang & Qiu 2006). Future developments in these groups could change our perspective of the relationships between habit and mycorrhizal affiliations.

It is important to point out that these conclusions are based on the assumption of relatively fast transitions from one type of mycorrhizal association to others, and relatively few episodes of change between the ancestral AM stage and novel acquisition strategies. Although it has been stated that switches in mycorrhizal affiliations are rare because of the complex genetic signaling involved in the establishment and the stabilizing selection associated with the mycorrhizal mutualism (Delaux *et al.* 2014), intermediate stages can be important as transitional steps in the acquisition of a novel strategy for nutrient acquisition (Maherali *et al.* 2016). For instance, plant species engaging in mutualisms with multiple mycorrhizal types (e.g., AM and ECM) are frequently reported (Gehring *et al.* 2006). If these lineages represent an intermediate between mycorrhizal states, studying them in more detail could assist in unraveling the relationship between root evolution and mycorrhizal evolution, and is therefore a promising field for future research.

An important task to develop in future work is to acquire integrated data for entire plants, including root

and leaf traits in the same individuals (Cordlandwehr *et al.* 2013, Medeiros *et al.* 2017), rather than efforts to compile information from independent studies. Leaf and root traits can be affected substantially due to local conditions, and that intraspecific variability can only be accounted if traits are measured in an integrated fashion (Violle *et al.* 2012). More importantly, this study detected an important information gap between root and leaf functional traits, particularly vein density, due to the historical focus on the venation pattern of broadleaf species (Boyce *et al.* 2009). Further studies in leaf venation patterns in groups with dominant parallel venation (i.e., Gymnosperms and Monocots) will help to elucidate how root and leaf traits evolved in these groups.

We also need more detailed reconstructions of the evolutionary pathway of root modifications over time for families with particularly diverse mycorrhizal strategies. For instance, families related to NM groups like Polygonaceae, Plumbaginaceae and Rhabdodendraceae (in Caryophyllales), Eupteleaceae (Ranunculales) or Sabiaceae (Protelales) also contain either AM or ECM associations, but we know little about the morphology of those root systems to infer the role of root morphology on the departure from the AM state. Similarly, more detail studies within closely related groups could help to elucidate the relationship between root morphology and mycorrhizal associations (Brundrett 2017). For instance, recent studies in botanical gardens showed large variation in root diameters within the genus *Rhododendron* (Ericaceae, Medeiros *et al.* 2017), a genus that is usually reported as forming ericoid associations (Wang & Qiu 2006) but can also associate with AM fungi (Chaurasia *et al.* 2005). Unfortunately, no studies so far paired mycorrhizal and root morphology information in a phylogenetically controlled experiment. Future studies that match mycorrhizal associations with morphological syndromes among closely related species in common gardens can vastly improve our understanding of the evolution between root morphology, mycorrhizal associations and ecological strategies in seed plants.

Evolution of root morphology and leaf vein density during the diversification of Angiosperms

Our study shows for the first time that root systems could have been subjected to strong selection and rapid modification during the diversification of Angiosperms during the Late Cretaceous. To date, the role of fine roots has not been fully included in the discussion about the diversification and expansion of flowering plants. The increases in vein density during Angiosperm radiation have been linked to increased gas exchange and thus higher photosynthetic rates, which could have been favored when climate became colder, drier and growing season length decreased in temperate regions (Franks & Beerling 2009). Higher vein densities and associated increases in hydraulic conductance also allow plants to maintain open stomata under drier soil conditions (Brodribb & Holbrook 2004). However, increases in leaf evaporation due to higher stomatal conductance (Hetherington & Woodward 2003; Boyce & Lee 2010) requires more efficient conductance in vessel anatomy (Christman & Sperry 2010, Feild & Brodribb 2013) and xylem traits (Morris *et al.* 2016, Nardini & Jansen 2013). Our findings suggest that higher SRL, smaller diameters, and higher tissue density modifications in the Rosids was coordinated with other modifications to achieve more efficient photosynthesis and may help to explain the dominance of Rosids as major components of forests globally (Wang *et al.* 2009). Other groups adapted to colder and drier conditions by reducing their lifespan and acquiring an herbaceous habit (Zanne *et al.* 2016). Asterids and Monocots, both groups largely represented by herbs, increased SRL but evolved lower RTD, possibly as an adaptation to limit root investments in herbs with shorter lifespan.

