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Mycorrhizal symbiosis pathway and edaphic fertility frame root economics space among tree species

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Summary

- The root economics space (RES) is multidimensional and largely shaped by belowground biotic and abiotic influences. However, how root-fungal symbioses and edaphic fertility drive this complexity remains unclear.
- Here, we measured absorptive root traits of 112 tree species in temperate and subtropical forests of China, including traits linked to functional differences between arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) hosts.
- Our data, from known mycorrhizal tree species, revealed a 'fungal-symbiosis' dimension distinguishing AM from ECM species. This divergence likely resulted from the contrasting mycorrhizal evolutionary development of AM versus ECM associations. Increased root tissue cortical space facilitates AM symbiosis, whereas increased root branching favors ECM symbiosis. Irrespective of mycorrhizal type, a 'root-lifespan' dimension reflecting aspects of root construction cost and defense was controlled by variation in specific root length and root tissue density, which was fully independent of root nitrogen content. Within this function-based RES, we observed a substantial covariation of axes with soil phosphorus and nitrate levels, highlighting the role played by these two axes in nutrient acquisition and conservation.
- Overall, our findings demonstrate the importance of evolved mycorrhizal symbiosis pathway and edaphic fertility in framing the RES, and provide theoretical and mechanistic insights into the complexity of root economics.

Keywords: Absorptive root, trait-function linkage, trait interplay, root economics, root trait variation, arbuscular mycorrhizas and ectomycorrhizas

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Introduction

Roots have evolved diverse resource-acquisition strategies since the emergence of the first land plants (Ma *et al.*, 2018; Bergmann *et al.*, 2020). Belowground, multiple plant traits, representing aspects of root architecture, morphology, anatomy, physiology, or associations with other organisms, concomitantly define the capacity of plants to acquire resources (Freschet *et al.*, 2021a). Although a number of trade-offs define and limit the set of trait values possible to ensure plant viability and success (Weigelt *et al.*, 2021), and these trade-offs are further modulated by environmental constraints (Laughlin *et al.*, 2021), plants show a remarkable diversity of trait values and trait combinations to achieve similar levels of resource acquisition and growth (Freschet *et al.*, 2018; Guerrero-Ramirez *et al.*, 2021). Some of this diversity can nonetheless be summarized using trait-based root economics frameworks (Kong *et al.*, 2014; Valverde-Barrantes *et al.*, 2015; Bergmann *et al.*, 2020). Recent advances have demonstrated the merits of considering a multi-dimensional root economics space (RES), yet the proposed patterns are highly variable among studies (Kong *et al.*, 2014; Valverde-Barrantes *et al.*, 2015; Weemstra *et al.*, 2016; Liese *et al.*, 2017; Bergmann *et al.*, 2020). This is because roots inhabit in complex soil matrix and interact with both biotic (e.g., mycorrhizal fungi) and abiotic (e.g., nutrient availability) factors that shape root economics in contrasting ways across plant functional types, environments and types of plant-fungi and plant-bacteria symbioses (Bardgett *et al.*, 2014; Valverde-Barrantes *et al.*, 2015; Weemstra *et al.*, 2020). Our conceptual and practical understanding of how such factors, particularly mycorrhizal symbioses and edaphic fertility, drive the complexity of root economics remains largely incomplete (Bergmann *et al.*, 2020; Ding *et al.*, 2020).

Over 90% of woody plant species on Earth have developed symbioses with mycorrhizal fungi (Brundrett, 2009; Bonfante & Genre, 2010). Arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) roots are the two most common types of symbioses with contrasting evolutionary history and symbiotic (intracellular vs. intercellular) pathways (Brundrett, 2002; Tedersoo *et al.*, 2020). For AM species, mycorrhizal associations evolved in early land plants via colonization of primitive roots by fungal hyphae of early glomeromycota and endogonomycetes, where hyphae

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penetrated cortical cells to form intracellular arbuscules (Brundrett, 2002; Tedersoo & Baharam, 2019; Strullu-Derrien *et al.*, 2018). By contrast, the more recently evolved ECM fungal association forms a sheathing mantle and Hartig net, where fungal hyphae grow around and between epidermal and cortical cells of roots (Smith & Read, 2008; Martin *et al.*, 2016), although, for ECM gymnosperms (e.g., Pinaceae) the inner hyphae can reach deeper into cortical cells (Brundrett, 2002; Genre *et al.*, 2020). Ectomycorrhizal fungi have partly evolved from their saprotrophic ancestors (i.e., basidiomycetes and ascomycetes) and exude hormones such as ethylene and auxin (Raudaskoski & Kothe, 2015; Martin *et al.*, 2016; Tedersoo & Brundrett, 2017), which enable roots to proliferate and branch for increased fungal colonization (Fig. **1**; Comas *et al.*, 2014; van der Heijden *et al.*, 2015). In contrast, AM species mainly regulate the extent of root cortical tissue to provide the main habitat for AM fungi (Fig. **1**). This fundamental divergence implies contrasting influences of mycorrhizal type on root economics. For example, while a trade-off between mycorrhizal colonization intensity and mean root diameter has been clearly established for AM species, such evidence is scarce for ECM species (Kong *et al.*, 2014; Bergmann *et al.*, 2020; Wambsganss *et al.*, 2021). As such, it has been hypothesized instead that a higher branching intensity (BI, i.e. the number of root tips on a given length unit of parent root) of absorptive roots may be the key trait responsible for an increased probability of colonization by ECM fungi, but this remains to be tested on a comprehensive range of species and traits associated with symbiotic pathways.

At the global scale, plant roots differ in their strategies to acquire and conserve edaphic resources along at least two major axes (or functions, Fig. **2a**) (Bergmann *et al.* 2020). A first axis is the 'conservation gradient' ranging from roots with high root tissue density (RTD) that show a slow resource return on investment but are long-lived and well-protected, to roots with a high nitrogen (N) content and metabolic rate for fast resource return on investment but a short life span. A second is the 'collaboration gradient' ranging from a "do-it-yourself" soil exploration strategy, characterized by high specific root length (SRL) to an "outsourcing" resource strategy where carbon (C) is allocated to the mycorrhizal partner, typified by greater root diameter and cortical

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fraction. We propose that this simple view of the 'collaboration' gradient does not adequately reflect the root functional differentiation among woody AM and ECM species, and therefore propose a more refined 'fungal symbiosis' gradient. Particularly, while AM associations tend to rely on increasing root diameter and cortex space, ECM associations are typically represented by thin, highly-branched roots with many root tips (Comas *et al.*, 2014). Therefore, a trade-off in symbiotic root traits can be envisioned to exist between the "symbiotic traits" of BI and root diameter with opposite trends between symbiotic groups.

