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Research

Coordination between compound-specific chemistry and morphology in plant roots aligns with ancestral mycorrhizal association in woody angiosperms

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Summary

- Recent studies on fine root functional traits proposed a root economics hypothesis where adaptations associated with mycorrhizal dependency strongly influence the organization of root traits, forming a dominant axis of trait covariation unique to roots. This conclusion, however, is based on tradeoffs of a few widely studied root traits. It is unknown how other functional traits fit into this mycorrhizal-collaboration gradient. Here, we provide a significant extension to the field of root ecology by examining how fine root secondary compounds coordinate with other root traits.
- We analyzed a dataset integrating compound-specific chemistry, morphology and anatomy of fine roots and leaves from 34 temperate tree species spanning major angiosperm lineages.
- Our data uncovered previously undocumented coordination where root chemistry, morphology and anatomy covary with each other. This coordination, aligned with mycorrhizal colonization, reflects tradeoffs between chemical protection and mycorrhizal dependency, and provides mechanistic support for the mycorrhizal-collaboration gradient. We also found remarkable phylogenetic structuring in root chemistry. These patterns were not mirrored by leaves. Furthermore, chemical protection was largely decoupled from the leaf economics spectrum.
- Our results unveil broad organization of root chemistry, demonstrate unique belowground adaptions, and suggest that root strategies and phylogeny could impact biogeochemical cycles through their links with root chemistry.

Introduction

One major goal of plant ecology is to elucidate the organization of the enormous variation in plant traits, which is key to understanding feedbacks between plant biodiversity, community assembly and ecosystem processes (Díaz *et al.*, 2016). Leaves and fine (or absorptive) roots have been focuses of this subject, as they both are resource-acquiring organs essential for plant fitness and critical contributors to biogeochemical cycles (Wright *et al.*, 2004; Ma *et al.*, 2018). Comparison of the morphology, physiology and elemental composition of leaves over the last decade has revealed a trait coordination axis that ranges from leaves with high nitrogen concentrations (N%), high photosynthetic rates, high specific leaf area (SLA, the light-intercepting area per unit mass invested) and short lifespans, to leaves with low metabolic activity but expensive tissue construction and long lifespans (Wright *et al.*, 2004; Reich, 2014). This axis was referred to as the leaf economics spectrum (LES) because it demonstrates how plants manage limited resources, where investments require trading off one goal for another, and thus are inherently economic (Bloom *et al.*, 1985; Wright *et al.*, 2004; Mankiw, 2014). The LES reflects tradeoffs between resource acquisition and conservation, constituting a framework for explaining leaf trait diversity and plant strategies globally.

Ongoing progress regarding fine root trait coordination has uncovered a more complex, multidimensional economics space that reflects multiple evolutionary pressures and tradeoffs below ground (Kong *et al.*, 2014; Weemstra *et al.*, 2016). A recent global synthesis demonstrated that the variation of root functional traits was mostly explained by a root–mycorrhizal collaboration gradient, along which specific root length (SRL, the absorptive length per unit mass invested) and root diameter (a proxy for symbiont habitat) covary in opposite directions, representing tradeoffs between 'do-it-yourself' to 'outsourcing' for resource uptake (Bergmann *et al.*, 2020). This collaboration gradient is largely independent from a root conservation axis, which is analogous to the LES and driven by tradeoffs between tissue density and N%. These highly generalizable patterns represent

major advances in understanding root trait diversity and adaptation below ground. However, current knowledge on root trait organization was developed using a few easily measured functional proxies, which have been questioned on their functional implications due to their indirect, ambiguous relationships with root functions in complex soil environments (Freschet *et al.*, 2020). The current paradigm of two-dimensional root economics space could provide insight into adaptive modifications of other traits that have more defined functions for plant fitness and ecosystem processes. Exploring root traits that have a more direct, clearer functional significance from a resource economics perspective would help disentangle the mechanistic basis for the root trait coordination.

Here, we aimed to add a significant expansion to the framework of root resource economics by using this approach to assess the organization of compound-specific chemistry that regulates both plant functions and biogeochemical cycles. Many protective compounds are known to mediate tissue integrity, chemical defense and interactions with fungal partners, and thus are integral to tissue growth, protection and resource strategies. For example, in roots, structural polyphenols (e.g. lignin and cross-linking phenols) increase cell-wall mechanical strength, which contributes to tissue stability and allows roots to penetrate soil matrix for resource acquisition (Santiago *et al.*, 2013; Schneider *et al.*, 2021). Furthermore, both structural and nonstructural polyphenols (e.g. tannins and flavonoids) regulate biotic interactions of roots: they add to chemical protection against biotic stresses but could negatively affect root–mycorrhizal collaboration due to their antifungal properties (Bhuiyan *et al.*, 2009; Orhan *et al.*, 2010; Daglia, 2012; Santiago *et al.*, 2013; Solaiman & Senoo, 2018; Suseela *et al.*, 2020). While delivering important functions, these protective phenolic compounds account for up to 30% of root biomass (Xia *et al.*, 2015; Sun *et al.*, 2018) and are biosynthetically costly (Chapin, 1989; Hemingway & Karchesy, 1989), thus representing substantial C allocation to their associated functions. At ecosystem scales, lignin and condensed tannins (CTs) impact biogeochemical cycling by affecting litter decomposition and N cycling (Berg, 2000; Tharayil *et al.*, 2013; Sun *et al.*, 2018).

Because protective compounds are integral to resource strategies (e.g. tissue conservation, mycorrhizal association) and represent significant C investments, our overarching hypothesis is that the significant selection that shapes the previously identified root trait tradeoff axes may also impact the organization of protective compounds. The novelty of our approach is in the integration of the variation in protective compounds as a root function. So far, the chemical aspect of resource economics has been largely limited to elemental concentrations, while higher resolution chemistry has remained unexplored. Integrating compound-specific features into the framework of plant functional tradeoffs allows for the fine-tuning of chemical adaptations to be linked to species-level strategies and ecosystem-level biogeochemical processes.

Our hypothesis provides a set of testable predictions for the relationships between tissue chemistry and other functional traits, as well as the coordination between roots and leaves. There was substantial evidence that root trait variation was dominated by the mycorrhizal-collaboration axis that was driven by the inverse relationship between SRL and root diameter and linked to a mycorrhizal association (Ma *et al.*, 2018; Bergmann *et al.*, 2020). If the relationship of protective compounds and other root traits reflects this collaboration gradient, we predict (1) that the species bearing fine roots with large diameter and low SRL will maintain wide cortex (habitat for symbionts) and low levels of secondary protective compounds, enhancing the collaboration with mycorrhizae. By contrast, thinner, more branched root systems would allow substantial accumulation of defense compounds as plants become less reliant on fungal partners while increasing surface area would make them more susceptible to pathogen infections. The rationale is that, if roots depend heavily on mycorrhizal fungi, they could face selection to become a better host (Brundrett, 2002), which may be at odds with the abundance of secondary compounds exhibiting antifungal properties. If mycorrhizal symbiosis indeed plays a key role in shaping the proposed trait coordination, we predict that (1.1) the coordination of root chemistry and morphology should be coupled with variation in mycorrhizal colonization, and that (1.2) chemical protective features will be less coordinated between roots and leaves, as leaves do not sustain an analogous symbiotic relationship. On the other hand, if protective investments in roots are driven by a conservation axis that is often orthogonal to the collaboration gradient (Bergmann *et al.*, 2020), we predict that (2) roots with high tissue density but low N%, in tandem with conservative leaves from the LES framework, should show higher amounts of compounds that provide protection and structural stability but are biosynthetically expensive.

We test these predictions by analyzing a unique dataset integrating compound-specific chemistry, morphology, and anatomy in fine roots and leaves across 34 tree species spanning major angiosperm clades (Supporting Information Fig. S1) and replicated in two living tree collections (Fig. S2). This dataset: includes chemical traits that characterize both the abundance and the molecular composition of ecologically important secondary protective compounds that occur widely in trees, are relatively abundant in roots and leaves (i.e. generalized defense, in contrast to highly specialized defense), and perform explicit ecological functions (Table S1); focuses on species forming arbuscular mycorrhizal (AM) associations (found in 72% of vascular plants, Brundrett & Tedersoo, 2018), allowing us to control for confounding chemical/morphological modifications associated with alternative mycorrhizal types; and covers trees from basal to later-derived lineages, enabling us to investigate the structuring role of evolution history in plant traits.