We propose that the mechanism causing the decrease in root diameter and increase in SRL was a reduction in root cortical tissue (Kong *et al.* 2017). Physiological studies in fine roots have shown that hydraulic resistance substantially increases with the thickness of cortical tissue (Steudle & Peterson 1998, Huang & Eissenstat 2000); and the production of thin, ephemeral roots is a coping mechanism for periodic droughts (Chen *et al.* 2013). Decreases in diameter and increases in SRL are also mechanisms associated with the increase in root surface area in poor soils (Prieto *et al.* 2015). Moreover, comparisons across angiosperm trees have shown that reductions in cortical tissue were much more pronounced than stele diameter (Valverde-Barrantes *et al.* 2016), suggesting a strong selection for the reduction in cortex as the main contributor to the evolution of finer roots, rather than an even decrease in all root tissues (Kong *et al.* 2019). Then, increase the capacity to capture water and move it efficiently into the vascular tissue to cope with higher photosynthetic

demands. In any case, the acceleration of belowground metabolic activity during the diversification of Angiosperms may have contributed largely to the increased weathering of parent material and decreased atmospheric CO₂ levels during Cretaceous period (Raven and Edwards 2001, Palike *et al.* 2012).

These patterns in root modifications indicate that the development of a more efficient root system was an adaptation to cope with more unstable and drastic climatic conditions, possibly enhanced by a later adoption of ECM fungi in colder areas (Brundett and Tedersoo 2018). Interestingly, the increase in diameter in the Magnoliids and other families seem a derived condition from an ancestral intermediate value, suggesting that the “magnoliid” type of root is a specialized adaptation for nutrient foraging. Albeit the loss of the mycorrhizal symbiosis was associated with finer roots in some clades, it is unlikely that the two variables were causally related because of underlying covariation with plant habit (i.e., finer roots in NM species may have evolved because of herbaceousness). The hydraulic and metabolic shifts experienced by flowering plants during angiosperm radiation in the Late Cretaceous, resulting in the combination of highly efficient roots and leaves, could have allowed Eudicots to become dominant in temperate and boreal areas, whereas other Angiosperm groups with thicker root systems were favored in warmer biomes (Wang *et al.* 2009, Chen *et al.* 2013). Further studies in low latitude ecosystems are urgent in order to understand the foraging activity of plants in these ecosystems, and how they may change in future climatic scenarios.

Our analysis can also help to create more robust predictions about future distributions of plant groups. Future increase in atmospheric CO₂ concentration, coupled with a warming climate, could shift the geographic distribution of particular groups of plants. In the past, climatic changes may have favored a subset of species that already possessed fine roots that facilitate soil foraging when nutrients became increasingly retained in inorganic matter in colder environments (Ostonen *et al.* 2007). Future climate changes may benefit species with thicker roots that could benefit to a greater extent from enhanced mineralization rates under a warmer climate. Since changes in root morphology seem highly conserved across phylogenies (Ma *et al.* 2017), it is possible that arboreal groups in the Magnoliids and Asterids, currently limited by climatic conditions, could start to expand their natural ranges to higher latitudes and altitudes (Fadrigue *et al.* 2018). Future synthesis and modelling work will need to integrate roots as an important component in global plant vegetation models to better understand how plant communities may react to expected climatic changes (Verheijen *et al.* 2013).

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sSTATEMENT OF AUTHORSHIP

O.J.V.B., and C.B.B. conceived the idea, O.J.V.B and H.M. collected the data, and O.J.V.B., C.B. and H.M. conducted the statistical analyses and graph configuration. All authors interpreted results and contributed to the writing of the manuscript.