Here, we contend that the relevance of certain root traits to multiple functions within the RES (e.g., root diameter and SRL can be conceptually linked to both RES axes) not only limits our capacity to apply a singular interpretation to axes of RES variation but also likely leads to substantial differences in local representations of the RES (e.g., across biomes or plant functional types). Particularly, the degree of variation observed in root trait strategies along axes on the RES seems to depend on the degree of environmental variation studied. Along the conservation gradient, root lifespan has been linked to changing availability of soil nutrients, particularly N (Eissenstat & Yanai, 1997; McCormack & Guo, 2014), suggesting an often ignored but positive role of root lifespan (as well as associated changes towards high RTD, root diameter and C/N, and low SRL) in nutrient acquisition (Ryser 1996; Eissenstat *et al.*, 2000; Withington *et al.*, 2006; McCormack *et al.*, 2012). Along the proposed 'fungal symbiosis' gradient, plants would rely on different symbiotic pathways with mycorrhizal fungi to forage immobile soil P (van der Heijden *et al.*, 2015; Liu *et al.*, 2018), via regulating root cortex space for AM species and branching tips for ECM species (Comas *et al.*, 2014). Thus, the diversity of belowground strategies along RES axes depends to some extent on the availability and mobility of limiting nutrients, such as N and P in soil, which also interacts with mycorrhizal association type.

To reveal how root-fungal symbiotic pathways and edaphic fertility drive root economics of woody plants, we sampled absorptive fine roots (the resource-acquiring first and second order roots within root systems) of 112 tree species consisting of 77 AM species and 35 ECM species

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spanning two temperate forests and two subtropical forests in China. We considered seven root economics traits (SRL, RTD, root diameter, cortex thickness (CT), BI, root N concentration (RN), and root carbon concentration (RC)) that are representative of root construction strategy, root-fungal symbiotic pathway, and resource return on investment (i.e., soil derived nutrient acquisition per plant-derived C allocation) at the root-system level (Kong *et al.*, 2014; Bergmann *et al.*, 2020). Compared to intraspecific variation, interspecific variation in root traits is often large (Hogan *et al.*, 2020). Moreover, among taxa root traits are highly phylogenetically structured, evidencing how evolution has largely determined root functional differences (Valverde-Barrantes *et al.*, 2013; Valverde-Barrantes *et al*., 2017). Based on phylogenetically informed analyses, we hypothesized that both AM and ECM tree species would fit within a bidimensional RES, represented by:

(i) a resource 'conservation' gradient describing the trade-off between slow versus fast resource return on investment dominated by traits associated with root lifespan, independent of mycorrhizal types;

(ii) a 'fungal-symbiosis' dimension that not only accounts for the dichotomy between species adopting a 'do-it-yourself' versus an 'outsourcing' strategy of soil resource acquisition, but also represents differences between mycorrhizal types, with strategies ranging from large root diameters and CTs for AM species to high BIs for ECM species.

Based on current knowledge of tree species root trait distributions along soil resource gradients, we further hypothesized that:

(iii) interspecific root trait variation along the 'root-lifespan' dimension is more associated with soil N availability, while variation along the 'fungal-symbiosis' dimension is more associated with soil P availability for both mycorrhizal types.

Materials and Methods

Study site and species selection

This study was conducted in four temperate and subtropical forests of China. The four forests were located at: (1) Mengluan forestry station (41°52′- 42°01′N, 116°49′- 116°59′E) in Hebei province; (2) northern slopes of Taibai Mountain (33°49′- 34°10′N, 107°19′- 107°58′E) in Shaanxi

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province; (3) Qianyanzhou Ecological Station (26°44′39″ N, 115°03′33″ E) in Jiangxi province; (4) Yangming Mountain (25°39′26″N, 114°18′49″E) in Jiangxi Province. The sampling sites range from 102 m to 2100 m. a. s. l. in elevation, with mean annual temperature ranging from 2 °C to 17.9 °C, and mean annual precipitation of 470-1587 mm (Kou *et al.*, 2019; Jiang *et al.*, 2021). The species we selected are representative of the local plant community at each site. In total, we sampled 366 individuals of 112 woody species, and then categorized these species into 77 AM (from 58 genera of 34 families) and 35 ECM species (from 17 genera of seven families) regarding symbiotic guild assignments of Steidinger *et al.* (2019). Each site has a unique set of species, as described in Table **S1**.

Field sampling and processing

The field sampling was carried out in autumn of 2018 and 2020. Samples from each species were collected from at least three mature healthy trees with similar diameters at breast height (DBH) to minimize the potential influences of intraspecific difference (< 5 cm in DBH) on root traits. The individuals for each species were spaced at least 10-m-apart to ensure sampling independency. Surface soil (0-20 cm) was excavated around the stems of the trees to expose lateral roots. Intact roots were cut from the main lateral roots, and the attached soil was cleared following the procedure described by Guo *et al.* (2008a). At least eight intact distal root branches, including more than five root orders, were sampled. Each root sample was then divided into two subsamples. One subsample was gently washed in deionized water to remove adhering organic matter and was immediately fixed in formalin-acetic acid-alcohol solution (90 ml 50% ethanol v/v, 5 ml 100% glacial acetic acid v/v, and 5 ml 37% methanol v/v) for measuring anatomical traits. The other subsample was placed into valve bags, incubated with ice bags, and then stored at -20 °C for later dissection and analyses of morphological and chemical trait Guo *et al.* (2008a). Approximately 1 kg of soil was also collected during root sampling (0-20 cm) under each tree using a shovel. All soil samples were sieved (2-mm mesh) to remove roots and visible organic particles and stored at 4 °C for later laboratory analysis. Soil samples were generally extracted and analyzed within 1-2 weeks after sampling to minimize the influence of storage time on available nutrients (Bailey *et*

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al., 2021).

Root trait measurements

The first set of root subsamples were dissected based on the protocol described by Pregitzer *et al.* (2002) for measuring morphological root traits. Specifically, three root clusters, including at least five root orders, were randomly chosen and dissected with fine forceps on a plate filled with deionized water as described by Guo *et al.* (2008). The absorptive roots (1st- and 2nd-order roots) were used in this study because they are belowground resource-acquiring units (Guo *et al.*, 2008a; McCormack *et al.*, 2015; Kou *et al.*, 2018). Root diameter was measured using WinRHIZO Arabido version 2012b (Regents Instruments Inc., Quebec City, Canada) by analysing images of root samples scanned on an Epson Expression 10000 XL scanner (Seiko Epson Corporation, Japan) at a resolution of 400 dpi. Given the difficulties in distinguishing mycorrhizal structures from roots, the mycorrhizal mantle was included in the measurement of root diameter for ECM species. SRL was calculated as the ratio of root length to root dry mass, and RTD was calculated as the ratio of root dry mass to root volume. Root volume was recalculated from WinRHIZO outputs as the sum of all diameter classes' averages (Freschet *et al.*, 2021b). Referring to the calculation of BI for the 1st-order roots (Kong *et al.*, 2014), we calculated BI of absorptive roots as the number of their root tips divided by the total root length of their parent roots (i.e. the 3rd-order roots). All absorptive roots were weighed, oven-dried, and then ground to fine powders using a SPEX 8000-D mixer mill (SPEX, Edison, USA) for measuring the concentrations of C and N using a Vario EL Cube elemental analyser (Elementar, Hanau, Germany). Root trait values were averaged across all sampled replicates for each species.