Materials and Methods

Tissue sampling and processing

We collected samples from 34 tree species replicated at two sites (Fig. S2): the Holden Arboretum, Ohio, and Boone County Arboretum, Kentucky. The species assemblage was designed to represent three key angiosperm lineages (magnoliids, asterids and

rosids) for temperate trees forming AM symbiosis (Fig. S1). The Holden site receives an average annual precipitation of 1160 mm and an average annual temperature of 8°C. This site is within the broader zone of mixed mesophytic and beech–maple forest on fine-silty, mixed, active, mesic Aeric Fragiaqualfs from the Platea series. The Boone site has an average annual precipitation of 1050–1120 mm with an average annual temperature of 11°C. The soils are fine-silty Aquic Fragiudalfs in the Rossmoyne series.

Roots and leaves of each species were collected during mid-summer of 2010 and 2011. Samples from each species were taken from two healthy adult trees within a site as shown in Valverde-Barrantes *et al.* (2015). Briefly, roots were identified by tracing back to the main stem and collected from two soil cores (10 cm diameter × 15 cm deep) within 2 m of the main stem. More than five fully expanded sunlit leaves were sampled from the most distal first-year shoots from the same trees where roots were collected. The distal two orders of roots, which are predominantly responsible for resource acquisition and thus the belowground counterpart of leaves (Xia *et al.*, 2010; McCormack *et al.*, 2015), were used for analysis. In cases where the amount of sample was limited, roots from replicates of a species were combined within a site. Thirty-one species (excluding *Koelreuteria paniculata*, *Magnolia macrophylla* and *Magnolia kobus* due to sample limitations), collected from the Boone site, were analyzed for leaf traits, where leaves from two individual trees of a species were analyzed separately as biological replicates in chemical/morphological analysis.

Chemical, morphological and anatomical analysis

Bound phenols (BPs) and lignin were determined for all samples with a sequential extraction and CuO oxidation, followed by GC-MS (Wang *et al.*, 2015). BPs were released with base hydrolysis at 85°C from methanol-extracted pellets. *Trans*-cinnamic acid (CiAD) and ethyl vanillin (EVAL) were added to the hydrolysates as internal standards (ISs). The sediments after base hydrolysis were washed, dried and depolymerized in Acid Digestion Vessels model 4749 (Parr Instrument Co., Moline, IL, USA) with CuO and $Fe(NH_4)_2(SO_4)_2.6H_2O$ in N_2-sparged 2 M NaOH. The vessels were incubated at 155°C for 160 min and spiked with ISs. After adjusting the pH to < 2, the released bound phenols and the depolymerized lignin phenols were extracted with ethyl acetate, respectively. Twelve major phenolic monomers plus ISs were analyzed with GC-MS (Agilent 7890 system + Agilent 5975C mass detector; Santa Clara, CA, USA) after derivatization by N-methyl-N methyl-N-(trimethylsilyl)-trifluoroacetamide with 1% trimethylchlorosilane (MSTFA + 1% TMCS; Kaiser & Benner, 2012). These phenolic monomers were identified and quantified based on retention time, mass spectra and detector response of authentic standards (Table S2). The total yield of BPs and lignin phenols were calculated, respectively, as the added concentrations of all 12 phenolic monomers. The proportion of syringyl (S) and vanillyl (V) monomers in lignin (S/Lig and V/Lig) were calculated as the total yield of each type divided by lignin, respectively. The CT concentration was determined with an acid–butanol assay using the purified tannins from *Acer rubrum* leaves as standards (Tharayil *et al.*, 2011). The concentrations of soluble phenolics (SPs) were determined using a Folin–Ciocalteu assay (Singleton & Rossi, 1965). Hydrolysable tannins (gallotannins + ellagitannins, HTs) and flavonoids were subject to acid hydrolysis and determined following the procedure of Tharayil *et al.* (2011). Briefly, upon acid hydrolysis, gallotannins released gallic acids while ellagitannins underwent lactonization to produce ellagic acids. Gallic acids and ellagic acids were analyzed using an Ultimate 3000 HPLC device (Thermo Scientific, Waltham, MA, USA) coupled to an Orbitrap Fusion (Thermo Scientific) Tribrid mass spectrometer (Bowers *et al.*, 2018) and identified based on retention time, accurate mass, MS^2 fragmentation patterns from the literature (for ellagic acids) or authentic standards (gallic acids; Sigma-Aldrich). The abundance of gallotannins and ellagitannins was estimated as peak intensities, and the added peak intensities of gallotannins and ellagitannins were used as a proxy for HTs. Similarly, flavonoids were acid-hydrolyzed to produce aglycones, which were analyzed with UHPLC–Orbitrap MS. Ten types of aglycones were tentatively identified based on retention time, accurate mass and MS^2 fragmentation patterns from the literature or authentic standards (quercetin, kaempferol, myricetin; Sigma-Aldrich). The abundance of each aglycone was estimated as peak intensities, and the yield of total flavonoids was approximated as the added peak intensities of all aglycones. See Methods S1 for instrumental parameters.

Elemental composition, morphology, anatomy and mycorrhizal colonization were determined as shown in 2016). Briefly, carbon concentrations (C%) and N% were determined using an elemental analyzer (Model 4010; Costech Analytical Valencia, CA, USA). Root morphology parameters were acquired by root image analysis using WINRHIZO software (2007 Pro version; Regent Instruments, Quebec, QC, Canada). Leaf dry matter content (DMC) and SLA were measured following Wilson *et al.* (1999). Leaf thickness was measured *in situ* from 10 replicates at three points each using an electronic thickness gauge (Eagle Technology, Mequon, WI, USA). Mycorrhizal colonization was determined by both quantitative PCR with primers specific for 18S ribosomal RNA of AM fungi and trypan-blue staining followed by gridline intersect counting.

Data analysis

Data analysis was performed with R software (v.3.6.3). The phylogenetic tree was constructed with the package PHYLOMATIC using a dated molecular phylogeny (Zanne *et al.*, 2014). The effects of clade, site and their interactions on plant traits were tested with linear mixed models using the package LME4. The relationships between chemistry, N%, morphology and anatomy were examined by constructing a Pearson's correlation coefficient matrix using the package HMISC after variables were square-root transformed to improve homoscedasticity and normality of residual errors. To test the robustness of the relationships indicated by Pearson's correlation analysis, we also performed a nonparametric Spearman's rank correlation analysis with the package HMSIC. We next performed a principal component analysis (PCA) using the package STATS after the variable