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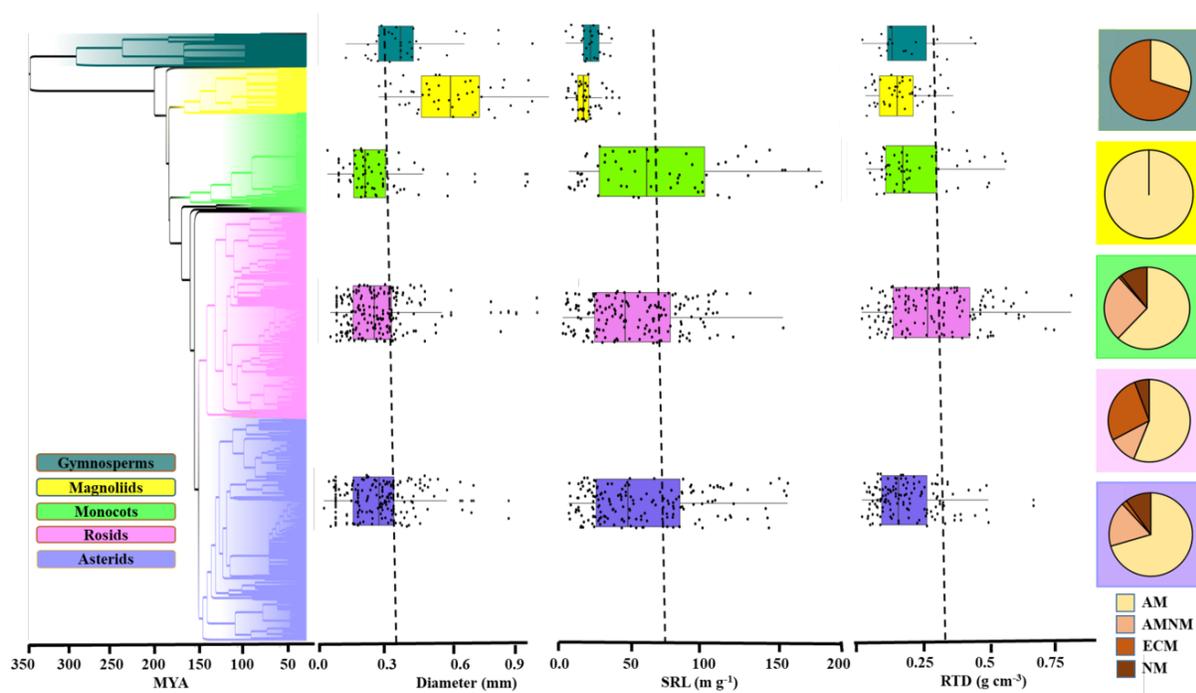
Table 1. Summary of results from phylogenetic logistic regression (PLR) analyses testing the effect of transitions in mycorrhizal affiliations (AM = Arbuscular mycorrhizal; AMNM = Facultative Non-mycorrhizal, EM= Ecto and Ericoid mycorrhizal, NM= Non-mycorrhizal) on root diameter, specific root length (SRL) and leaf vein density for seed plants and its major clades. Numbers represent the estimated slope for each regression, bold values represent significant regressions after phylogenetic correction ($p < 0.05$). For each transition, the first row shows the coefficient (standard error) for the logistic regression slope and the second row shows the phylogenetic correlation α -value.