The second subsample set was also dissected in the same manner as morphology and chemistry for measuring anatomical traits. More than 20 segments were randomly selected from the absorptive roots, stained with safranin-fast green, dehydrated in a set of alcohol solutions, and embedded in paraffin. Root sections 8 μm thick were prepared, photographed using a compound microscope (DM2500, DFC450, Leica, Weztlar, Germany), and measured for CT using ImageJ

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(NIH Image, Bethesda, USA).

Soil measurements

Soil total C and N concentrations were determined using the Vario EL Cube elemental analyser described above. Approximately 13-15 g of soil was used for the extraction (50 ml of 2 mol L^{-1} KCl) of mineral soil N (NH_4^+ and NO_3^-). The concentrations of NH_4^+ and NO_3^- were determined using a Flow Auto Analyser (Autoanalyser III, Bran + Luebbe, Germany). Available P from acidic and alkaline soil samples was extracted respectively using HCl/NH_4F and $NaHCO_3$, and then determined colorimetrically using an ascorbic acid molybdate analysis on a Flow Auto Analyser. Soil properties were averaged across all soil samples taken from each tree replicates.

Data analyses

The taxonomic binomials of all species were verified using The Plant List (TPL, http://www.theplantlist.org/). Plant family-level clades were determined according to APG4 (Angiosperm Phylogeny Group 2016) for angiosperms and TPL for gymnosperms. Phylogenetic trees were constructed respectively for all species, AM species and ECM species using "V.PhyloMaker", a package designed to generate phylogenies for vascular plants in R software (v. 3.6.3, R Core Team, 2020), and V. PhyloMaker uses the mega-tree derived primarily from GBOTB (Gene Bank taxa with backbone provided by Open Tree of Life version 9.1) for seed plants (Smith & Brown, 2018) and Zanne *et al.*'s (2014) phylogeny for pteridophytes (Jin & Qian, 2019). The phylogenetic tree across all species was then visualized with the annotation of plant phylogeny, mycorrhizal type and distribution of core traits (BI, CT, root diameter, RTD and SRL) using the "ggtree" package (Yu *et al.*, 2017) in R. Phylogenetic polytomies were resolved by the 'multi2di' function using "ape" package (Paradis & Schliep, 2019) in R. We calculated Blomberg's parametric *K* to evaluate the phylogenetic conservatism for each absorptive-root trait using the "picante" R package (Blomberg *et al.*, 2003; Münkemüller *et al.*, 2012). A significant phylogenetic signal indicates that the trait is constrained by phylogeny, and a higher value of Blomberg's *K* indicates higher phylogenetic conservatism (Blomberg *et al.*, 2003). The variations

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in each root trait across all species was estimated by calculating the means, minima, and maxima and the coefficient of variation (CV). Additionally, bivariate relationships among root traits were assessed using both linear and phylogenetic generalized least square models in the "stats" and "caper" R packages (Freckleton *et al.*, 2002; Orme *et al.*, 2018) for all species, and for AM and ECM species, separately. Relationships between root trait and soil nutrient availability were assessed using Pearson's correlations for all species and the two mycorrhizal groups.

Given the influence of phylogeny on traits, we performed phylogenetically-informed principal component analysis (pPCA) to account for the dependency in root trait variations for all 112 species across the phylogeny. The pPCA was implemented with Pagel's lambda correlation structure using the 'phyl.pca' function of the "phytools" package (Revell 2009; Uyeda *et al.*, 2015) in R. The pPCA was conducted for seven key root traits: BI, CT, root diameter, RTD, SRL, RN and RC that are previously reported as drivers of the tradeoffs between fungal depedency and root construction strategies at global levels (Brundrett, 2002; Kong *et al.*, 2014; Bergmann *et al.*, 2020). To check for the effect of potential contamination of roots by soil minerals (potentially different among species, e.g. between ECM and AM species) on the general trends observed in this study, we carried out the same analyses with RTD data corrected for C concentration (i.e. expressed on a root C mass basis rather than root dry mass basis). Because the observed trends were the same, only the non-corrected data were displayed. Factor loadings of pPCA on corrected data can be found in Table **S2**.

To test for the difference between AM and ECM species on "fungal-symbiosis" dimension, we first performed an ANOVA analysis on the second axis scores of AM versus ECM species based on the result of pPCA for all species. We then repeated pPCA for AM (n = 77) and ECM (n = 35) species, respectively. Given the contrasting symbiosis pathways between ECM angiosperms and ECM gymnosperms from Pinaceae family (Brundrett, 2002), we further performed this analysis for ECM angiosperms (n = 28) by removing Pinaceae species (n = 7). Lastly, linear regressions were used to examine the relationships of soil nutrients with pPCA loadings on the

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'root-lifespan' dimension (PC1) and the 'fungal-symbiosis' dimension (PC2) for all species, AM species, and ECM species. To meet the assumption of normality, we $\log_{10}$-transformed data for each root trait in bivariate-correlation analyses and pPCAs.

Results

Variations in root traits and plant phylogeny influences

Across 112 tree species (Table **S1**), we found patterns of variation in absorptive root traits (Table **1**) that differ from previously-published global patterns of trait variation, as available from the GRooT database (Guerrero-Ramírez *et al.*, 2021) (Table **S3**). The symbiotic root traits (BI and CT) varied considerably, with BI ranging from a minimum of 0.77 cm^{-1} of *Cinnamomum porrectum* (AM species) to a maximum of 12.80 cm^{-1} of *Castanea mollissima* (ECM species) (coefficient of variation (CV) = 63.9% vs. 60.2% in the GRooT database). There was an eight-fold variation in CT, ranging from 0.04 mm of *C. mollissima* (ECM species) to 0.32 mm of *Diospyros lotus* (AM species) with a CV of 36.8% vs. 59.5% in the GRooT database. For morphological root traits, there were greater variations in RTD (CV = 80.2% vs. 68.5% in the GRooT database) and SRL (CV = 77.6% vs. 181.5% in the GRooT database) than RD (CV = 16.0% in our dataset vs. 45.1% in the GRooT database). For root tissue C and N, the variation in RC (CV = 10.4% vs. 16.6% in the GRooT database) was smaller than that in RN (CV = 25.3% vs. 37.3% in the GRooT database). The very low value of RC observed in some species suggests that roots may have been contaminated with soil minerals, especially in ECM roots that are harder to clean, with potential effects on associated values of those mass-related traits.

There were no significant phylogenetic signals for the two symbiotic traits that are most indicative of root construction trade-offs with fungal association (CT for AM trees vs. BI for ECM trees) when all species were analyzed as well as when analyses were run within mycorrhizal types based on Blomberg's *K* values ($P > 0.05$; Table **1**). Root diameter and RN were influenced by plant phylogeny and exhibited higher phylogenetic conservatism than other root traits (Table **1**). For instance, gymnosperms and species in the Magnoliids tended to have thicker roots with higher

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RN, while species belonging to the more-recently evolved clades like the Rosids showed the opposite patterns (Fig. **3**). Also, similar patterns of phylogenetic influence were observed when separating all species into different mycorrhizal groups, except for root diameter which had a marginally significant phylogenetic signals for AM species ($P = 0.073$, Table **S4**).