matrix was transformed with a Box-Cox procedure (Box & Cox, 1964) using the package MASS to improve linearity and reduce the skewness of the trait distribution in linear multivariate models (Legendre & Borcard, 2018) and standardized to remove scale effects. To test the significance of clustering by clade, we performed a permutational multivariate analysis of variance (PERMANOVA, Euclidean distance) based on PCA scores from the first two axes, using the package VEGAN. We further examined how root chemistry covaries with root diameter and N% by fitting linear, allometric and piecewise models, using the package STATS and segmented. The allometric models describe nonlinear relationships between the fraction of root cross-sections occupied by the stele (pStel) and root diameter (x): $\text{pStel} = (1-2k-2cx^{-1})^2$, where k and c describe the relationship between cortex thickness (rC) and x as in ($rC = kx + c$, $c < 0$; Kong *et al.*, 2019). The piecewise models assessed ecological thresholds by fitting two lines joining at a threshold point, where an abrupt change of slope occurs (Muggeo, 2008). Davies' test was used to test the significance of the threshold (Muggeo, 2008). We included this model because relationships between root traits often changed abruptly (Chen *et al.*, 2013; Kong *et al.*, 2014). Model performance is shown in Table S3. The variance partitioning of mycorrhizal colonization by four variable categories followed the Legendre method (Legendre & Legendre, 2012) using the package VEGAN. Prior to the variance partitioning, we reduced the number of chemical variables by assessing the importance of each chemical trait to mycorrhizal status independently, followed by redundancy analysis (RDA) constrained by mycorrhizal status to further remove nonsignificant factors (Blanchet *et al.*, 2008). The chemical traits that entered the variance partitioning model were lignin, HT, CT, SP, flavonoids (quercetin and hesperidin). Morphology included Diameter, SRL, density, SRTA, Fractal and Link length. N status included N% and C : N ratios. Anatomy included cortex thickness and pStel. The net effect of a variable category was tested using RDA models in the package VEGAN. Relationships between leaf and root traits from standardized major axis tests and the slopes (or scaling exponents) were estimated by the package SMART. Phylogenetic nonindependence (tendency of closely related species to resemble each other) was estimated with four commonly used phylogenetic signal indices (Münkemüller *et al.*, 2012): Abouheif's C_{mean}, Moran's I, Pagel's λ and Blomberg's K, using the packages PHYTOOLS, APE and ADEPHYLO. We consider a trait as being phylogenetically structured if all four tests detect a significant phylogenetic signal ($P < 0.05$, Tables S4, S5), to minimize the chance of type I error. Because Abouheif's C_{mean} and Moran's I did not provide information on phylogenetic signal strength, Pagel's λ and Blomberg's K were used to assess the strength of phylogenetic signal (Münkemüller *et al.*, 2012). We fitted phylogenetic generalized least square models for pairwise relationships in root and leaf traits using the package CAPER. The phylogenetically corrected correlations were calculated from the model r^2 as in Bergmann *et al.* (2020). We then performed a phylogenetically informed PCA (pPCA) based on the phylogenetically corrected correlation matrix (Bergmann *et al.*, 2020).

Results

Trait variation across sites and phylogenetic lineages

Our dataset showed a broad range of variation in fine roots (Fig. 1; Table S6), from thick roots with high N% and low lignification (e.g. those from *Magnolia macrophylla* and *Liriodendron tulipifera*)

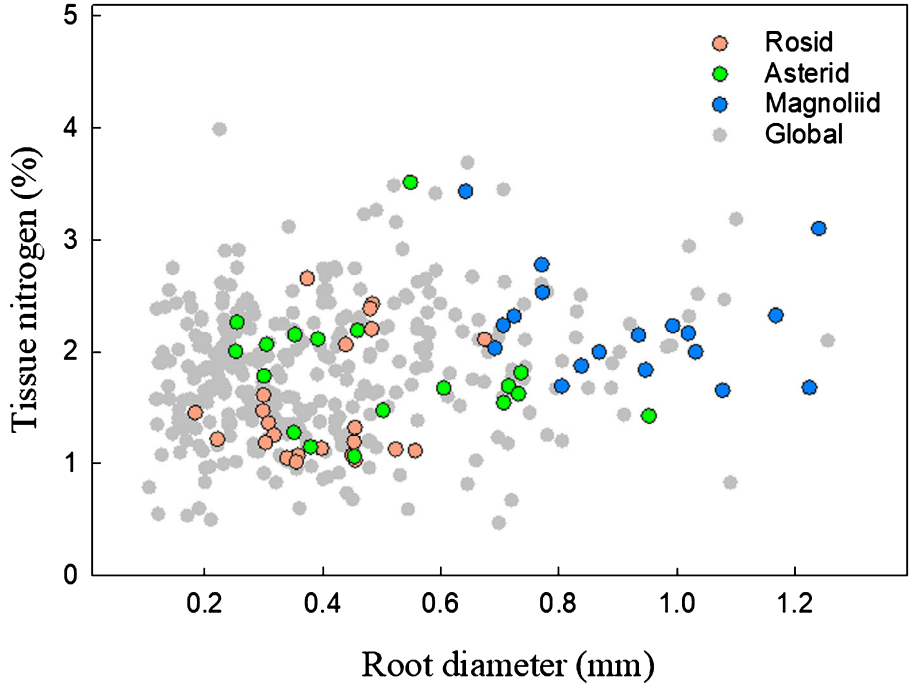

Fig. 1 Distribution of absorptive root diameter and tissue N concentration (%) across tree species spanning major angiosperm clades replicated in two study sites and that from a global data set of angiosperm woody species collected from multiple biomes (Kong *et al.*, 2019).

to very thin roots abundant in secondary protective compounds (e.g. *Ulmus americana* and *Acer saccharum*). Our collection encompassed a large proportion of the variation in fine root diameter and N% observed in a global woody species dataset (Fig. 1), suggesting that our sampling represents the natural variation in root traits found in plant communities across biomes and global climate gradients.

Phylogenetic division at superorder levels (clade) was an important factor explaining root trait diversity, while root traits were less influenced by sites (Table S6). Clade was a significant factor explaining the variations in most root traits (31 out of the 53 chemical features, all morphological traits and two anatomical traits, $P < 0.048$) after accounting for the site effect and the species-driven variation. Rosids tended to develop thinner, more branched (i.e. higher in specific root tip abundance (SRTA) and fractal dimension) roots with smaller cortex and more abundant protective compounds than magnoliids. Cell-wall components also differed by clade. Lignins in rosid roots tended to be more abundant in V moieties which form aryl–aryl bonds that are more resistant to depolymerization (Talbot *et al.*, 2012), while asterids developed roots high in bound ferulic acids (FADs), the dominant form of cell-wall cross-linkers that cement the cell-wall matrix (Santiago *et al.*, 2013). Such variations indicated fundamental differences in the construction of the root cell-wall matrix by clade with implications for tissue decomposition. Leaf traits were comparatively less affected by clade: nine out of 53 leaf chemical traits differed significantly by clade ($P < 0.043$; Table S7).

Coordination of chemistry, morphology and anatomy

We observed a close relationship between chemistry, morphology and anatomy in fine roots across species: protective compounds were generally lower in thicker, less branched roots with a lower SRL and larger cortex, supporting Prediction 1 (i.e. protective investments align with the collaboration gradient). Both the abundance ($P < 0.004$, $r^2 > 0.228$) and composition of lignin ($P < 0.061$, $r^2 > 0.106$) were linked with morphological and anatomical traits (Fig. 2a): thinner, more branched roots with a higher SRL and smaller cortex were associated with higher abundance of lignin richer in V moieties, suggesting greater protection. Similarly, other chemical protective features such as the abundance of CTs, SPs and total flavonoids also exhibited negative relationships with root diameter ($P < 0.018$, $r^2 > 0.162$) and cortex thickness ($P < 0.046$, $r^2 > 0.118$). By contrast, these protective features increased when pStel was higher ($P < 0.004$, $r^2 > 0.231$) and when root systems became more branched ($P < 0.096$, $r^2 > 0.084$). Spearman's rank correlation analysis examining the monotonic relationships between root traits confirmed a similar pattern to those detected by Pearson's correlation tests (Fig. S3).

The PCA exhibited a primary axis (PC1) similar to the dominant collaboration gradient observed previously (Ma *et al.*, 2018; Bergmann *et al.*, 2020), which was closely associated with SRL and diameter in opposite directions (Fig. 2c). This axis further supported the coupling between root chemistry, morphology and anatomy that is consistent with Prediction 1: PC1 concentrated 42.31% of the trait variation from 19 root traits and was contributed relatively evenly by a suite of chemical, morphological and anatomical traits (Table S8). Almost all secondary protective features were negatively related to PC1 that had a positive relationship with root diameter and cortex but negatively correlated with SRL and branching intensity (Fig. 2c; Table S8). This root chemical–morphological gradient was differentiated by clade (pseudo $F = 19.32$; $P < 0.001$). Magnoliid roots tended to span a narrow space at one side that is associated with larger root diameter but low chemical protection, whereas rosid roots tended to occupy the opposite side of the spectrum but varied more widely (Fig. 2c).