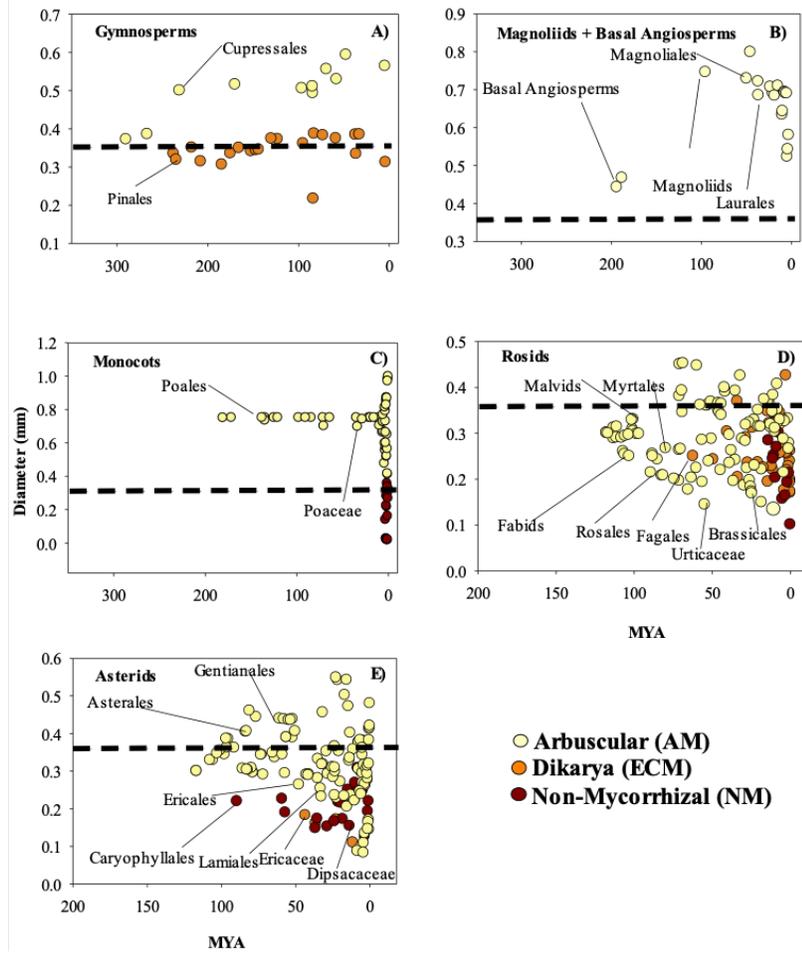
Clade	Seed plants	Gymnosperms	Monocots	Rosids	Asterids
Diameter					
AM EM	-0.272 (0.85)	-3.33 (2.24)	NA	0.20 (0.73)	NA
α	0.008	0.0009	NA	0.003	NA
AM AMNM	-3.20 (0.99)	NA	-4.93 (3.48)	-1.45 (2.05)	-4.31 (1.82)
α	0.135	NA	0.002	0.46	0.493
AM NM	-1.87 (0.32)	NA	NA	-3.97 (4.84)	NA
α	0.01	NA	NA	0.0216	NA
AM (AMNM+NM)	-4.917 (0.98)	NA	-6.69 (2.58)	-2.912 (2.03)	-4.51 (1.91)
α	0.134	NA	0.317	0.075	0.503
SRL					
AM EM	-0.85 (2.19)	2.66 (5.58)	NA	0.13 (0.23)	NA
α	0.002	0.001	NA	0.021	NA
AM AMNM	0.74 (0.24)	NA	0.414 (0.42)	-0.269 (0.398)	0.519 (0.25)
α	0.135	NA	0.399	0.455	0.004
AM NM	0.53 (0.25)	NA	NA	-0.02 (0.51)	0.204 (0.17)
α	0.02	NA	NA	0.022	0.001
AM (AMNM+NM)	0.68 (0.19)	NA	0.175 (0.366)	0.634 (0.390)	0.419 (0.19)
α	0.136	NA	0.396	0.441	0.003
RTD					
AM EM	0.0002 (0.0001)	-0.004 (2.04)	NA	0.007 (0.39)	NA
α	0.001	0.001	NA	0.007	NA
AM AMNM	-1.22 (1.37)	NA	8.03 (5.04)	-2.96 (2.39)	-2.43 (1.32)
α	0.005	NA	0.39	0.45	0.001
AM NM	-2.20 (1.25)	NA	NA	-0.05 (2.51)	NA
α	0.015	NA	NA	0.025	NA
AM (AMNM+NM)	-3.86 (1. 53)	NA	-10.87 (4.36)	-1.11 (1.16)	-2.58 (1.21)
α	0.052	NA	0.38	0.045	0.001

Table 2. Summary of the phylogenetic ANOVA analysis testing the relationship between leaf vein density, habit (herb, shrub or tree) and mycorrhizal state (AM, EM, AMNM and NM) on fine root diameter and specific root length. F and p-values were calculated following a phylogenetic informed general linear model (PGLS) for seed plants and its major clades. Number in parentheses indicates the number of species included in each model. Lambda values indicate the level of phylogenetic dependence of the residuals in the linear model with 0 indicating complete independence and values close to 1 indicating stronger phylogenetic dependence. Monocots and Gymnosperms were not included due to the paucity of vein density data for these groups. waste

	DF	F-value	p-value	DF	F-value	p-value	DF
Diameter (mm)	All (103)	All (103)	All (103)	Magnoliids (24)	Magnoliids (24)	Magnoliids (24)	Rosids (54)
Veins	1	6.21	0.01	1	0.02	0.98	1