Bi-variate relationships among root traits

Our results from the phylogenetically-corrected bivariate trait relationships, particularly for those related to symbiotic interactions and RN, showed striking differences when performed on all species versus on separate mycorrhizal groups (Figs. **4**, **S1**-**S2**). Statistically significant correlations were found among BI, CT and root diameter, with BI decreasing linearly with both CT and root diameter for all species ($r^2 = 0.04$, $P < 0.05$ and $r^2 = 0.40$, $P < 0.001$, respectively; Fig. **4**) and for AM species only ($r^2 = 0.07$, $P < 0.05$ and $r^2 = 0.22$, $P < 0.001$, respectively; Fig. **S1**). However, for ECM species, we found negative relationships between BI and root diameter ($r^2 = 0.15$, $P < 0.05$) and between CT and SRL ($r^2 = 0.12$, $P < 0.05$) (Fig. **S2**). Additionally, RTD was negatively correlated with SRL ($r^2 = 0.95$, $P < 0.001$; Fig. **4**) for all species, and this correlation showed similar patterns for both AM ($r^2 = 0.93$, $P < 0.001$; Fig. **S1**) and ECM ($r^2 = 0.96$, $P <0.001$; Fig. **S2**) species, separately. RN was negatively correlated with BI ($r^2 = 0.04$, $P < 0.05$) for all species, but had no significant relationships with BI when taking a close look at mycorrhizal type.

Multivariate ordination RES

In accordance with other research on the RES, the pattern of multivariate ordination was represented by two independent axes (dimensions) since the eigenvalues of the first two principal components were > 1 for all species (Table **S5**). The results of phylogenetically-informed principal component analysis (pPCA) showed that the first two axes accounted for 55.5% of total variation for all species. The first axis was best described by SRL and RTD, while the second axis was represented by root diameter, BI and CT (Fig. **5a, b**; Table **S5**). The results of the ANOVA analysis on the second axis scores of AM versus ECM species from the result of pPCA for all species indicated a significant influence of mycorrhizal type on RES axis too ($P < 0.001$; Table

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S6). We found a similar pattern of RES among AM species to that among all species with the first two axes explaining 31.3% and 27.3% of total variations (Fig. **5c, d**; Table **S5**). For ECM species, the first axis was also driven by SRL and RTD, explaining 32.5% of total variation, while the second axis was mostly driven by BI, RD and RN, explaining 19.6% of total variation (Fig. **5e, f**; Table **S5**). A similar pattern was found for ECM angiosperms (i.e., when excluding gymnosperms from the ECM group) with a shift in the location of root diameter from the second axis to the first axis (Table **S5**; Fig. **S3**).

Linkages of root traits and RES with edaphic nutrients

We found divergent correlations between root traits and soil nutrient availability for all species and for species belonging to the different mycorrhizal groups (Table **S7**). BI was positively correlated with soil nitrate concentrations for AM species (r = 0.38, P < 0.001; Table **S7**), but was negatively correlated with soil available P and nitrate concentrations for ECM species (r = -0.48, P < 0.01 and r = -0.43, P < 0.01, respectively; Table **S7**). CT was negatively correlated with concentrations of available P and nitrate for both all species (r = -0.23, P < 0.05 and r = -0.24, P < 0.05, respectively; Table **S7**) and AM species (r = -0.34, P < 0.01 and r = -0.37, P < 0.001, respectively; Table **S7**). BI and CT were unrelated to soil ammonium availability for all species (Table **S7**).

Consistent with these relationships between soil available nutrients and root traits, we observed several significant relationships between our pPCA axes and soil nutrient availability (Fig. **5**; Table **S8**). For AM species, we found a significant tendency towards greater CT and root diameter on the second axis with decreasing soil availability of P (r^2 = 0.05, P < 0.05, Fig. **5d**; Table **S8**) and nitrate (r^2= 0.08, P < 0.05). By contrast, ECM species showed significant tendency towards increased BI on the second axis with decreasing soil availability of P (r^2 = 0.17, P < 0.05, Fig. **5f**; Table **S8**) and nitrate (r^2 = 0.15, P < 0.05). We found no significant relationships of the second axis with any soil nutrient factor for all species (Fig. **5b**; Table **S8**). Additionally, we found a significant tendency towards higher RTD on the first axis with decreasing soil nitrate availability

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for all species and AM species ($r^2 = 0.06$, $P < 0.01$ and $r^2 = 0.06$, $P < 0.05$, respectively; Fig. **5a, c**; Table **S8**), but decreasing soil P availability for ECM species ($r^2 = 0.13$, $P < 0.05$, Fig. **5e**; Table **S8**). Soil ammonium availability was not correlated with either of the two axes for all species and both mycorrhizal types (Table **S8**).

Discussion

Building on a recent global representation of the RES, we demonstrate the context-dependency of the RES as a conceptual framework. Specifically, we reveal two functionally-independent ('root-lifespan' vs. 'fungal-symbiosis') dimensions that differ from previous representations of the RES. First, a dimension reflecting aspects of resource 'conservation', hereafter called the 'root-lifespan' dimension to differentiate it from the 'conservation' axis described by Bergmann *et al.* (2020), which is characterized by a trade-off between SRL and RTD—two key traits that are associated with root construction strategy—rather than a trade-off between RTD and RN. Second, the 'fungal-symbiosis' dimension, which is best characterized by the direct (BI and CT) and indirect (root diameter) symbiotic traits, rather than a simple collaboration axis characterized by a trade-off between SRL and root diameter (Bergmann *et al.* 2020). This refined perspective of the RES adds two traits which differentiate AM and ECM mycorrhizal pathways (Fig. **S4**) on root functional morphologies. Additionally, two major changes distinguish our findings from these of Bergmann *et al.* (2020): the absence of RN along our 'root-lifespan' axis and the sharp decoupling of SRL with the 'fungal-symbiosis' axis.

At the root-system level, root economics operate on plant-derived C ('capital') and soil-derived nutrients ('commodities'), leading to a trade-off between C investment and nutrient return (Bloom *et al.*, 1985). Although both SRL and RTD are associated with the rate of return per unit of investment, they represent opposite directions based on the theoretical formula: $SRL = 4/(\pi \times RTD \times \text{root diameter}^2)$ (Ryser, 1996; Ostonen *et al.*, 2007) or empirical trade-off relationships (Zhou *et al.*, 2018; Ma *et al.*, 2018; Han & Zhu, 2020). These traits have traditionally been regarded as the belowground equivalent of aboveground specific leaf area and leaf tissue density

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that locate at the two ends of the one-dimensional 'fast-slow' leaf economics spectrum (Ryser, 1996; Wright *et al.*, 2004; Reich, 2012). Belowground, species with an 'acquisitive root-strategy' tend to develop absorptive roots with higher SRL and shorter lifespan (McCormack *et al.*, 2012), while species with a conservative root-strategy tend to develop absorptive roots with higher RTD and longer lifespan (Ryser 1996; Eissenstat et al., 2000). Therefore, the 'root-lifespan' dimension of the RES represents a meaningful shift from high SRL to high RTD and depicts a belowground acquisition-conservation trade-off for tree species. The strong relationship between SRL and RTD could be due to the low variability in root diameter, with SRL being mostly defined by RTD (Fig. **2b**). Patterns of variation in absorptive root diameter from the current study (Table **1**) differ markedly from previously published global patterns of variation in fine root (< 2 mm) diameter for woody species (CV = 45.1%, Table **S3**; Fig. **S5**) in the GRooT database (Guerrero-Ramírez *et al.*, 2021), which may cause the contrasting RES patterns. As such, the 'root-lifespan' dimension could be sensitive to root diameter variation, particularly when incorporating multiple root categorizations for woody species. Given that only a small share (*ca*. 37%) of data in the GRooT database comes from absorptive roots, further studies with more standardized root categorization (e.g., absorptive roots) are needed to determine the general pattern of the RES.