Our analysis did not show an orthogonal conservation gradient linked to root density and N%, which is the foundation for Prediction 2 (i.e. protective investments align with the conservation gradient). Rather, density was strongly and negatively related to root diameter and cortex thickness ($P < 0.001$, $r^2 > 0.570$) while N% was associated with root diameter ($P = 0.009$, $r^2 = 0.197$, Fig. 2a). These two traits varied largely along PC1 that was linked with root diameter and SRL (Fig. 2c; Table S8), while PC2 were more associated with an inverse relationship between structural (BP, FAD) and nonstructural phenolics (e.g. HT, SP). Therefore, although roots with higher density and lower N% did show higher amounts of protective compounds as we predicted for a conservative strategy, caution is needed for interpretating these patterns.

Prediction 2 was also not supported in leaves. Leaf chemistry in general showed less covariance with other leaf traits (Fig. 2b, d). Consistent with the LES, leaf N% and C : N ratios varied with SLA ($P < 0.001$, $r^2 > 0.357$; Fig. 2b). Surprisingly, being the key driver of the LES, SLA did not correlate with any chemical protective features ($P > 0.251$). PCA showed that SLA aligned with N% and C : N, representing the LES, and primarily associated with PC2 (Fig. 2d; Table S8). PC1 was more closely linked to leaf density, DMC and chemistry. Interestingly, this leaf axis also exhibited an inverse relationship between structural (lignin, BP, FAD) and nonstructural compounds (CT, HT, SP, flavonoids). These chemical traits varied along an axis almost orthogonal to the LES (Fig. 2d), indicating that these chemical protection traits were largely decoupled from the LES.

We further examined the relationship between root chemistry and diameter, the dominant driver of the collaboration axis in previous global syntheses (Ma *et al.*, 2018; Bergmann *et al.*, 2020), by employing linear, allometric and piecewise models. The best-fitting piecewise models showed that lignin, CT, SP, and flavonoids tended to decrease sharply when root diameter increased from 0.2 to *c.* 0.6 mm, but this trend disappeared when diameter became larger (Fig. 3). Similarly, CT and N% also exhibited a two-phase relationship (Fig. 3). Within the rosid lineage, lignin, SP, CT and flavonoids were all negatively correlated with root diameter ($P < 0.046$; Fig. 3). In asterids, these protective features generally remained at a lower level in roots with larger diameter, but this negative relationship was only significant between lignin and diameter ($P = 0.035$, $r^2 = 0.235$). The decrease of these compounds with diameter was more a reflection of chemical modification than anatomical difference, as nonstructural compounds accumulated in roots with smaller cortex and lignin exhibited a stronger relationship with diameter than with pStel (Figs 2, 3). In magnoliid roots, these chemical groups did

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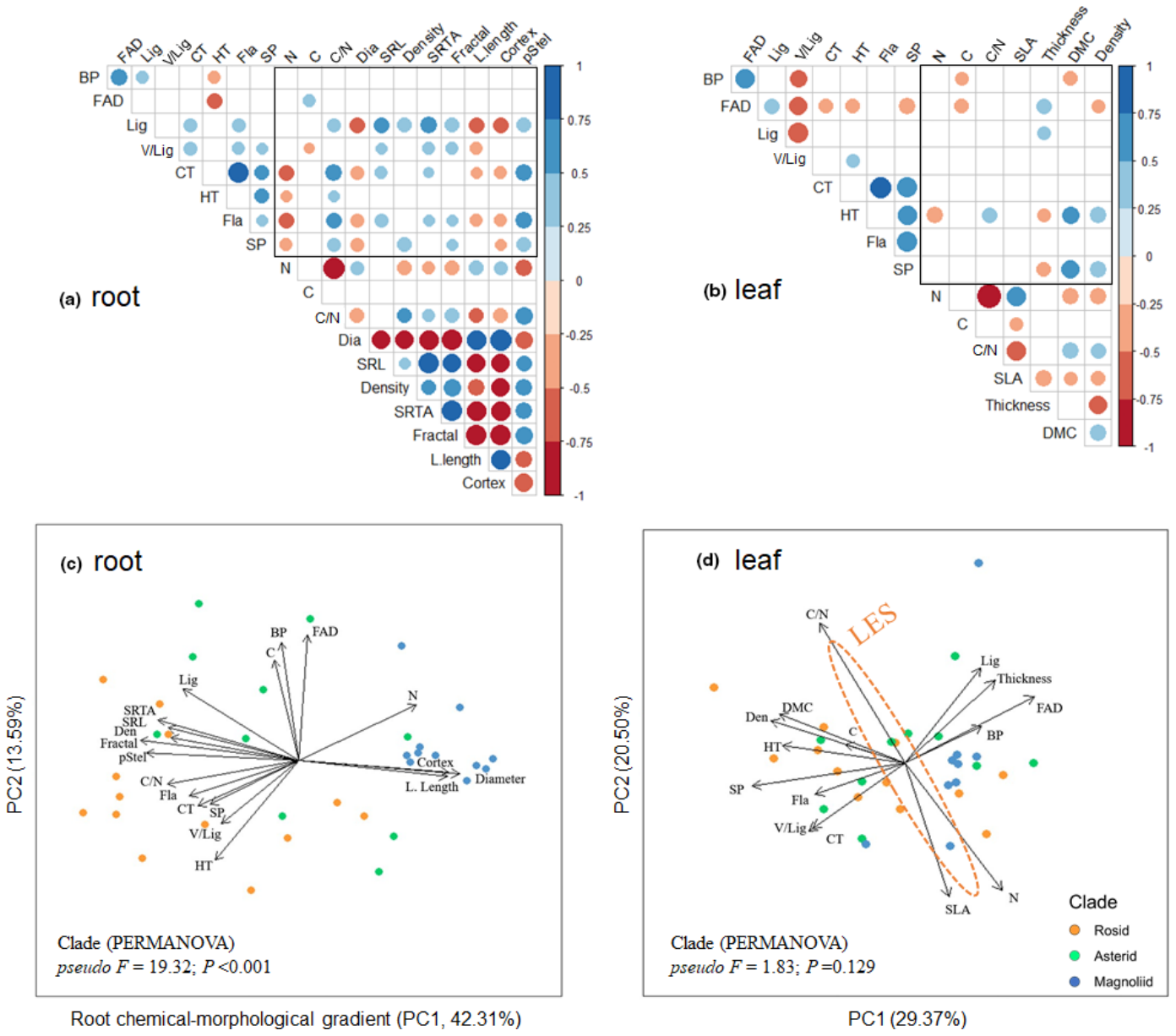

Fig. 2 Relationship between plant traits across angiosperm trees. (a, b) Correlation coefficient matrix of chemistry, morphology and anatomy for root and leaf traits. We focussed on eight chemical traits that extract key information from the measured chemical traits (for full list see Supporting Information Table S1): lignin and its composition (proportion of vanillyl moieties in lignin, V/Lig), bound phenols (BP) and the major cross linker in this pool (ferulic acids, FAD), three classes of nonstructural defense polyphenols (condensed tannins, CT; hydrolysable tannins, HT; flavonoids, Fla) and total soluble phenolics (SP). Specific root tip abundance (SRTA) and fractal dimension (Fractal) described root branching intensity while link length (L.length) estimates root length between two bifurcations. Significant ($P < 0.1$) coefficients shown with colors correspond to the legend indicating the strength and direction of the relationships. The squares highlight the relationship between compound-specific chemistry and other commonly reported functional traits. (c, d) Trait coordination in roots and leaves visualized by a principal component analysis (PCA, see Table S8 for trait loadings). Den, density; LES, leaf economics spectrum; pStel, fraction of root cross-sections occupied by the stele; SLA, specific leaf area; SRL, specific root length.

not show a significant relationship or slightly increased with root diameter (Fig. 3). We note that such biphasic relationships could be driven by the intrinsic difference between clades and/or a general effect of root diameter; however, these effects cannot be distinguished by our dataset as the large root diameters in magnoliids did not overlap significantly with those from later-derived angiosperms and thus need further research attention.