	DF	F-value	p-value	DF	F-value	p-value	DF
Habit	3	4.32	0.02	2	1.99	0.17	3
Mycorrhizal state	3	3.04	0.03	NA	NA	NA	3
Veins X Habit	2	0.70	0.50	1	0.76	0.39	2
SRL							
Veins	1	14.70	0.0002	1	0.52	0.47	1
Habit	3	6.36	0.002	2	4.50	0.04	3
Mycorrhizal state	3	3.84	0.01	NA	NA	NA	3
Veins X Habit	2	0.83	0.44	1	0.01	0.97	2
RTD							
Veins	1	0.73	0.39	1	0.13	0.72	1
Habit	2	6.98	0.002	1	2.23	0.15	2
Mycorrhizal state	3	1.27	0.29	NA	NA	NA	3
Veins X Habit	2	0.70	0.50	1	5.30	0.03	2





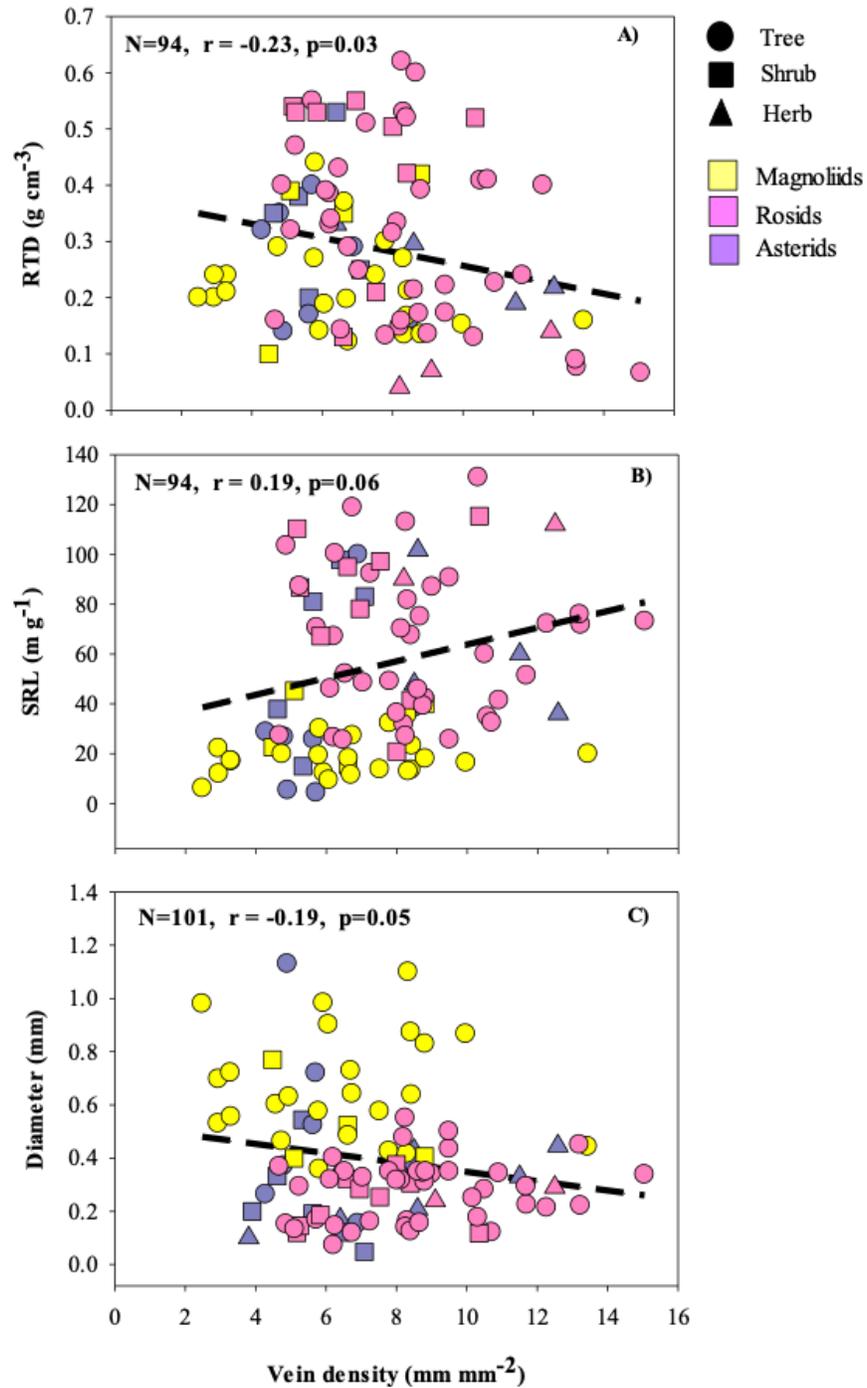


Fig 3. Relationships between leaf venation and root traits in current species. Panels illustrate the correlation between A) Root tissue density (RTD), B) Specific root length (SRL) and C) Diameter with leaf vein density for species where both traits were measured. Colors represent different seed plant clades along the phylogeny and shapes different habits.