The reasons why RN does not align with the axis of RTD variation, as observed across the global GRooT database (Guerrero-Ramírez *et al.* 2021) used by Bergmann *et al.* (2020) accounting for 7% shared variation between RN and RTD, remains unclear to us as this trend appeared consistent across their subset of woody species. Moreover, RN varied substantially in our data, although variation was smaller than in the GRooT dataset (CV = 37.3%, Table **S3**; Fig. **S5**), One potential reason may relate to our focus on absorptive roots of the first and second orders, according to the most recent functional categorization of roots (McCormack *et al.* 2015), whereas the GRooT database includes a majority of root data representative of the historical focus on roots < 2 mm. Since roots of the most distal orders are known to be the most metabolically active and display higher RN concentratrions than higher-order roots (Iversen *et al.* 2017; Freschet *et al.* 2021), the RN gradient in Bergmann *et al.* (2020) could represent to some extent a gradient

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variation in diameter of absorptive roots can be explained by CT in woody species (Gu *et al.*, 2014; Kong *et al.*, 2019). This pattern was also confirmed by the positive relationship between root diameter and CT (Fig. **4**), indicating that within the data analyzed here, root diameter tends to be more directly linked to symbiotic traits than to other morphological traits (i.e., SRL and RTD).

Although CT diverged from root diameter on the 'fungal-symbiosis' dimension for ECM species, a trade-off appeared between root diameter and BI (Fig. **5e, f**; Fig. **S2**), implying that thinner-rooted ECM species had more branching and vice versa. Ectomycorrhizal fungi have co-evolved divergent soil exploration and nutrient use strategies in complementarity with root morphologies (Agerer, 2001, Tedersoo & Smith, 2013). ECM fungal groups with a long-distance exploration (i.e., long hyphae) are commonly associated with thicker-root species to compensate for the deficiency in nutrient exploitation of these species (Liu *et al.*, 2015). By contrast, ECM species with thinner roots and more branching tend to associate with short-distance soil exploring fungal taxa (i.e., those with short fungal hyphae), which demand less construction and maintenance costs (Chen *et al.*, 2018). For instance, trees in the Pinaceae family have relatively thick roots, and can associate with rhizomorphic hyphae that explore longer distance into the surrounding soil (Agerer, 2001). Consistently, ECM gymnosperms (i.e., Pinaceae in this study) had larger root diameters and lower BIs than ECM angiosperms (Table **S9**). The trade-off between root diameter and BI persisted when excluding gymnosperms from the ECM group (Table **S5**; Fig. **S3**), implying the robustness of this trade-off among ECM species. Overall, these findings jointly highlight that the type of mycorrhizal symbiosis acts as a critical driver to the complexity of root economics owing to the contrasting evolutionary history and symbiosis pathways between AM and ECM species.

The symbiosis intensity lies in the resource needs of the symbiotic partners, which is fundamentally mediated by the soil matrix. Plants can establish symbioses with mycorrhizal fungi to forage immobile soil P (van der Heijden *et al.*, 2015; Liu *et al.*, 2018). The tight linkages between the 'fungal-symbiosis' dimension and soil P availability for both AM and ECM species

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(Fig. **5d, f**; Table **S8**), support that edaphic fertility drives, at least to some extent, the position of plant species within the multidimensional RES (Ding *et al.*, 2020). These relationships were clearly dominated by symbiotic root traits, as revealed by the negative relationships of soil available P concentration with CT for AM species and with BI for ECM species (Table **S7**). Thus, soil P availability can shape the 'fungal-symbiosis' dimension of the RES for both mycorrhizal types. Soil nitrate availability presented similar correlations with this dimension for both AM and ECM species (Fig. **5d, f**; Table **S7**). This synergy could relate to the close linkage between soil nitrate and P concentrations (Fig. **5**) or highlight the importance of mycorrhizal fungi in plant acquisition of N (Hodge, 2003; Treseder, 2003; Miller & Cramer, 2004). We included species from subtropical and temperate forests where soil ammonium and nitrate availability varied greatly (Table **S10**). The young soils that originate from volcanic parent material in temperate forests typically have high levels of nitrate, while the older highly-weathered soils in subtropical forests show higher levels of ammonium than nitrate (Table **S10**). Despite this, it seems that the dominant form of N has limited effect on the RES.

The 'root-lifespan' dimension of the RES was driven by soil P availability in ECM species but by soil nitrate availability in AM species (Fig. **5c**; Table **S8**). The observed negative relationships between the 'root-lifespan' dimension and soil fertility suggest that plants tend to employ a conservative strategy by increasing cell wall fraction and thus RTD for extending root lifespan under infertile habitats (Eissenstat & Yanai, 1997; Ding *et al.*, 2020). The distinct controls of soil nitrate and P availability over the 'root-lifespan' dimension of AM and ECM species could be related to the contrasting nutrient economies between mycorrhizal types (Phillips *et al.*, 2013). Different from the organic-nutrient ECM species, AM species are assumed to specialize in inorganic nutrient uptake, given the limited saprotrophic ability of most AM fungi (Smith & Read, 2008). In addition, our results suggest that, independent of plant reliance on mycorrhizal symbiosis intensity, AM species may increasingly rely on thin non-mycorrhizal roots to acquire nitrate in conditions of high nitrate availability, whereas ECM species may rely more largely on mycorrhizal symbiosis. Although AM and ECM trees exhibit similar abilities to acquire mineral P, ECM fungi

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may have an advantage of immobile P acquisition over longer distances and from organic sources (Tedersoo & Bahram, 2019), which may offset the costly maintenance of the ECM structures (Phillips *et al.*, 2013). One may thus expect that ECM species tend to construct roots with higher RTD to extend root lifespan and symbiotic partnership with soil fungi for maximizing P acquisition in P-deficient soils (Kramer-Walter *et al.*, 2016; Kou *et al.*, 2017). However, the relationships of edaphic fertility with morphological traits and the RES were overall weak (Fig. **5**; Tables **S7, S8**), which may point towards the influence of other belowground interactions on the RES. For example, interspecific root interactions, root-pathogen-herbivore interactions, and root phosphatase (Valverde-Barrantes *et al.*, 2013; Bardgett *et al.*, 2014; Han *et al*. 2021) may likewise shape the structure of the RES.