Because biosynthesis of lignins and CTs are more expensive than that of polysaccharides and proteins (Chapin, 1989; Hemingway & Karchesy, 1989), thin roots were estimated to divert considerably more energy for biosynthesizing these polymers than thick roots (Fig. S4). Species that develop thin root systems (< 0.6 mm in diameter) on average invested about two-fold higher energy to construct protective polymers than those bearing thicker roots (> 0.6 mm, $P <$ 0.001; Fig. S4), with the very thin roots from *Koelreuteria paniculata* investing up to eight-fold more energy than a typical magnoliid root, suggesting a significantly greater

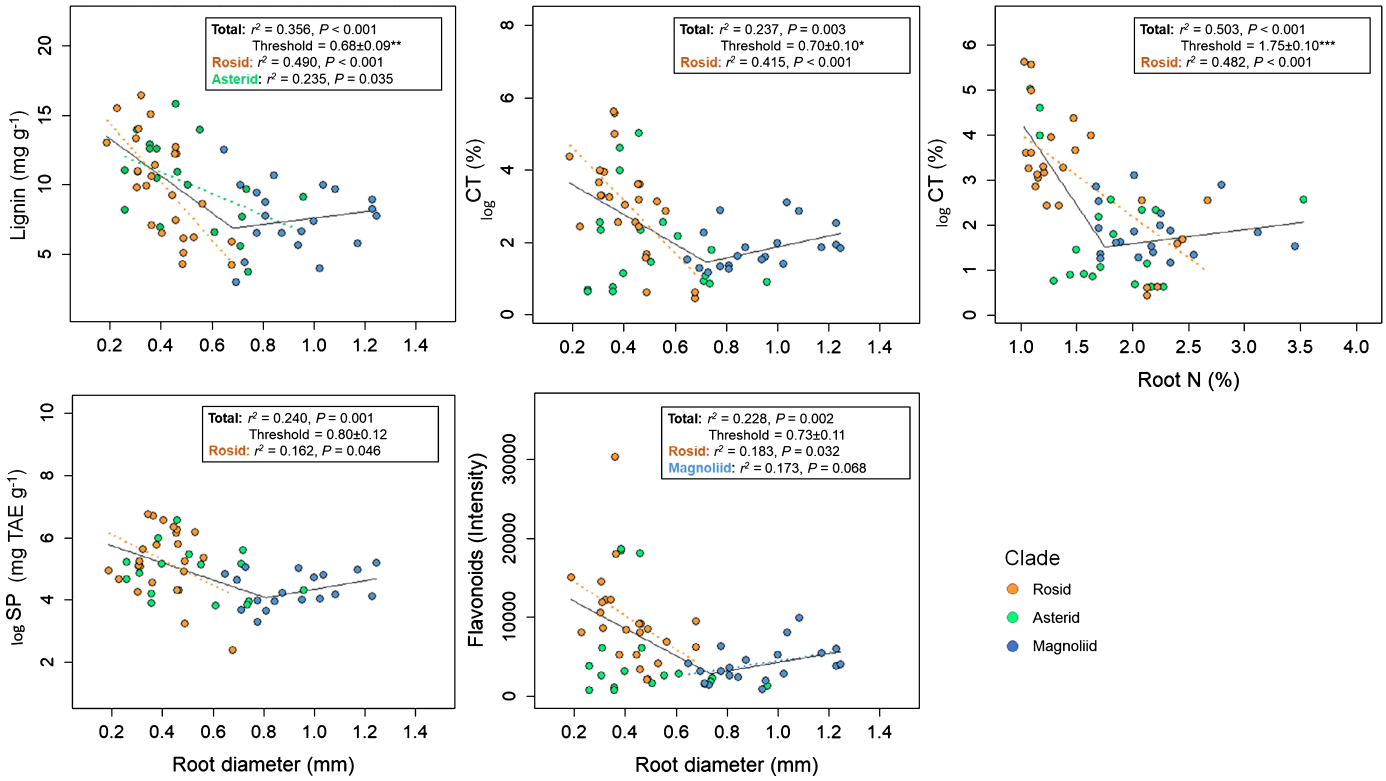

Fig. 3 Two-phase relationships between root traits across angiosperm trees fitted by a piecewise model (solid black lines). The significance threshold was tested with Davies' test: ***, $P < 0.001$; **, $P < 0.05$; *, $P < 0.1$. Soluble phenolics (SP) are expressed as tannic acid equivalents (TAE). The peak intensity of Flavonoids was mass-normalized and square root-scaled before data analysis. CT, condensed tannins.

amount of investment shifted away from growth and mycorrhizal fungi towards tissue preservation.

Linking trait coordination with mycorrhizal dependence

Consistent with Prediction 1.1, the coordination of root chemistry, morphology and anatomy was coupled with shifts in mycorrhizal colonization (Fig. 4), supporting the functional interpretation of the mycorrhizal-collaboration axis. PC1 (from Fig. 2c), which represents a gradient of root chemical, morphological and anatomical syndromes, correlated positively with the two proxies for mycorrhizal dependency: the percentage root length colonized by AM (microscopic quantification, $P < 0.001$, $r^2 > 0.500$) and AM fungal gene copies per mg dry mass (qPCR, $P < 0.001$, $r^2 > 0.525$; Fig. 4a). Higher PC1 values that represent thicker roots with lower protective compounds were linked to greater mycorrhizal associations. These positive relationships were also manifest within rosids (microscopic: $P = 0.078$, $r^2 = 0.254$) and asterids (microscopic: $P = 0.064$, $r^2 = 0.408$), but not within magnoliids that generally maintained high mycorrhizal colonization ($P > 0.300$). When included in the PCA, mycorrhizal colonization was indeed associated with the outsourcing side of the collaboration gradient (Fig. 4b; Table S8). By contrast, mycorrhizal status did not show a meaningful relationship with PC1 extracted from leaf traits that was linked to chemistry, nor with PC2 (the LES, $P > 0.164$; Fig. S5), indicating the organization of leaf traits in plants was largely decoupled from their association with mycorrhizae below ground.

Variance partitioning analysis summarized relationships of different variable categories to mycorrhization (Fig. 4c). Together, chemical protection, morphology, N status and anatomy accounted for 62% of the variation in mycorrhizal colonization. Although each category showed a large simple effect (> 32%), most of the explained variation (*c.* 86%) was attributed to the joint effects of two or more categories, with the largest effect intersection being the intercept of all four categories (22%; Fig. 4c). These large intersections cannot be unambiguously assigned to any specific category and further support that the coordination of a suite of roots traits spanning chemical protection, morphology and anatomy played an important role accommodating mycorrhizal growth in roots. Chemical protection (42%) and anatomy (54%) showed higher simple effects than morphology and N (< 35%), with chemical protection exhibiting a unique net effect (7%, $P = 0.081$; Fig. 4c). Although mycorrhizal dependency has been primarily linked to root morphology, our observations suggest a central role of root chemistry and anatomy in shaping the root–mycorrhiza partnership.

Coordination between roots and leaves

Consistent with Prediction 1.2, cell-wall chemical traits (BP, FAD, lignin and V/Lig) did not show significant relationships