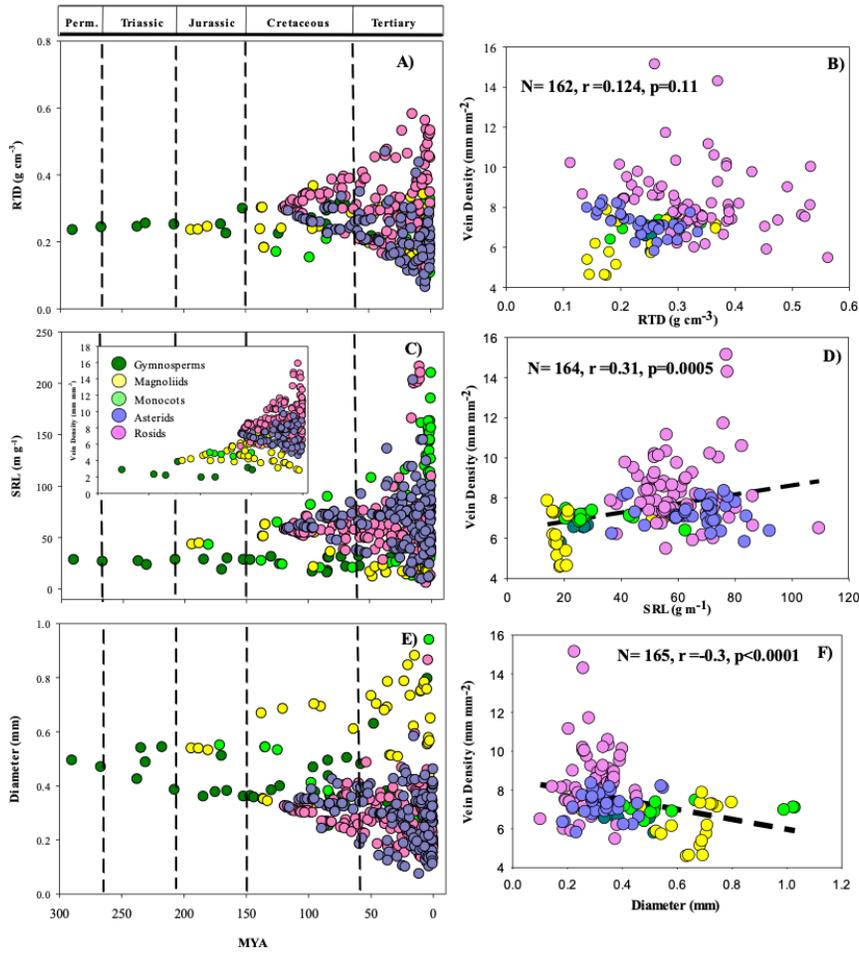


Fig.4. Coordination in trait modification in leaf vein density, specific root length (SRL) and root diameter across seed plants. Points represent reconstructed node values for each trait along the phylogeny based on extant species. Colors represent main clades within seed plants. Panels A), C) and D) represent the reconstructed changes across time (MYA) in RTD, SL and Diameter respectively, with the insert in panel C) representing the reconstructed changes for leaf vein density as a comparison. Panels B), D) and F) represent the correlation between leaf venation and root traits from shared nodes between phylogenetic trees after phylogenetic reconstruction.

TableS1. Comparison of evolutionary models using Akaike Information Criterion (AIC) values on three root functional traits.