In conclusion, by elaborating on root trait-function linkage and fertility-driven root trait interplay, we demonstrate a bi-dimensional RES based on root functions: root-lifespan vs. fungal-symbiosis, and provide a refined framework for theoretical and mechanistic understanding of the complexity of root economics within tree species. Irrespective of mycorrhizal type, the 'root-lifespan' dimension rendered by SRL and RTD depicts a resource acquisition-conservation trade-off representing the maintenance of root lifespan along the edaphic fertility gradient. Interestingly, the 'fungal-symbiosis' dimension characterized by the direct (BI and CT) and indirect (root diameter) symbiotic traits differs between AM and ECM species or even possibly between angiosperms and gymnosperms within ECM groups. These fundamental divergences have likely resulted from the contrasting evolutionary history and symbiosis pathways between mycorrhizal groups. Arbuscular fungi co-evolved with a large habitat dependence of a thicker root cortex, but increased root branching was prioritized for ECM angiosperms. Importantly, the availability of soil P and nitrate are key determinants for shaping the RES, particularly the 'fungal-symbiosis' dimension via potentially differentiating root-fungal symbiosis intensity among species. Formal testing of the hypothesis that increased root colonization by ECM fungi is related to higher root branching should therefore be a key priority of future research in this area. Overall, our findings suggest that root-fungal symbiosis pathways and soil fertility have jointly influenced root trait

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variation, which underpins the role of complex belowground interactions in framing root economics.

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Author contributions

L.K. and H.Y. conceived the ideas and designed the study. H.Y. performed the experiments, collected the data, performed data analyses and drew the graphics. L.K., H.Y. and G.T.F. led the writing of the manuscript. H.W., J.A.H., S.L., O.J.V., X.F., R.W., X.D, L.J., S.M., F.Y. and M.Z. substantially contributed to writing the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data Availability Statement

All data supporting the findings of this study can be accessed in Dryad: https://doi.org/10.5061/dryad.j0zpc869s (Yan et al., 2022).

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Table 1 Summary of absorptive-root traits and phylogenetic signals (Blomberg's *K*) for 112 tree species.

Trait	Abbreviation	Units	Min	Max	Mean	CV%	*K* value
Branching intensity	BI	branches cm^{-1}	0.77	12.80	4.25	63.9	0.029
Cortex thickness	CT	mm	0.04	0.32	0.18	36.8	0.044
Root diameter	RD	mm	0.31	0.62	0.38	16.0	**0.080**
Root tissue density	RTD	g cm^{-3}	0.04	0.97	0.25	80.2	0.018
Specific root length	SRL	m g^{-1}	7.78	303.96	69.27	77.6	0.014
Root nitrogen concentration	RN	%	0.81	2.63	1.78	25.3	**0.080**
Root carbon concentration	RC	%	28.28	50.73	42.30	10.4	0.016

Values (mean, $n = 112$); Min, minimum value of the trait; Max, maximum value of the trait. The significant *K* values are in bold ($P < 0.05$).

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Figures

Fig. 1 Schematic depiction of divergent symbiosis pathway of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) roots. a, In AM roots, fungal hyphae (depicted in red) principally from glomeromycota and endogonomycetes grow between and penetrate root cortical cells to form intracellular arbuscules and vesicles. Thus, AM plant roots tend to facilitate mycorrhizal symbiosis by expanding cortical space for increased habitat availability for AM fungi. In ECM roots, fungal hyphae (depicted in blue) principally from basidiomycetes and ascomycetes, stemming from saprotrophic ancestors grow attached to the epidermal cell of lateral roots to form a sheathing mantle, then develop an intercellular 'Hartig net' around root cortical cells (ECM angiosperms) or around and into cortical cells (ECM gymnosperms). **b**, For AM species, mycorrhizal colonization intensity can be approximated by the proportion of total absorptive-root length colonized. **c**, For ECM species, mycorrhizal colonization intensity is rather a fungal occurrence frequency on root tips. ECM fungal taxa exudate hormones such as ethylene and auxin to stimulate proliferation of lateral roots, thereby ECM species tend to regulate root branching structure hosting mycorrhizal fungi. The left diagram is inspired by Bonfante & Genre, (2010).

Fig. 2 Conceptual framework for root trait-function linkage and trait interplay. a, At the root-system level, root economics run on plant-derived carbon ('capital') and soil-derived nutrients ('commodities'), reflecting resource return (nutrient acquisition) on investment (carbon expense). Root diameter (RD) and specific root length (SRL) simultaneously link to two major root functions—root-lifespan and fungal-symbiosis. This multifunctionality connects root tissue density (RTD) and root nitrogen concentration (RN), both related to root-lifespan, with symbiotic traits (cortex thickness (CT) and branching intensity (BI); related to fungal-symbiosis). Thus, root-lifespan is represented by interactions among RD, SRL, RTD, and RN, while fungal-symbiosis is represented by interactions among RD, SRL, BI, and CT. The role of RD and SRL in root economics is determined by the interplay among root traits. **b**, By using the term 'trait interplay' in root economics, we here illustrate that root trait trade-offs are not necessarily bivariate but can be more complex, involving multiple traits simultaneously at the root-system level. The mathematical causal trait relationships (solid lines) and expected trait covariations (dotted lines) are displayed by red arrow and blue arrow that denote positive and negative relationships, respectively. Following Freschet *et al.* (2021a), trait position along the vertical axis represents trait hierarchy, with upper levels representing 'basal' traits and lower levels representing 'composite' traits. The chemical

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root traits are not displayed in root trait interplay for brevity.

Fig. 3 Plant phylogeny influences on key root economics traits. The inside diagram displays the phylogenetic tree of 112 tree species. The bottom colors for latin name represent different clades, and circles at the tip of the phylogenetic branch denote mycorrhizal type colored in blue for arbuscular mycorrhizal (AM) species or in orange for ectomycorrhizal (ECM) species. For the outside diagram, the standardized values of root economics traits involved in trait interplay are mapped onto each species with values ranging from low (green) to high (purple). Root diameter (RD) exhibits the highest phylogenetic conservatism among these root traits (see Table **1**). Specific information about clade and mycorrhizal type for each species is exhibited in Table **S1**. SRL, specific root length; RTD, root tissue density; BI, branching intensity; CT, cortex thickness.

Fig. 4 Pairwise correlations among seven root economics traits for all species. The bivariate relationships among root traits using general linear and phylogenetically corrected regressions for all tree species (n = 112). Significant correlations are represented by regression lines colored in blue for general linear regression and in red for phylogenetically corrected regression in scatter plots (upper triangle). Correlation coefficients are displayed in the same colors for the two regression analyses (lower triangle). BI, branching intensity; CT, cortex thickness; RD, root diameter; RTD, root tissue density; SRL, specific root length; RN, root nitrogen concentration; RC root carbon concentration.