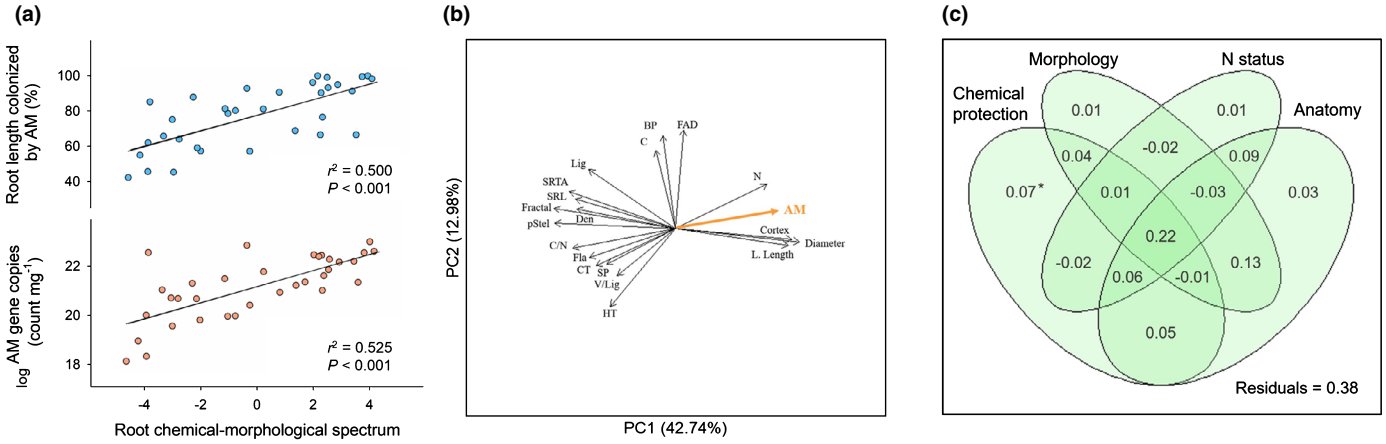

Fig. 4 Link between trait coordination and mycorrhizal association in roots across angiosperm trees. (a) The relationship between the root chemical–morphological spectrum (PC1 from Fig. 2c) and mycorrhizal colonization. (b) Coordination of root functional traits and arbuscular mycorrhizal (AM) association visualized by a principal component analysis (PCA, see Supporting Information Table S8 for trait loadings). AM was the first principal component extracted by a separate PCA from the two AM association proxies and explained 86.3% of their variations. (c) Venn diagram summarizing the variation in mycorrhizal association (gauged by both microscopic and qPCR evidence) explained by chemical protection, morphology, N status and anatomy of roots. The intersections represent variation that is jointly explained by two or more variable categories and cannot be unambiguously linked to a specific category. The net effect of chemical protection was marginally significant (*, $P = 0.081$). Note that intersections cannot be tested statistically.

between roots and leaves ($P > 0.136$; Table 1), indicating the decoupling of cell-wall construction between above- and belowground organs. Diameter, SRL and tissue density in roots also did not scale with leaf thickness, SLA or density ($P > 0.327$). However, the abundance of CTs, HTs, SPs, flavonoids and N% between leaves and roots showed significant relationships ($P < 0.011$), ranging from relatively weak (e.g. flavonoids, SPs) to strong (HTs), suggesting whole-plant regulation on these chemical classes and N utilization at various degrees. The scaling exponent for flavonoids was 0.267, indicating a disproportionate relationship between roots and leaves: a 10-fold increase of total abundance of flavonoids in roots only corresponds to a 1.8-fold increase in leaves. These observations demonstrated that the morphological adjustment, cell-wall strengthening and accumulation of flavonoids in roots were tissue-specific and not mirrored by leaves. In addition, the chemical–morphological gradient of roots (PC1 from Fig. 2c) was decoupled from leaf tradeoffs along the LES ($P = 0.819$, two-tailed Pearson's test).

Phylogenetic structuring of root and leaf traits and their covariance

Root traits were in general phylogenetically structured, whereas evolutionary history plays a lesser role on leaf traits (Fig. 5). Phylogeny structured root cell-wall features such as FAD, V/Lig and S/Lig ($K > 0.562$, $\lambda > 0.725$) and nonstructural protective compounds such as CTs, HPs, SPs, total flavonoids and seven individual flavonoids ($\lambda > 0.492$; $K > 0.524$, Figs 5, S4). Not only were individual root traits phylogenetically distributed, the root chemical–morphological gradient as well as mycorrhizal association also exhibited strong phylogenetic signals (Table S4), indicating that root strategies were structured by shared ancestry. In leaves, cell-wall features, elemental composition, morphology and the LES axis were not influenced by phylogenetic relationships; however, 37.5% of traits in nonstructural secondary compounds exhibited similar phylogenetic patterns to those found in roots

Table 1 Scaling relationships based on standardized major axis tests between root and leaf traits on log–log scales.

Traits	*P* value	r^2	Slope, or scaling exponents (95% confidence intervals)	PGLS *P*	PGLS r^2
log BP	0.136				
log FAD	0.275				
log lignin	0.780				
log V/Lig	0.333				
log CT	**< 0.001**	**0.524**	1.082 (0.835, 1.401)	**0.005**	**0.210**
log HT	**< 0.001**	**0.693**	0.867 (0.703, 1.068)	**< 0.001**	**0.603**
log flavonoids	**0.010**	**0.207**	0.267 (0.191, 0.372)	0.253	0.012
log SP	**0.011**	**0.202**	0.890 (0.638, 1.242)	0.288	0.006
log N	**< 0.001**	**0.384**	0.803 (0.599, 1.078)	**< 0.001**	**0.329**
log SLA vs log SRL	0.327				
log Thick vs log Dia	0.775				
log Tissue density	0.862				

The slopes represent 'scaling exponents' (Wright *et al*., 2004) of leaf traits vs root traits (95% confidence intervals in parentheses) that describe the proportionality (when slopes are close to +1 or −1) or disproportionality (when slopes are far from +1 or −1) between variations of leaf and root traits. We also estimated the relationships between roots and leaves after removing phylogenetic components from trait variation using phylogenetic generalized least squares (PGLS) analysis. Bold numbers: $P < 0.05$. BP, bound phenols; CT, condensed tannins; Dia, diameter of roots; FAD, bound ferulic acids; HT, hydrolysable tannins; SP, soluble phenolics; SLA, specific leaf area; SRL, specific root length; Thick, leaf thickness; V/Lig, the proportion of vanillyl moieties in lignin.

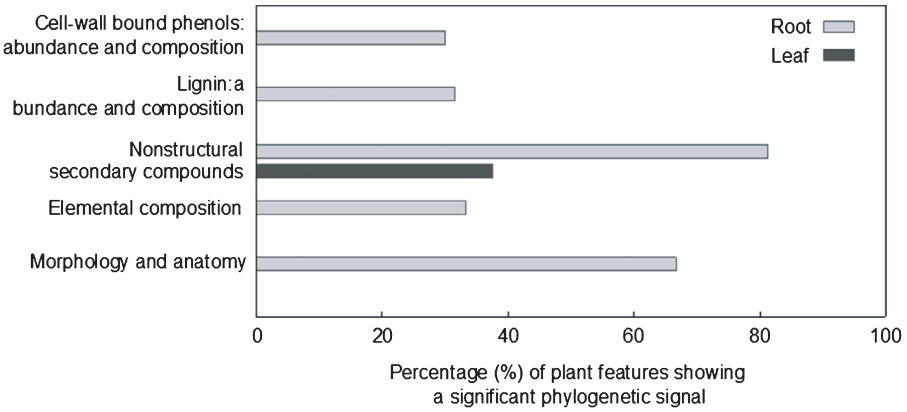

Fig. 5 Percentage of the traits showing significant phylogenetic signals in cell-wall-bound phenols (15 features), lignin (19 features), nonstructural secondary compounds (16 features), elemental composition (three features), morphology and anatomy (nine features for roots; four features for leaves) in roots and leaves. The list of traits, the significance and strength of phylogenetic signals are shown in Supporting Information Table S4 and S5.

(Fig. 5; Table S5). Interestingly, these compounds also showed correlations between roots and leaves (Table 1). After removing phylogenetic effects, these relationships were lower or insignificant (except for HT, Table 1), suggesting an important role of phylogeny shaping the coordination of nonstructural defense compounds between roots and leaves.

We next removed the phylogenetic component from trait variation using phylogenetically informed analysis. The phylogenetically corrected correlation analysis indicates an important role of shared ancestry structuring root trait coordination while the general tradeoffs between chemical protection, morphology and anatomy in roots were still manifest after removing phylogenetic effects. The relationships between root chemistry and other traits were generally reduced after controlling for phylogeny (Fig. S6), suggesting that evolutionary divergence at high levels had an organizing role on root trait covariance. However, the relationships between lignin and morphological/anatomical traits remained relatively strong after removing phylogenetic effects ($P < 0.049$, r^2 values ranged from 0.115 to 0.307). Flavonoids and CT were still positively and strongly related with pStel ($P < 0.002$, $r^2 > 0.253$) and less strongly with SRL ($P < 0.078$, $r^2 > 0.094$); SP also maintained a positive relationship with pStel ($P = 0.045$, $r^2 > 0.120$, Fig. S6a). Similarly, pPCA, which removes phylogenetic covariance from the axes, still showed a primary axis associated with SRL, diameter and lignin, with the loadings of FAD and BP strengthened, indicating a strengthening of the cell-wall matrix with thinner roots regardless of phylogenetic structure (Fig. S6c; Table S8). The loadings of nonstructural compounds (CT, Fla, SP) and N% to the primary axis, maintaining the same directions, were lower after phylogenetic correction; these traits clustered along tradeoffs between N% and nonstructural phenolics. However, higher AM colonization was still associated with lower amounts of both structural and nonstructural protective compounds (Fig. S6c; Table S8). These observations supported a general adaptation of cell-wall strengthening and accumulation of protective compounds when tree roots devoted less area to cortex and depended less on outsourcing regardless of their ancestry. For leaves, the two main axes (the LES and the axis demonstrating the inverse relationship between nonstructural and structural protective compounds) maintained similar patterns after controlling for phylogeny (Fig. S6; Table S8).