Model	RTD	SRL	Diameter
Null	144.3	237.17	205.55
Brownian	205.09	492.58	245.30
Orstein-Uhlenbeck	142.77	245.77	200.05
Pagel	142.29	194.41	149.17
Bloomberg	182.3	458.28	217.97

Table S2. Summary of results from ordinary logistic regression (OLR) analyses testing the effect of transitions in mycorrhizal affiliations (AM = Arbuscular mycorrhizal; AMNM = Facultative Non-mycorrhizal, EM= Ecto and Ericoid mycorrhizal, NM= Non-mycorrhizal) on root diameter, specific root length (SRL) and leaf vein density. Numbers represent the estimated slope for each regression, bold values represent slope was significantly different from zero. Numbers in line with the transition represent the coefficient (standard error) for the logistic regression slope. Gray section in the table indicated the number of species used for each logistic regression model (Table 1).

	Seed plants	Gymnosperms	Monocots	Rosids	Asterids
Root Diameter					
AM EM	-2.25 (0.85)	-27.98 (5.56)	NA	-0.387 (1.64)	NA
AM AMNM	-5.47 (1.14)	NA	-3.92 (2.53)	-1.45 (2.05)	-4.58 (2.12)
AM NM	-8.87 (2.30)	NA	NA	-11.85 (5.52)	NA
AM (AMNM+NM)	-6.23 (1.06)	NA	-3.91 (2.32)	-2.625 (1.93)	-4.63 (2.01)
SRL					
AM EM	-0.085 (0.19)	8.62 (4.04)	NA	0.213 (0.25)	NA
AM AMNM	0.85 (0.26)	NA	0.84 (0.49)	-0.09 (0.43)	0.94 (0.52)
AM NM	0.82 (0.31)	NA	NA	2.715 (0.99)	0.376 (0.51)
AM (AMNM+NM)	0.814 (0.21)	NA	0.38 (0.36)	0.667 (0.40)	0.636 (0.36)
RTD					
AM EM	3.68 (0.95)	0.64 (3.85)	NA	2.16 (1.21)	NA
AM AMNM	-1.82 (1.63)	NA	7.73 (5.71)	-2.08 (2.40)	-2.46 (3.56)
AM NM	-3.76 (1.59)	NA	NA	2.61 (2.47)	NA
AM (AMNM+NM)	-1.04 (1.09)	NA	-10.91(4.37)	-0.54 (0.73)	-5.34 (3.47)
Root Diameter					
AM EM	280 → 60	8 → 19	NA	72 → 38	NA
AM AMNM	280 → 67	NA	31 → 17	72 → 20	84 → 16
AM NM	280 → 24	NA	NA	72 → 7	NA
AM (AMNM+NM)	280 → 91	NA	31 → 22	72 → 27	84 → 19
SRL					
AM EM	243 → 43	7 → 15	NA	65 → 42	NA
AM AMNM	243 → 28	NA	28 → 10	65 → 9	71 → 9
AM NM	243 → 19	NA	28 → 5	65 → 6	71 → 6
AM (AMNM+NM)	243 → 47	NA	28 → 15	65 → 15	71 → 15
RTD					
AM EM	259 → 42	9 → 10	NA	88 → 29	NA
AM AMNM	259 → 25	NA	30 → 7	88 → 9	81 → 9
AM NM	259 → 15	NA	30 → 5	88 → 6	NA
AM (AMNM+NM)	259 → 40	NA	30 → 12	88 → 15	81 → 13

Table S3. Summary of results from phylogenetic (PLR) and ordinary logistic regression (OLR) analyses testing the effect of transitions in mycorrhizal affiliations (AM = Arbuscular mycorrhizal; AMNM = Facultative Non-mycorrhizal, EM= Ecto and Ericoid mycorrhizal, NM= Non-mycorrhizal) leaf vein density. Numbers represent the estimated slope for each regression. Gray section in the table indicated the number of species used for each logistic regression model, including the PLG (Table 1).

PLR	Seed plants	Gymnosperms	Monocots	Rosids	Asterids
AM EM	-0.035 (0.22)	NA	NA	-0.01 (0.04)	NA
α	0.004	NA	NA	0.006	NA