Fig. 5 Fertility-driven root economics pattern varies with mycorrhizal type. Root trait biplots of phylogenetically-informed principal component analyses (leaf diagram) for **a and b**, all 112 tree species; **c and d**, 77 arbuscular mycorrhizal (AM) species (blue circle); **e and f**, 35 ectomycorrhizal (ECM) species (orange circle). The 'root-lifespan' dimension is characterized by specific root length (SRL) and root tissue density (RTD), while the 'fungal-symbiosis' dimension is predominated by cortex thickness (CT) and/or branching intensity (BI), depending on mycorrhizal type. Along with the two direct symbiotic traits, root diameter (RD) also loads on the 'fungal-symbiosis' dimension representing the outcome of the interplay between morphological and symbiotic root traits. Edaphic fertility is embedded into the biplots to indicate the influences of different soil nutrients on root economics with color gradients from dark green (low NO_3^-) to light green (high NO_3^-), from dark grey (low AP) to light grey (High AP), and from dark brown

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(low AP + NO_3^-) to light brown (low AP + NO_3^-). The linear regressions are fitted (right diagram) between soil nutrient availability and axis loadings along the 'root-lifespan' dimension (PC1) for all species (**a**), AM species alone (**c**), ECM species alone (**e**) as well as along 'fungal-symbiosis' dimension (PC2) for all species (**b**), AM species alone (**d**), ECM species alone (**f**). See Table **S5** for the loading scores of the two dimensions and Table **S8** for more details about the linear regression results. RN, root nitrogen concentration; RC root carbon concentration; NO_3^-, soil nitrate concentration; AP, available soil phosphorus concentration. PC, principal component.

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Supporting information

Figure S1 Pairwise correlations among seven root economics traits for arbuscular mycorrhizal species.

Figure S2 Pairwise correlations among seven root economics traits for ectomycorrhizal species.

Figure S3 Root economics pattern for ectomycorrhizal angiosperms.

Figure S4 The test of the root economics space (RES) using the four traits RD, RTD, SRL and RN in Bergmann *et al.*, 2020.

Figure S5 Density histograms of woody species root trait distribution of our own data on absorptive roots as compared to these of fine root (diameter < 2 mm) traits from the GRooT database.

Table S1 Species information in the study.

Table S2 Loading scores of phylogenetically-informed principal component analysis of root traits for all species, arbuscular mycorrhizal (AM) species, and ectomycorrhizal (ECM) species based on the corrected RTD data (carbon basis).

Table S3 Summary of the seven root traits used in this study based on the GRooT database.

Table S4 Blomberg's *K* value of root traits for arbuscular mycorrhizal (AM, $n = 77$) and ectomycorrhizal (ECM, $n = 35$) species.

Table S5 Results of phylogenetically-informed principal component analysis of root traits for all species, arbuscular mycorrhizal (AM) species, ectomycorrhizal (ECM) species, and ECM angiosperms.

Table S6 One-way ANOVA analysis on the effect of mycorrhizal type on scores of "fungal-symbiosis" dimension of 112 tree species.

Table S7 Coefficients of Pearson's correlation for for linkages of root traits with edaphic nutrients for all species, arbuscular mycorrhizal (AM) species, and ectomycorrhizal (ECM) species.

Table S8 Results of the general linear regression between principal component (PC) scores and edaphic nutrients.

Table S9 Student's *t*-test evaluating the differences in root traits between angiosperms ($n = 28$) and gymnosperms ($n = 7$) of ectomycorrhizal (ECM) species.

Table S10 Student's *t*-test evaluating differences in soil nutrient availability between species from subtropical ($n = 64$) and temperate ($n = 48$) forests.

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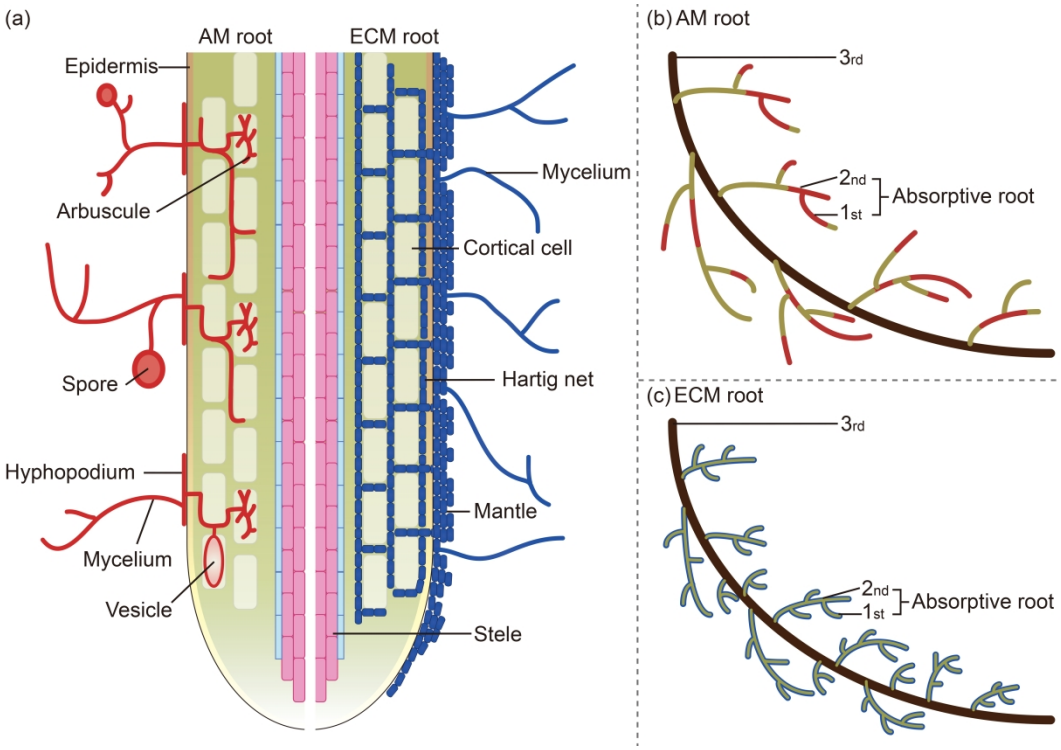

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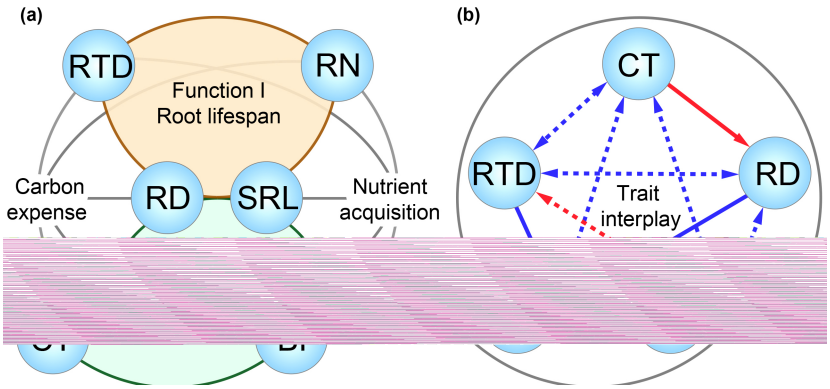