Discussion

Here we tested the generalizability of leaf and root resource economics hypotheses after the incorporation of compound-specific chemistry and examined whether the ecological strategy was coordinated at whole-plant levels or specific to above- and belowground organs. Our data showed that chemical protective features were not associated with the LES in leaves but aligned with a mycorrhizal-collaboration gradient in fine roots where greater chemical protection was associated with thinner roots with a smaller cortex and lower mycorrhizal dependency. This mycorrhiza-aligned trait syndrome was unique to roots and not mirrored in leaves, which resonates with previous comparisons between leaves and roots of woody plants (2015, 2020) and suggests strongly that root and leaf traits evolve independently due to very different selective pressures.

Although our data confirmed the LES, which reflects acquisitive–conservative tradeoffs in leaves, conservative leaves did not accumulate higher amounts of compounds that provide tissue protection. Rather, protective compounds generally showed a weak relationship with SLA and varied along an axis largely independent from the LES (Fig. 2), suggesting that the driving force underlying these compounds was decoupled from the photosynthetic tradeoff described by the LES. Interestingly, this leaf chemical axis demonstrated a tradeoff between structural and soluble phenolic investments in leaves irrespective of phylogenetic structure (Figs 2, S6), seemingly reflecting their competition for the common precursors in the phenylpropanoid pathway. A study of tropical canopy trees also found a similar defense spectrum that was orthogonal to the LES and associated with varying levels of SPs (Chauvin *et al.*, 2018). This and our study suggest a general defense tradeoff in leaves that may be widespread in trees.

Roots exhibited a very different trait organization from leaves. Consistent with global synthesis studies (Ma *et al.*, 2018; McCormack & Iversen, 2019; Bergmann *et al.*, 2020), we observed a primary collaboration gradient linked to SRL and diameter (Fig. 2).

More interestingly, we found a close relationship between root chemistry, morphology and anatomy in a manner that aligns with the collaboration gradient (Figs 2, 3). Roots that were thicker and lower in SRL, corresponding to a 'outsourcing' strategy, tended to branch less, devote more volume to cortex, and maintain a lower level of protective compounds such as CTs, SPs, flavonoids and lignin, which also tended to have a lower proportion of V moieties that can form resistant aryl–aryl linkages (Talbot *et al.*, 2012). By contrast, thinner, more branched roots tended to accumulate a high level of V-rich lignin and nonstructural protective compounds and invest more energy to construct costly protective polymers (Figs 2, 3, S4). This unique chemical–morphological–anatomical coordination in roots may arise from the selective pressure that generates a particular tradeoff between enhancing chemical protection and optimizing for hosting symbionts (*sensu* Brundrett, 2002). Indeed, this gradient aligns with mycorrhizal colonization (Fig. 4), supporting the functional interpretation of the collaboration axis and suggesting that roots were fine-tuning chemistry, morphology and anatomy along a trait syndrome to achieve different levels of mycorrhizal association (Figs 2–4). Thick-rooted species that depended strongly on outsourcing generally have a large cortex and sustain a reduced chemical defense to maintain a suitable habitat for symbionts. In addition, high costs of mycorrhization that may draw plant allocation at the expense of chemical defense (Barazani *et al.*, 2005; De Deyn *et al.*, 2009) could further stabilize the low level of secondary compounds. By contrast, thin, more branched roots maximize surface area to efficiently explore soil volume and intercept nutrients, becoming efficient root systems themselves and rendering symbiosis less necessary. The lower mycorrhizal dependency would allow a smaller cortex and spiking of protective compounds that could benefit a 'do-it-yourself' strategy: higher lignin was linked to greater mechanical strength that helps roots to penetrate the soil matrix (Schneider *et al.*, 2021), while exploring of larger soil volume poses a greater chance of contact with herbivores and pathogens and may require enhanced defense.

We did not observe an orthogonal density–N% axis that was expected to be independent from the collaboration axis; rather, root tissue density was closely related to root diameter and SRL (Fig. 2). Previous work showed that this proposed conservation axis was less consistent across plant types than the collaboration axis (Bergmann *et al.*, 2020). The observed relationship between density and diameter is consistent with previous studies that also showed significant negative relationships between these two traits across more comprehensive sets of species (Kong *et al.*, 2014, 2019; Ma *et al.*, 2018). Such a negative relationship aligns with the shift in root anatomy. As diameter decreased, pStel increased (Fig. 2); because stele tissues have higher dry weight than cortex (Hummel *et al.*, 2007), the increased pStel could lead to a higher tissue density. In addition, root N% showed low or no correlations with SRL, diameter and density but was more closely related to nonstructural phenolics (Figs 2, S6). The link between root N% and 'acquisitive' potential seems less clear compared to leaf N% (Freschet *et al.*, 2020), as root N% plays multiple functions (e.g. assimilation, transport, storage, defense) and may be confounded with the N deposited in fungal tissues. The relationship between N% and nonstructural phenolics may reflect tradeoffs along a conservative–acquisitive gradient, shifts between phenolic and N-based defenses, or be driven by their relationships with AM colonization. Taken together, the coordination of N% and density to other root traits appears more sensitive to the scope/type of trait data than the SRL–diameter gradient, and their functional implications may be more complex than expected from the conservative gradient.

Our data confirmed the previously observed phylogenetic effects on root morphology (Ma *et al.*, 2018) and revealed that phylogeny also shaped the distribution of many protective compounds (e.g. FADs, SPs, CTs, HTs, flavonoids; Fig. 5; Table S4). Phylogenetic structuring was not only widespread in individual root traits, but also present in the root chemical–morphological gradient and mycorrhizal colonization (Fig. 5; Table S4). Roots optimized their chemistry, morphology and anatomy in a coordinated manner towards two distinct root strategies that clustered in different evolutionary lineages: becoming good hosts for fungal partners or growing efficient root systems (Figs 2–4, 6). The good hosts appear to be common in basal angiosperms, although similar roots may occur in more-derived families (Valverde-Barrantes *et al.*, 2020). Efficient root systems are more common in the rosid clade, which also showed a remarkable diversity in chemical composition. Rosids went through rapid radiation in the Cretaceous when they formed novel partnerships with other microbial groups (Wang *et al.*, 2009). Our data showed that the large diversification in chemical composition and other root traits had already occurred in rosids that form AM symbiosis, suggesting that this chemical/morphological diversification may have set the stage for novel microbial partnerships. Although root traits were phylogenetically structured in general, accounting for phylogeny only notably reduced the covariance between morphology and nonstructural phenolics, while the relationships between lignin and morphology, and those between root anatomy, protective compounds and AM colonization persisted (Fig. S6), suggesting the tradeoff between chemical protection and mycorrhizal association observed in this study may be a general factor shaping root trait diversity in trees regardless of their phylogenetic relationships. Further studies linking chemical composition, morphology and mycorrhizal colonization across a wider set of species, particularly from subtropical and tropical latitudes, is warranted.