PLR	Seed plants	Gymnosperms	Monocots	Rosids	Asterids
AM AMNM	0.422 (0.55)	NA	NA	0.001 (0.08)	NA
α	0.154	NA	NA	0.45	NA
AM NM	NA	NA	NA	NA	NA
α	NA	NA	NA	NA	NA
AM (AMNM+NM)	0.35 (0.51)	NA	NA	-0.101 (0.09)	0.189 (0.19)
α	0.154	NA	NA	0.46	0.02
ORL					
AM EM	-0.08 (0.19)	NA	NA	0.837 (0.68)	NA
AM AMNM	0.94(0.61)	NA	NA	0.345 (0.86)	NA
AM NM	NA	NA	NA	NA	NA
AM (AMNM+NM)	0.68 (0.55)	NA	NA	-0.27 (0.79)	1.79 (1.87)
N					
AM EM	125 \rightarrow 29	NA	NA	57 \rightarrow 25	NA
AM AMNM	125 \rightarrow 22	NA	NA	57 \rightarrow 16	NA
AM NM	NA	NA	NA	NA	NA
AM (AMNM+NM)	125 \rightarrow 25	NA	NA	57 \rightarrow 19	29 \rightarrow 9

Table S4. Summary of the ANOVA analysis testing the relationship between leaf vein density, habit (herb, shrub or tree) and mycorrhizal state (AM, EM, AMNM and NM) on fine root diameter and specific root length. F and p-values were calculated following an ordinary linear model (OLS) for all species and separated into major clades. Number in parentheses indicates the number of species included in each model. Lambda values indicate the level of phylogenetic independence of the data with values close to 1 indicating stronger dependence. Monocots were not included due to the paucity of vein density data for the group

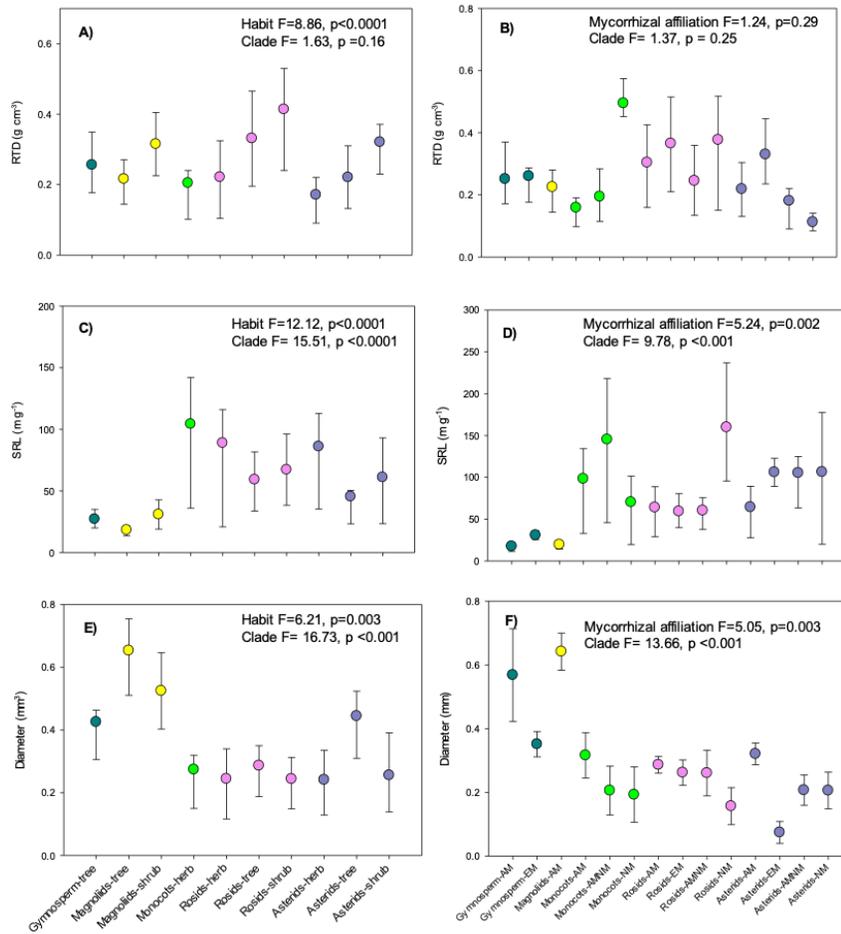


Fig S1. Distribution of fine root trait values as observed across different phylogenetic groups. Panels A), C) and E) represent root tissue density (RTD), specific root length (SRL) and diameter among growth habits respectively. Panels B), D) and F) represent the same traits across mycorrhizal affiliations. Circles represent mean values and the two whiskers the 10th and 90th percentiles. Statistical values represent an ANOVA test with the phylogenetic clade and the categorical classifications as the fixed factors.