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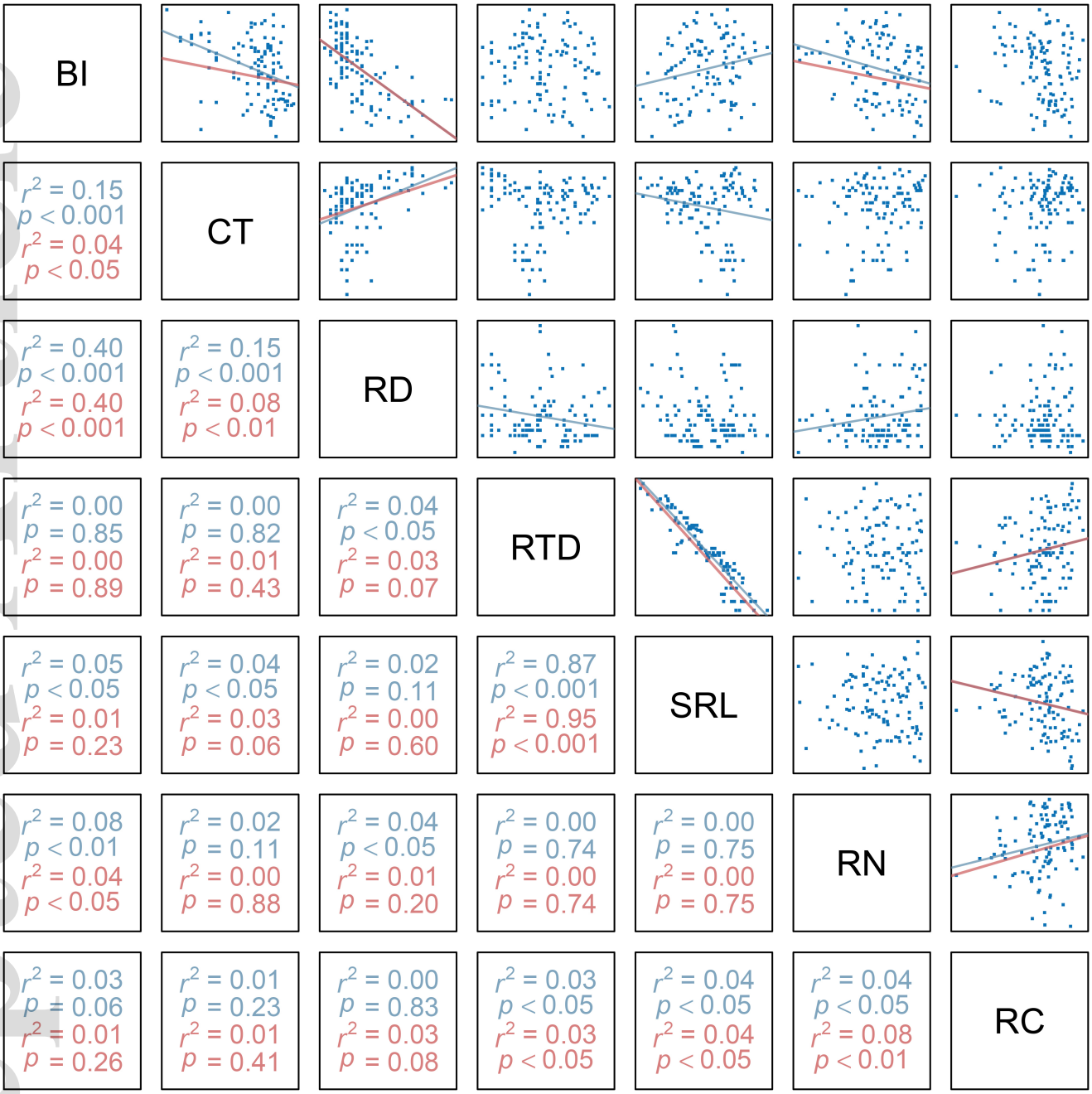

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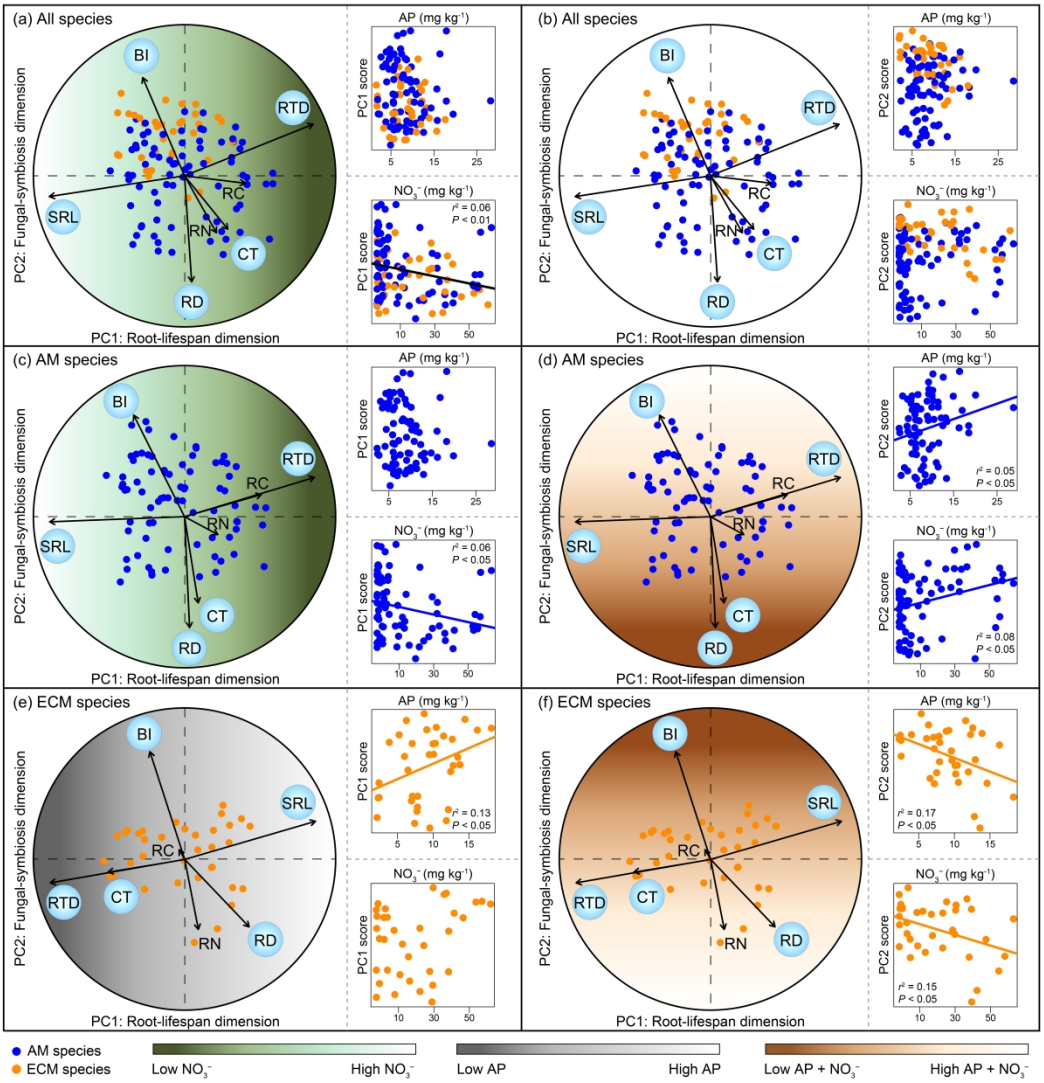

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