Together, we found coordination between root chemical protection, morphology and anatomy that aligns with mycorrhizal association, strengthening the idea of a coordinated ensemble of strategies along a functional gradient of outsourcing with mycorrhizal partners. We also confirmed the role of phylogenetic structure clustering these syndromes along angiosperm lineages. These contrasting strategies may further impose divergent consequences on biogeochemical cycles (Fig. 6). Because secondary compounds impact soil C and N transformations, our observations shed new light on linking species identities and root strategies with ecosystem-level biogeochemical processes. Previous studies have revealed the prominent influence of secondary compounds on litter degradability, where phenolic features such as the abundance of lignin, CTs and BPs controlled litter decomposition (Berg, 2000; Sun *et al.*, 2018). CTs also inhibit N mineralization by

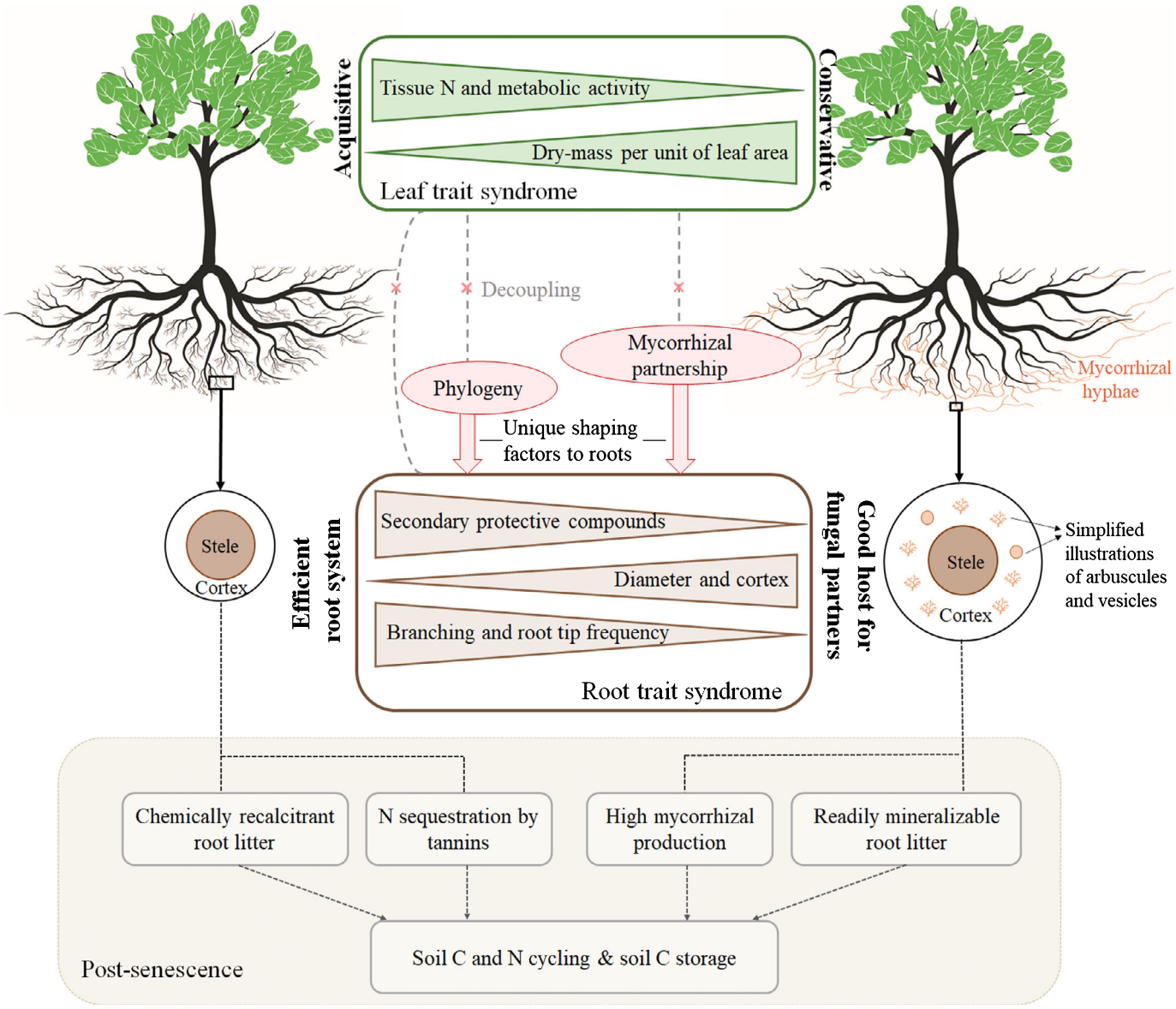

Fig. 6 Schematic representation of leaf and root trait coordination that reflects above- and belowground ecological strategies. While leaf traits follow a broad pattern ranging from 'acquisitive' to 'conservative', roots shift from being good hosts for arbuscular mycorrhizal fungi to developing efficient root systems with better chemical protection. The organization of root traits is closely linked to degrees of mycorrhizal association and structured by phylogeny, while these two factors do not play a significant role in shaping leaf acquisitive–conservative tradeoffs. Root strategies may further influence soil carbon (C) and nitrogen (N) cycling via impacting chemical composition of root litter that enters the soil: efficient root systems input low-quality root litter to the soil and may produce high amounts of tannins that sequester N in a mineralization-resistant organic form, while good hosts tend to produce high-quality root litter that may undergo fast C mineralization and quickly releases N in inorganic forms.

sequestering organic N sources (Tharayil *et al.*, 2013). Efficient root systems, often from the rosid lineage, tended to produce abundant V-rich lignin and CTs. Therefore, we expect efficient roots to exhibit chemical recalcitrance and thus slow litter degradation or greater importance of a physical route (i.e. fragmentation), rather than a microbial route on degradation (Cotrufo *et al.*, 2015; Minerovic *et al.*, 2018). Because roots represent 46% of terrestrial C fixation globally (Gherardi & Sala, 2020) and contribute two-fold more to soil organic C than shoots (Rasse *et al.*, 2005), we expect that root strategies and evolutionary history have a far-reaching consequence on terrestrial biogeochemical cycles by shaping the chemical composition of root litter.

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Author contributions

MX, OJV-B, CBB and NT conceived the idea, and OJV-B and CBB designed the collection of species included in this study, collected the samples, and performed analysis for morphology, anatomy, and mycorrhizal colonization. MX and NT performed

chemical analysis. MX performed data analysis and wrote the draft of the manuscript with critical feedback from OJV-B, VS, CBB and NT.

Data availability

The database to generate phylogenetic relationships in this study is available at http://www.onezoom.org/tree_index.html. The global dataset in Fig. 1 is from Kong *et al.* (2019). The morphology and elemental composition data are deposited in the online data repository Dryad (doi: 10.5061/dryad.53mc6). Compound-specific chemistry, anatomy and mycorrhizal colonization data are available upon request from the corresponding authors.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Phylogenetic relationships for 34 tree species spanning three major angiosperm lineages.

Fig. S2 Locations of study sites.

Fig. S3 Matrix of Spearman's rank correlation coefficients of root and leaf traits across angiosperm trees.

Fig. S4 Estimated metabolic costs for the biosynthesis of protective polymers in roots across angiosperm trees that developed roots of different sizes.

Fig. S5 The lack of a relationship between mycorrhizal status and principal components of leaf trait variation.

Fig. S6 Phylogenetically informed relationships in root and leaf traits.

Methods S1 Instrumental parameters of GC-MS and UHPLC–Orbitrap MS.

Table S1 List of plant traits, abbreviations, and descriptions of properties and ecological functions of chemical traits.

Table S2 Retention times and characteristic mass fragments of authentic phenol standards.

Table S3 Model performance measured by mean square errors (MSEs), Akaike's information criterion (AIC) and r^2.

Table S4 Significance and strength of phylogenetic signal indices for root chemical traits, elemental composition, morphology and anatomy in angiosperm trees.

Table S5 Significance and strength of phylogenetic signal indices for leaf chemical traits, elemental composition and morphology in angiosperm trees.

Table S6 Linear mixed model analysis and summary description of root traits in trees spanning three major angiosperm lineages across two study sites.

Table S7 Linear mixed model analysis and summary description of leaf traits in trees spanning three major angiosperm lineages.

Table S8 Eigenvalues of principal components from the PCA and phylogenetically informed PCA and loadings of root and leaf traits.

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